

Article

Preparation, Characterizations and Biological evaluations of new Nickel Bis (Azo-Salphen) Complex.

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Abstract

A new bis azo-salphen ligand (Azo Salicylaldehyde phenylenediamine Schiff base) was synthesized through an initial preparation of the salphen Schiff base via the condensation reaction between salicylaldehyde and o-phenylenediamine, which was subsequently followed by a coupling reaction involving the diazonium salt derived from p-amino acetophenone with the Schiff base component to yield the desired ligand (1,1'-(((1E,1'E)-(((1E,1'E)-(1,2phenylenebis(azanylylidene))bis(methanylylidene))bis(4-hydroxy-3,1-phenylene))bis(diazene-2,1-diyl))bis(4,1-phenylene))bis(ethan-1-one), this new ligand was characterized with many spectroscopic techniques as (FTIR, Mass, NMR, Uv-Visible and CHN Elementary analysis).

Its nickel complex was prepared and characterized with the previous techniques , certainly, the aforementioned supplementary methodologies, such as molar conductivity and magnetic susceptibility, alongside X-ray diffraction, were employed to ascertain the morphology and to propose its geometric configuration.

The ligand and its nickel complex were biologically examined from three point views: antibacterial zone of inhibition, antioxidant ability and breast cancer MCF7 cell line assay. The results were appointed that the nickel complex more efficient in the inhibition of bacterial and cancer cells, while the azo-salphen ligand has more antioxidant ability than its Nickel Complex.

Keywords :- azo-Salphen Schiff, identification, bio-evaluation, TM complexes , 4,5-diphenyl imidazole ,beta -Naphthol.

Introduction:-

Azo compounds are classified as a category of organic substances characterized by the presence of a nitrogen-nitrogen double bond ($N=N$) as a functional group.¹ These chemical compounds are characterized by their intense hues and extensive range, encompassing yellow, red, and blue², and find a huge application as dyes and pigments across diverse industries, including food³, cosmetics⁴ and textiles⁵.

In addition to their application as colorants and chromatic substances, azo compounds have also found utility in the realm of pharmaceutical science as prodrugs⁶, which refer to inert compounds that undergo metabolic transformations within the organism to generate a bioactive pharmaceutical agent. Certain azo compounds have also undergone scrutiny for their promising capabilities as anticancer agents⁷, although further exploration in this field is warranted.

Salen azo compounds represent a distinct category of azo compounds, boasting the inclusion of a Schiff base as a captivating ligand⁸. Schiff bases, on the other hand, epitomize organic compounds harboring a nitrogen atom that gracefully establishes a double bond with a carbon atom. The artful synthesis of salen azo compounds is accomplished through the harmonious reaction between a salen ligand and an azo compound, thereby birthing a complex with a nitrogen-nitrogen double bond ($N=N$) as an enchanting functional group.⁹ Salen azo compounds have been explored as catalysts in a many chemical reactions, such in the oxidation of alcohols¹⁰ and polymerization of olefins. Furthermore, their captivating nature has captivated researchers as they delve into their potential as sensors and molecular switches¹¹, This captivating quality stems from their remarkable ability to undergo reversible color metamorphosis in response to alterations in their surrounding ambiance. indicating their compatibility with optical devices¹²

A variety of applications exist in different domains for Azo complexes, they have been the subject of investigation as antimicrobial agents, specifically for treating infections caused by MRSA antimicrobial-resistant pathogens¹³. Furthermore, Azo complexes have undergone scrutiny for their ability to hinder microbial growth, demonstrating promise as antibacterial and antifungal agents¹⁴. Moreover, these complexes have been explored for their potential usefulness in nonlinear optical (NLO) applications¹⁵, additionally, scientists are currently delving into the biomedical

applications of Azo compounds, particularly in the realm of cancer research, encompassing cancer diagnosis and therapy ¹⁶. Azo metal complexes have also been created to serve as charge control agents for toners, providing an alternative to trivalent chromium Azo complexes while minimizing environmental and human health concerns ¹⁷.

2.Experimental part.

2.1.Techniques and methods

The whole Chemicals and Solvents were commercially, and used without further purification . FTIR- ATR was used for obtaining FTIR spectra, the magnetic resonance spectra's was done using a Bruker spectrometer (400 MHz ⁻¹H NMR and 100 MHz ⁻¹³C NMR) using a deuterated d₆-DMSO as a solvent , The mass spectra of the synthesized compounds was developed using a ESI-MS technique with MSD Direct probe using ACQ method low energy M.A UV_6100PC double beam spectrophotometer was utilized for UV-Visible spectra's observation , the metal ion in complexes was determination by using Shimadzu 6300 AA spectrophotometer, Elementary analysis (C.H.N) were recorded using elemental analyzer EuRo VECTOR. The pH meter model WTW 315i was used for recording the PH values measurements, WTW Ino Lab Cond 720 conductivity meter was used for the determination of the molar conductivity at room temperature in DMF solvent, the magnetic susceptibility measurement of the complex was assigned using Auto Magnetic susceptibility Balance, Sherwood, The ligand was synthesized as the procedure described.

2.1.1 preparation of 2,2'-((1E,1'E)-(1,2-phenylenebis(azanylylidene))bis(methanylylidene)) diphenol Schiff base **Salphen**:

Salphen was prepared using the procedure of the furniss¹⁸ with some modification, that In a 250ml round-bottom flask, the reaction mixture comprising the solvation of (20mmole, 2.12mL) salicylaldehyde and o-phenylenediamine (10mmole, 1.08gm) in 50 mL of heated ethanol, with the incorporation of a 0.5ml of glacial ACOH as a

reaction activator, was subjected to reflux for a duration of 6 hours, while the progress of the reaction was monitored utilizing the TLC technique. Subsequently, the reaction mixture was cooled in an ice bath, resulting in the formation of the Schiff base, which was subsequently subjected to filtration and recrystallization through the application of hot ethanol prior to undergoing a drying process, Salphen was a curcumin color solid, with a formula of $C_{20}H_{16}N_2O_2$ and yield 80%; M.P. 101° - 103° ; (TLC, n-hexane: ethyl acetate 2:3, R_f : 0.81); IR data (cm^{-1}): 3358 (Sal OH), (3068) Azomethene proton, (1644) azomethene C=N group, (1590) aromatic C=C group and (1360) for ν C-N.

2.1.2 preparation of the azo-Schiff ligand (L)¹⁹:

Azo-Schiff ligand was prepared via the addition of the diazonium salt of *p*-aminoacetophenone and coupling it with **Salphen** Schiff base as in the following :- a (10 mmole, 1.35gm) *p*-aminoacetophenone in a cold 20% HCl solution in iced water bath ($0-5^{\circ}$), a (20mM, 1.38gm) sodium nitrite solution was dropwise to the acidic solution at $5^{\circ}C$ for 15 min., for complete the diazonium salt formation, the whole solution still a way for 10 min.

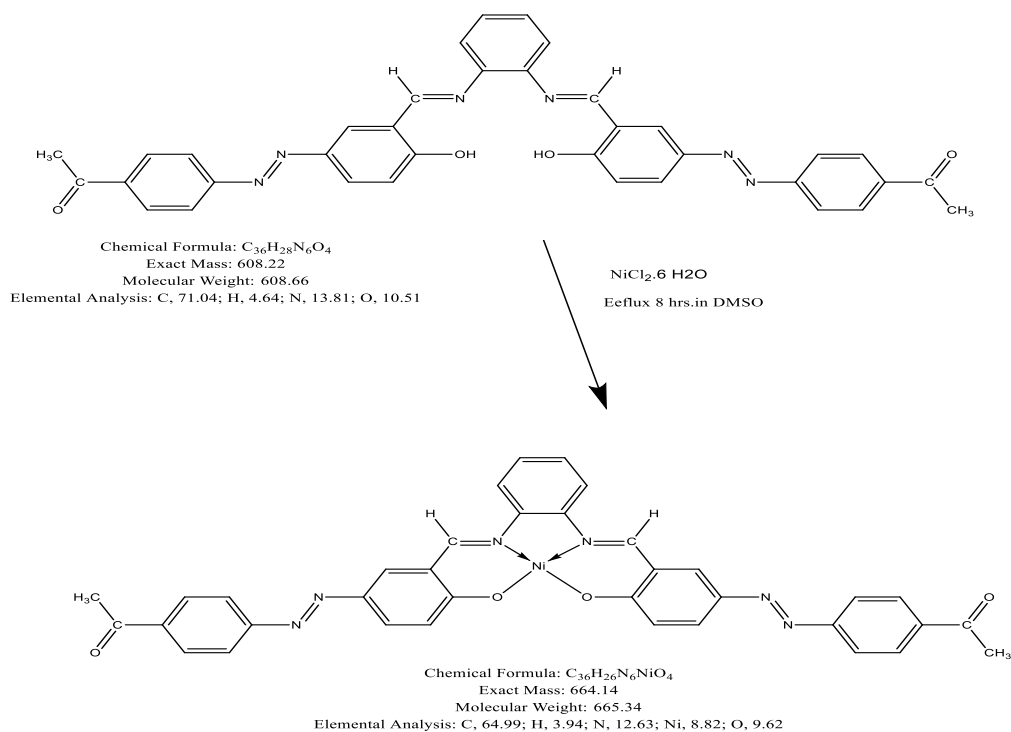
the coupling component of the Salphen (5 mmole, 1.58gm) was solvated in 10% alkali basic ethanolic solution and treated at the same temperature, coupling process was preformed via a slowly and gradual addition of the diazonium salt to the coupling component, taking in account the continuous stirring at the previously temperature, whereupon another color was developed due to the formation of azo compounds. Stirring was maintained for a duration of 30 minutes, after which the solution was neutralized. To ensure the completion of the diazotization process, an azo-Schiff colored precipitate was formed, which was then subjected to filtration and washed twice with deionized distilled water before being dried.

The azo-Salphen **L** was a yellowish orange solid with a ($C_{36}H_{28}N_6O_4$) formula, M.Wt 608.22 g/mole, Anal. Calc. C 71.04, N 13.81, H 4.64 and O 10.51 found C 71.77, N

18.31, H 4.22 and O10.32, yield 69 %, M.P. 203-205⁰C , **FTIR** data (cm⁻¹): 3315 (Sal O-H str.), 3063 (aldehyde C-H atom str.), 1657 (Ac-carbonyl), 1591 (C=N str.), 1577 (C=C ring str.), 1490 (azo N=N str.), 1398 (C-N str.), **¹H-NMR** (300 MHz, DMSO-*d*₆): δ 12.93 (s, 2 H, Sal.OH), 8.93 (s, 2H, Azomethine-H), 6.95-7.67 (m, aromatic ring protons) and 3.13 (s, 6 H, carbonyl H's), **¹³C - NMR** (75 MHz, DMSO-*d*₆): δ 197.29 (2C of carbonyl gps.), 158.41 (2C, C.OH atoms), 154.40 (2C, azomethine carbons), 122-138 (multiplet, Ar. Ring carbons), 26.95 (2C – carbonyl methyl groups CH₃). **Mass data Z/e** , M 608, M+1 609 .C₁₇H₁₈N₃O₂⁺ 316.

2.1.3 Synthesis of the Nickel Complexes

The nickel complex was synthesized²⁰ through the dissolution of (1 mmole, 0.608 g) of the azo-salphen ligand in 20 ml of ethanol and (1 mmole, 0.237 g) of NiCl₂·6 H₂O in 10 ml of ethanol, which was then gradually introduced into the aforementioned solution; subsequently, the resultant mixture was subjected to reflux for a duration of 8 hours. The incorporation of diethyl ether into the mixture at ambient temperature facilitated the formation of a brown precipitate, which was then filtered and thoroughly washed several times with ether before being dried under vacuum conditions. The NiL complex resulted in a brown solid characterized by a specific formula (NiC₃₆H₂₈N₆O₄) , MWt 664.14 g/mole , Anal. Calc.C 46.99, N 12.63, H 3.94, O 09.62 and Ni 8.82 found C 46.96, N 12.04, H 3.95, O 09.74 and Ni 8.95, yield 79%, M.P. 280-281⁰ C, **IR** data (cm⁻¹): 3089 (Ar-H), 2979, 2844 (C-H alkyl, sym. & assym.), 1580 (C=N str.) , 1508 (C=C ring str.), 1457 (N=N str.), 1391 (C-N str.), shown in fig.2 **¹H-NMR** (300 MHz, DMSO-*d*₆): δ 8.93 (s, 2 H, azomethine protons) 7.67 -6.95 (m, 14H, Ar-H), 3.13 (s, 6 H, Ac protons) , **Mass data Z/e** , M .665.607.



Scheme 1. Route of complexes preparation

2.2. Antibacterial test for metal complexes

The new ligand and its nickel complex antibacterial test (Minimum Inhibitory Concentration MIC and Minimum Bactericidal Concentration MBC) were done with the method of the approved guideline M26-A. that mentioned by Mahdieh et.al²¹.

2.3. antioxidant Assay

A reimagined Brand–Williams technique, unveiled by Miliauskas et al.²², was employed to assess the antioxidant potential through the DPPH radical-scavenging prowess of the crafted samples.

2.4. MCF7 Cell line protocol

The MTT Protocol that followed for MCF-7 cell line for human breast cancer ,was obtained from (ATCC, Manassas, VA, USA), the protocol of the cell line was described by Dhekra and Saad ²³ and the findings were presented as the average derived from three separate experiments.

The concentrations of samples exhibiting a 50% decline in cell viability (namely, IC₅₀ values) were subsequently determined.

3. Results and Discussion

3.1. synthesis

Each of the ligand and its nickel complex manifested as solid powders, exhibiting hygroscopic properties and an insoluble nature in water, yet they dissolved effortlessly in some organic solvents such as acetone, DMSO, dichloromethane, ethanol, DMF, methanol, and chloroform. They identified through many techniques such as Mass spectrometry, NMR, FTIR, UV-visible, indeed to magnetic susceptibility and molar conductance, the analytical findings harmonizing splendidly with the assumed values, where the expected formula [ML] consisted of M = Ni(II) demonstrating a [M:L] 1:1 stoichiometry, the analytical results alongside the physical characteristics have been meticulously displayed in the table.1

Table 1. physical properties of the synthesized compounds

Compound	FW gm/mole	m.p	yield	Elemantary analysis Found (cal.) %				$\Lambda_M \Omega^{-1} \text{cm}^{-1} \text{mol}^{-1}$	Electronic transition
				C	H	N	M		
C₃₆H₂₈N₆O₄	608.20	122-	85%	71.77	4.71	13.81		-----	380 nm
Azo-Salphen L		124		(71.04)	(4.64)	(13.18)	-----		
C₄₈H₄₀N₁₀Cl₄	664.14	239-	70%	65.44	3.98	12.94	9.12	18	448 nm
NiL		241		(64.99)	(3.94)	(12.63)	(8.82)		344 nm

3.2. Mass spectral study

A mass spectrometry technique represents a crucial instrument that elucidates the structural characteristics of a vast array of organic and inorganic compounds through the precise determination of their masses²⁴, the elucidation of their molecular

configurations, and the proposition of their fragmentation pathways, thereby facilitating the investigation of underlying mechanisms concurrently.

The mass spectra of the ligands and its Nickel complex were documented under ambient temperature condition, Utilizing them for the purpose of comparison with the proposed stoichiometric formulations, the ligand shows a molecular peak at 608 m/z and it agreed to the ligand mass and some spectra fragments were comparable to these of the in the fragmentations route suggested such 317, 119 and 77, this emphasizes the synthesis of the same compound.

while the nickel complex spectra shows a mother ion peak at 665 m/z that equivalence to its mass, and the ligand fragment was also observed at 607 m/z . indeed to the 317 fragment as in fig.1 and 2, that agreed with their masses.

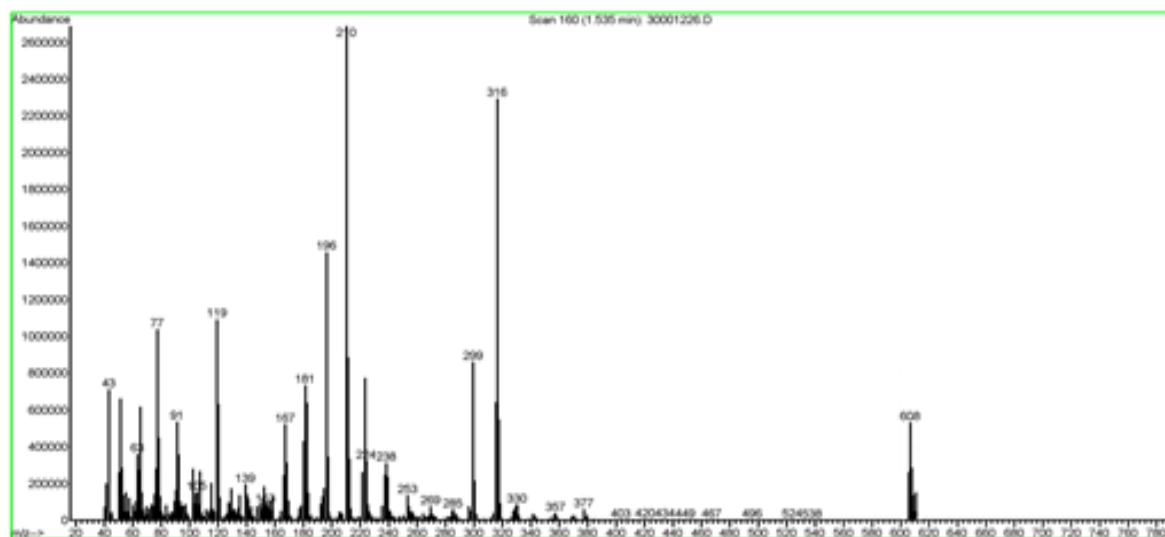
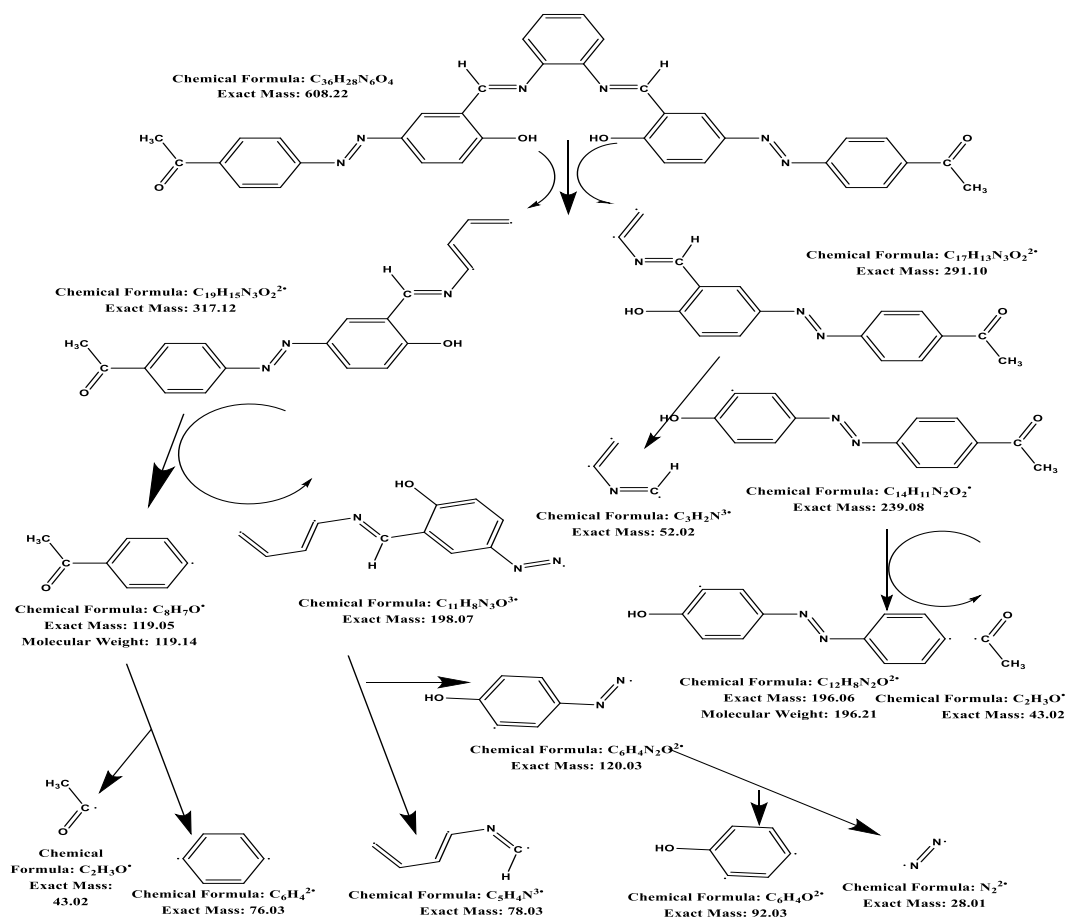


Fig.1 Mass spectra of the ligand



Scheme 2. Fragmentation route of the ligand.

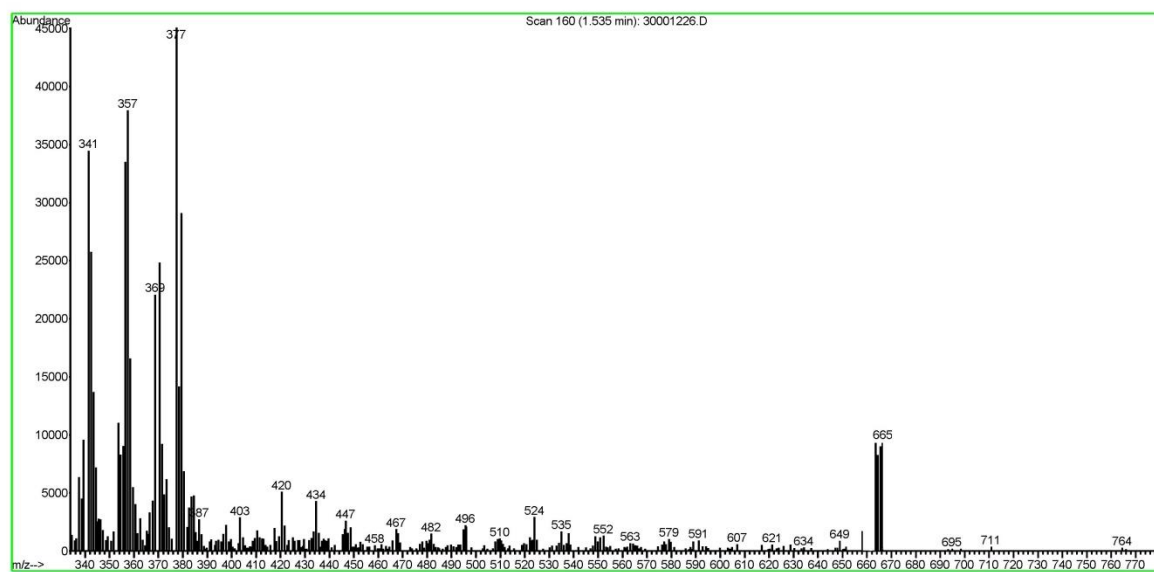


Fig. 2 Mass spectra of the Nickel complex.

3.3.FTIR spectral study

The FTIR technique provides essential and significant insights regarding the characteristics of ligand functional groups and their coordination in complexes, which can be obtained through IR spectroscopy. Furthermore, the comparative analysis between the ligands and their corresponding complexes is regarded as one of the critical pieces of evidence for the binding of functional groups in coordination to metal ions within these complexes²⁵.

Azo –salen derivatives type of ligand were prepared in two steps, the first one including the preparation of the Schiff base component (Salphen) by the condensation reaction between the *O*-phenylenediamine and salicylaldehyde, forming many characterized functional groups that enhanced the IR spectroscopy for determination as, the aromatic hydroxyl -OH group observed clearly at 3358 cm^{-1} and the azomethine C=N group at 1644 cm^{-1} . While the second part of the preparation included the synthesis of azo- salphen ligand via the diazoting of the *p*-aminoacetophenone and coupling it with the previously prepared salphen Schiff base, the resulted ligand was characterized via IR Spectroscopy and the main functional groups were determined as the vibration of the hydroxyl group at 3315 cm^{-1} , 3063 (Sal. H atom str.), the azomethine stretching C=N band 1591 , and the azo group N=N band²⁶ at 1490 cm^{-1} .

In the realm of Infrared (IR) Spectroscopy, the phenomenon of complexation can be inferred through the alteration, displacement, or absence of specific vibrational bands attributable to the formation of complexes, which occurs as a result of their involvement in the coordination of their lone electron pairs with the nickel ion vacant orbitals, exemplified by the lack of the hydroxyl group and the displacement of the azomethine vibrational frequencies consequent to coordination, meanwhile the (N = N) azo group doesn't precipitated in the coordination and its band still without shifting. FTIR spectra's can show the salphen (Schiff base), azo salphen and the nickel complex.

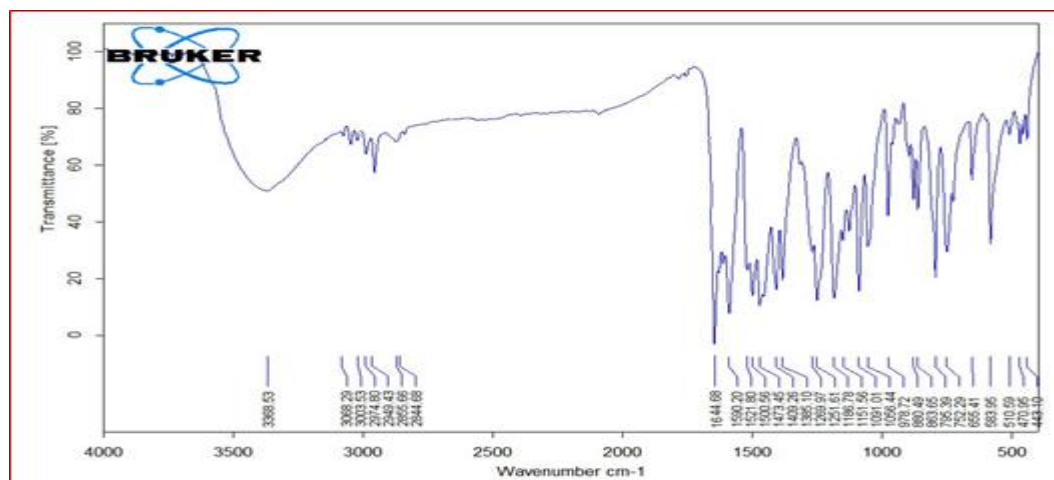


Fig.3 FTIR Spectra of Salphen

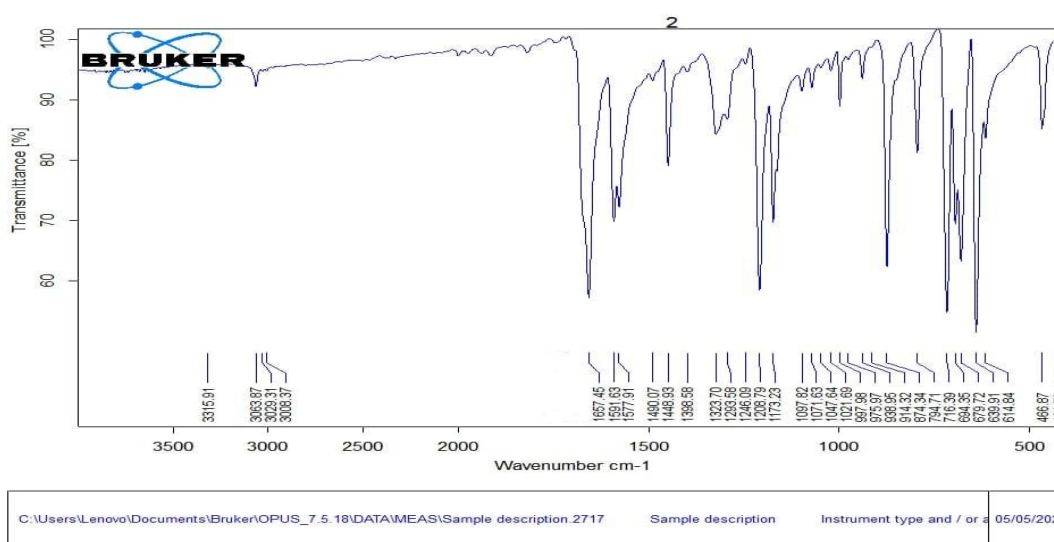


Fig. 4 FTIR Spectra of Azo-Salphen ligand

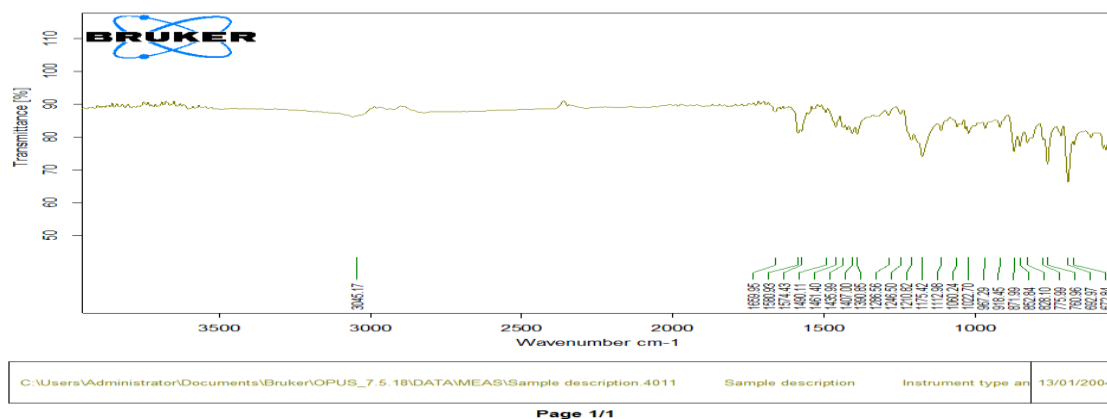


Fig.5 FTIR Spectra of Nickel complex

3.4. NMR spectroscopy

The nuclear magnetic resonance is a crucial method employed to identify organic compounds in a solution, while the FTIR reveals the existing functional groups, ^1H NMR offers invaluable perspectives on the number of magnetically distinctive hydrogen nuclei under examination, as well as the nature of their immediate environment for each specific type²⁴.

¹HNMR spectra of the azo-Schiff ligand ²⁷ observed a clear hydroxyl group OH proton was present at δ 12.93 ppm as a singlet signal, azomethine proton signals appear at 8.93 ppm, the aromatic rings protons were seen at 7.67 -6.95, methylenic group protons of the acetophenone were noted at chemical shift 2.65 ppm.

For the nickel complex the absence of the hydroxyl group signal is indicative of the occurrence of deprotonation and coordination of the oxygen lone pair in the nickel empty orbital, indeed to the shifting of the azomethine proton signal toward upfield region due the sharing of the nitrogen lone pair in coordination reach to 8.83 ppm., the ligand and its Nickel complex spectra shows in figs. 6-7.

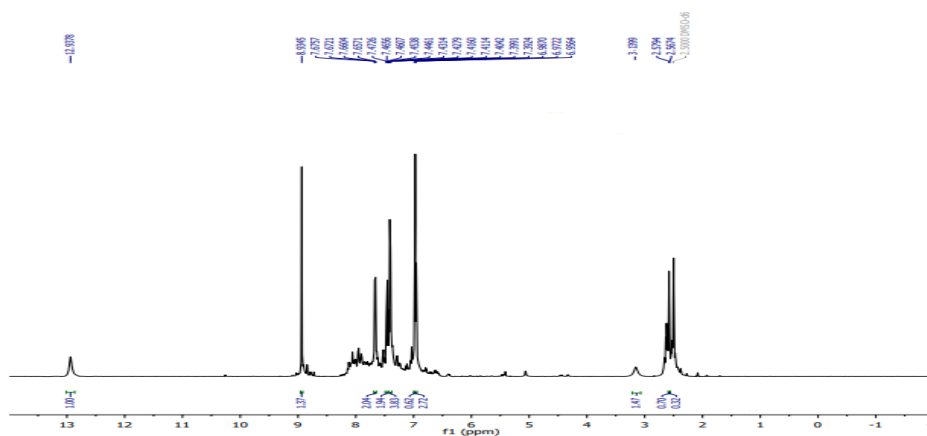


Fig. 6 ^1H NMR spectra of the ligand

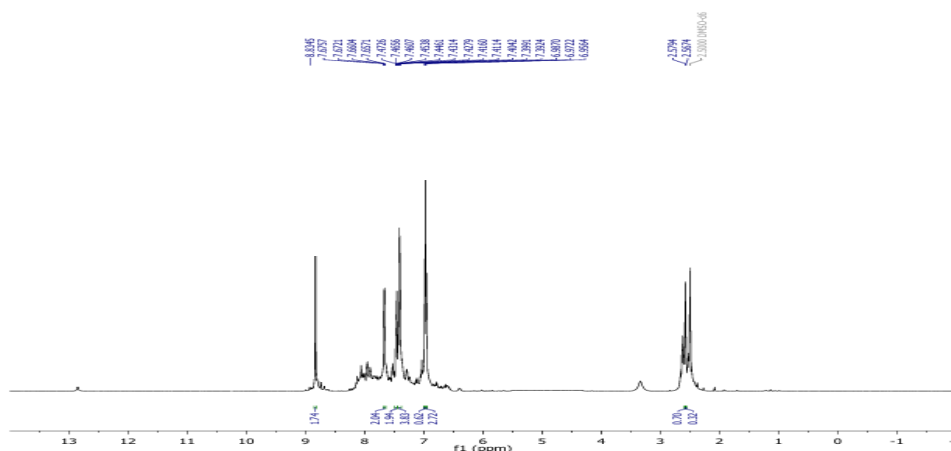


Fig.7 ^1H NMR spectra of the Nickel complex.

while ^{13}C NMR spectroscopy commonly referred to as Carbon Nuclear Magnetic Resonance, constitutes a specialized form of spectroscopy employed for the identification of distinct carbon atoms within a chemical compound. This methodology encompasses the utilization of radio frequency waves to ascertain the spatial distribution of carbon atoms in an organic molecule. In summary, the application of radio waves induces a change in the nuclear spin of specific carbon nuclei, leading to an energy transition that can be quantified²⁸.

The ^{13}C NMR of the ligand developed the carbonic environment of the prepared ligand and give many enhanced signals' that related to the ligand such the carbonyl carbon atom appeared at 197ppm, Azomethine C=N- Carbon 158ppm , Ar-C-OH 154ppm, Ar-C-N=N 138ppm , aromatic cabon atoms 129-122ppm and the methylene acetophenone carbon signal observed at 26ppm as shown in fig.8

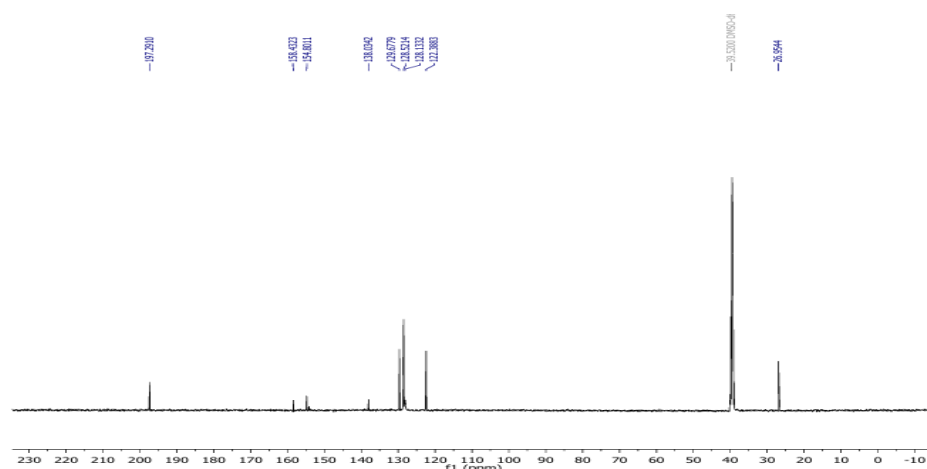


Fig. 8 ^{13}C NMR spectra of the ligand.

3.5. Electronic Spectroscopy , molecular Conductance and magnetic susceptibility

Electronic Spectroscopy represents a sophisticated analytical methodology employed for the identification of diverse compounds, including transition metals or ions and highly conjugated organic molecules; the resulting spectra provide both quantitative and qualitative insights regarding these compounds.²⁹

The Uv-visible spectra of the Azo -Salphen ligand and their Nickel complex were obtained in DMSO solvent at 5×10^{-3} M concentration , the ligand molecule has a maxima at 380 nm related to the (π - π^*) transition as shown in the spectra , while its Nickel complex exhibited two *d-d* electronic transition , the first one related to the $^1A_{1g} \rightarrow ^1A_{2g}$ at 22321 cm^{-1} (448nm), while the other related to the $^1A_{1g} \rightarrow ^1B_{1g}$ at 29069 cm^{-1} (344nm) , these electronic transition may interpreted the square planer geometry of the nickel complex and these can show in the following spectra's. the nickel complex reveal a neutral property due to its low molar conductance result in some organic solvents such ethanol, DMF and DMSO.

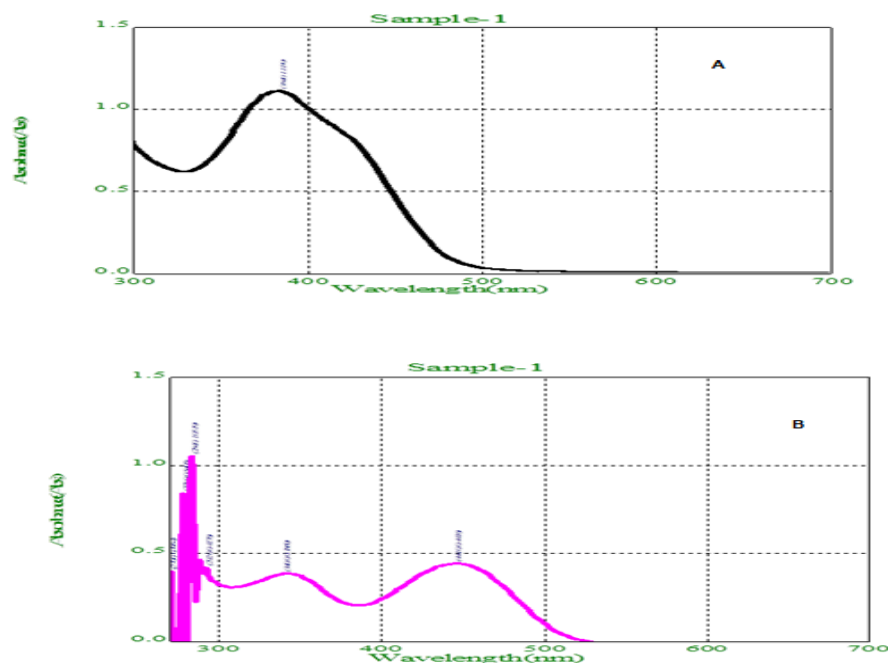


Fig.9 the electronic spectra of A-Azo salphen ligand , B- Nickel complexes .

The depression in magnetic susceptibility value for the nickel complex emphasis the diamagnetic in their electronic distribution of the d^8 complex and support the square planer for nickel complex geometry.³⁰

3.6. XRD data

The Nickel complex X- ray diffraction spectra show a cubic structure , according to the standard code data of the fcc Ni 01-088-2326 reference code³¹, the intensity of the 200 to the 111 peaks, which corresponded to the cubic crystalline structure, was distinctly resolved, and no other crystalline impurities such as sulfides or oxides were detected within the pattern, the average grain size of the nickel complex was 26.67nm , results can show in fig.10and table.2

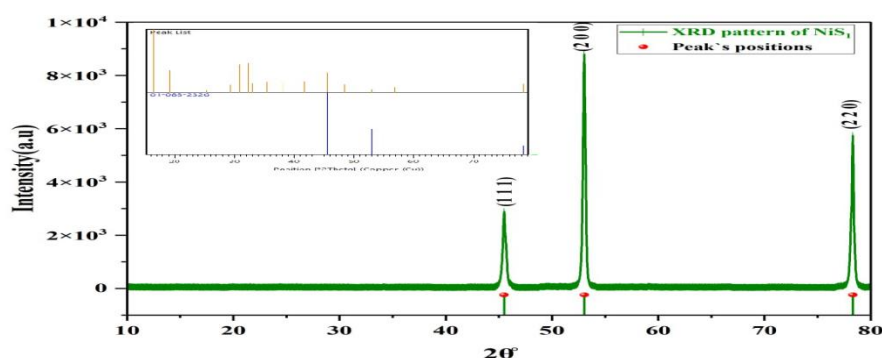


Fig.10 XRD of the nickel complex

Table.2 XRD Data of the nickel complex.

Peaks Positions ($2\theta^\circ$)	FWHM(β)	Grain Size(nm)	Average grain Size(nm)
45.5138	0.40376	21.32843211	26.67546367
53.03324	0.30277	29.31194731	
78.29179	0.34845	29.38601159	

3.7. Biological Evolutions

Within the study , a three biological point view were interested as :

1. Zone of inhibition (ZOI) antibacterial test was performed for the Azo-Salphen ligand and its Nickel complex utilizing two bacterial species *Staphylococcus aureus* ATCC 25923 (Gr+, Facultative Anaerobe) and *Escherichia coli* ATCC 25922 (Gr-,

Facultative Anaerobe), the results indicated that the overcoming of the nickel complex in the inhibition ability for these two species with an inhibition zone of 19 and 14 mm for the *S.aureus* and *E.Coli* respectively, meanwhile the ligand cannot inhibited for these species as mention in fig.

Sample	Diameter	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922
<i>Staphylococcus aureus</i>			
S4	0 mm		
NiS4	19 mm		
<i>Escherichia coli</i>			
S4	0 mm		
NiS4	14 mm		

Fig.11 ZOI Data of the ligand and its nickel complex.

2. antioxidant study

The DPPH methodology³² was employed for the assessment of the antioxidant capacity through the evaluation of radical scavenging abilities; the newly synthesized compounds were subjected to testing for their antioxidant scavenging properties, and the findings are presented as depicted in the following data .

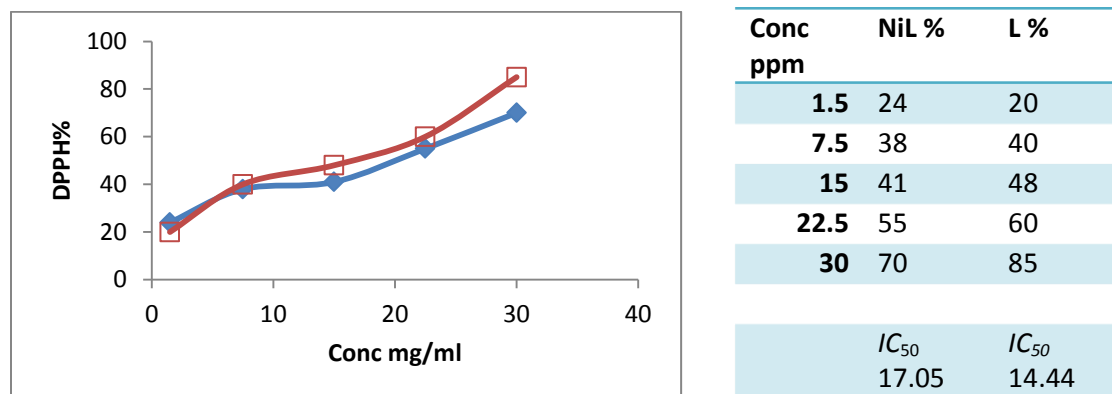


Fig.11 antioxidant data for the ligand and its nickel complex .

The data reveal that the azo-salphen ligand was more efficient as antioxidant than its nickel complex .

3.MCF7 Cell line study

Breast cancer cell line MCF7 was studied for the prepared ligand and its nickel complex for the comparing of these compounds effectiveness on breast malignant cells ³³, the obtained results observed that the ability of the nickel complex was more than the ligand giving an IC₅₀ value of 88.60 ppm, meanwhile a 141.15 ppm for the azo salphen ligand, The ability of nickel ion for penetrate the carcinogenic cells was more than ligand, due it complexed with the guanine nucleic acid of the DNA strands and preventing it from replication and hence cell death. Results can show as in the following tables 3-4 and fig 12-13.

Table 3 the viability percentage of the MCF7 cell line for the ligand and its nickel complex.

Conc.ppm	log Conc.	NiL viability%	L viability %
1000	3	39.27109974	32.31593039
500	2.69	56.8797954	58.94243641
250	2.39	66.60358056	66.97456493
125	2.09	71.20204604	77.05354752
62.5	1.79	83.1202046	84.47121821
31.254	1.49	88.68286445	97.14859438

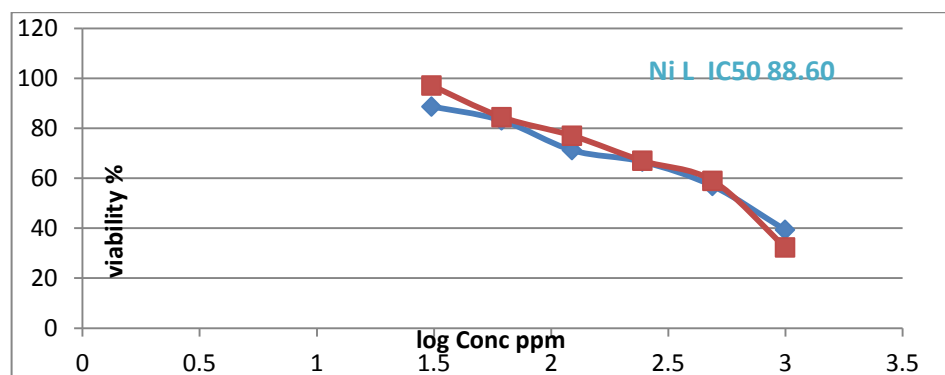


Fig.12 MCF7 Cell line for the prepared compounds.

Table .4 the inhibition rate of the ligand and its nickel complex.

	Conc. $\mu\text{g/mL}$	NiL inh. rate	L inh. rate
1	0	0	0
2	31.25	11.32	2.86
3	62.5	16.88	15.53
4	125	28.8	22.95
5	250	33.4	33.03
6	500	43.13	41.06
7	1000	60.73	67.69

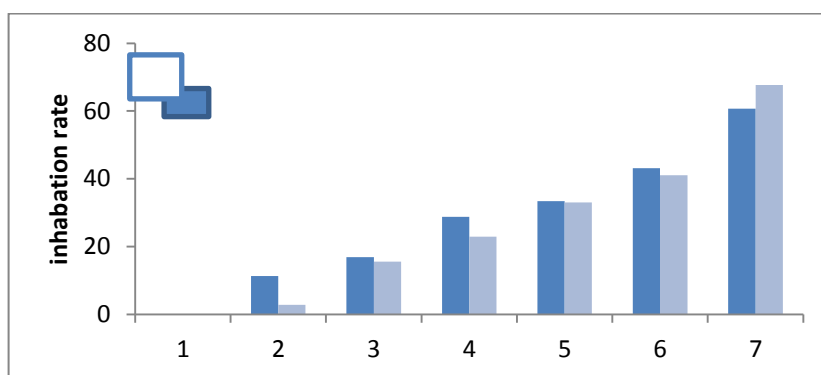


Fig.14 the rate of inhibition for the prepared compounds.

Conclusion

Novel bis azo Salphen (salicylaldehyde phenylene diamine) Schiff bases and its Nickel complex were prepared and subsequently identified by using some of spectroscopic techniques , such mass spectrometry , FTIR, NMR, UV-Visible spectroscopy, elemental analysis, , and X-ray diffraction . indeed to the assessments of molar conductivity and magnetic susceptibility.

Whole analytical and spectral data emphasis that the Nickel complex coordinate was occurs via the azomethine nitrogen atoms and the oxygen atoms of the deprotonated salicylaldehyde hydroxyl group and the ligand behave as a tetradentate NNOO chelating agents, which forming a square planar geometry with the nickel II ion complex. The examination of crystalline morphology and the determination of average

particle size demonstrate that the synthesized complexes were fabricated at the nanoscale.

The azo-salphen ligand, along with its nickel complex, underwent a comprehensive biological assessment concerning their antibacterial effectiveness, specifically the zone of inhibition (ZOI) against the gram-negative bacterium *Escherichia coli* ATCC 25922 and the gram-positive bacterium *Staphylococcus aureus* ATCC 25923. The findings revealed that the Nickel complex demonstrates superior antibacterial activity compared to the ligand. Conversely, in the DPPH antioxidant assay, it was observed that the ligand functions more as an oxidant than its nickel complex; however, the nickel complex exhibited a more pronounced capability in inhibiting malignant breast cells when evaluating the MCF7 cell line.

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