Research Article

Synthesis, Spectroscopic Characterization and Antibacterial Assessment of Cadmium and Molybdenum Complexes of *TcSal* Mixed Ligand

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ABSTRACT

The metal complexes of Cadmium and Molybdenum (II) were synthesized by refluxing equimolar mixtures of Tetracycline (Tc**)** as primary ligand and Salicylaldehyde (Sal**)** as a secondary ligand with M (II) salts (M = Cd & Mo) in ethanol media. The metallation of two exclusive ligands with Cd & Mo was monitored by the observation of change in color, surface tension, and conductivity. The stoichiometric ratio of the synthesized complexes was carried out using the physicochemical approach like elemental microanalysis (CHN), melting point, surface tension, conductivity measurement. The spectroscopic methods like FT-IR, ^{1}H & ^{13}C-NMR spectroscopy, UV/Vis, Mass spectrometry techniques. The surface structure of the complex was carried out through scanning electron microscopy (SEM). The thermodynamic and kinetic studies were carried out via TGA/DTA curves through which ΔE*, ΔH*, ΔS* & ΔG* parameters of several decomposition stages were evaluated by Coats-Redfern equation. The consequences of elemental microanalysis signify that these metal complexes of the mixed ligand are determined to have a 1:1 metal-to-ligand molar ratio. Molecular modeling gives geometry of the complex which was accomplished through Cs-Chem Office software program. The antibacterial activity study was done by Kirby- Bauer Paper disc diffusion techniques with the help of using *S. aureus, E. coli,* & *P. aeruginosa* clinical bacterial pathogens. All synthesized metal complexes showed good consequences in opposition to the selected clinical pathogenic bacterias.

Keywords: Antibacterial activity, metal complexes, molecular modeling, tetracycline, thermal analysis.

INTRODUCTION

In the field of coordination chemistry, development is widely expansive in recent years due to the easy chelation of the ligand with the metallic ions and it further requires significant study for the generation of novel metal complexes. So, the research primarily based on metal complexes of the mixed ligand is of great interest for medicinal and analytical chemists[1]. The modern-day antibiotics are underneath bacterial resistance due to their improper use in the therapy techniques and the world is going through a shortage of antibiotic drugs. This leads to accelerated mortality of the human population. So, there is the first need for antibiotic research to remedy the present trouble of drug resistance [2].

Metal complexes of Mixed ligand are of utmost significance in the discipline of coordination chemistry due to their varied applications in analytical, chemical and biomedical fields. Their functions in pollution, magneto and photochemistry, industrial and material science further decorate in the area of chemical research [3]. Tc is the 3rd generation broad-spectrum antibiotic which is used in the therapy of the various bacterial infections promoted using bacteria. The bacterial infection includes acne, cholera, brucellosis, plague, malaria, and syphilis, but due to cellular research in the bacterial pathogen, the current antibiotics are under bacterial resistance. This can also be triggered by way of anti-enzymatic actions on drug supplies [4]. Among transition metals, cadmium (d^{10}) forms a metal complex with coordination number ranging from four to eight. Presently a cadmium (II) ion acts as the catalytic center for carbonic anhydrase. The d^{10} metal ions are used as photoluminescence materials which can enhance the nature of fluorescence [5].

The combination of transition metal ions with antibiotics has focused our attention and forced us to interact their chemistry to established whether pharmacological properties of the ligand affects the complexation and also finds additional knowledge about the action of antibiotics[6]. There is fantastic endeavor in the discipline of coordination chemistry that cadmium is toxic metal and strong carcinogen in nature to the environment. So, the mobilization and immobilization of cadmium can be done with the help of some technical strategies such as in ligand exchange chromatography through complexation of the metal Center by way of chelating nitrogen donor ligands [7]. In the present work, we concentrated for the synthesis of Cd (II) & Mo (II) metal complexes of mixed ligand by taking tetracycline as primary ligand and salicylaldehyde as the secondary ligand. Besides of their spectroscopic characterizations, antibacterial susceptibility test was done by Kirby- Bauer Paper disc diffusion techniques using clinical pathogenic bacteria like *S.aureus, E. coli, P.aeruginosa*.

MATERIALS AND METHODS

The reagents, as well as solvents used in the laboratory work, were of analytical research-grade (AR). They were purified and dried under the standard procedure. The glassware used in the test was of high-grade and were used

for excessive precision overall performance experiment. The integral chemicals have been procured from various international chemical agencies such as Tetracycline (Sigma Aldrich), Salicylaldehyde, $CdCl_2.H_2O$, $ZrOCl_2.8H_2O$ (LobaChemie Pvt. Ltd), $PdCl_2$ and MHA (Himedia), and $MoCl_3$ (Alfa Aesar). Triple distilled water was used in the whole experiment for washing of the equipment and also for a solvent. Distilled ethanol was used for the experimental work.

Instruments

The C, H and N content in the synthesized metal complexes of mixed ligand were determined with Euro-E 3000 microanalyzer. Electronic spectral data (UV/Visible) of the complexes prepared in DMSO solution had been monitored in the length range of 200-2000 nm on Varian, Cary 5000. The FT-IR spectra had been done from the region between 4000-400 cm^{-1} through Perkin **Elmer Spectrum II** by use of KBr pellets. ^{1}H-NMR was carried out in presence of DMSO-d_6 solvent on a **Bruker AvII- 400**MHz spectrometer and Me_4Si acts as an internal reference. The kinetic and thermal behavior (TGA/DTA) curves were calculated under the nitrogen atmosphere to room temperature up to 860 °C at linear heating rate of 10 (°C/min). The ESI-MS evaluation has been determined through water UPLC-TQD mass spectrometer within the vary of 0-1000 m/z. The melting point was recorded in VEEGO ASD-10013 programmable apparatus. Conductivity measurement was done at 25°C in DMSO using Conductivity/ TDS Meter TCM 15+ digital conductivity meter. The surface tension data were taken from KRUSS Easy Dyne Tensiometer by Wilhelmy plate with du Nouy ring method. The morphology of the synthesized metal complex surface was done with the help of JEOL model JSM-6390 LV through Scanning Electron Microscopy technique. The complexes molecular structures were performed by using the help of CsChem Draw Ultra-12.0 Pro software. The anti-bacterial study was done with the help of Kirby Bauer paper disc diffusion technique. The stock solutions were prepared by mixing the metal complexes in DMSO at different concentration.

Antibacterial sensitivity assay

In vitro antibacterial screening test of the ligand and synthesized complexes were done on two bacterial strains:gram-negative (*Escherichia coli* & *Pseudomonas aeruginosa)* and gram-positive *(Staphylococcus aureus)* clinical pathogens by using Kirby-Bauer paper disc diffusion technique. Test solutions of the complexes were prepared at three different concentrations (25, 12.5 and 6.25 µg/µl) in 30% DMSO to measure their actual effectiveness. Initially, the fresh culture of organisms was prepared from broth and incubating them for 2 h at 37 °C for proper and total growth. Paper disc with 5 mm diameter size (Whatman no.1) was sterilized and loaded with test solutions. The sterile MHA media for testing of antibacterial sensitivity was prepared according to the reported literature. The fresh and revived organism slowly swab inside the sterile media and complexes of different concentration were injected on the paper disc. Amikacin of 30 mcg/disc was applied as a standard antibiotic drug which acts as a positive control[8]. After finishing all these tasks, the loaded Petri plates were placed in an incubator about 37 °C up to 36 hrs to observe the inhibition zone of bacterial growth.

Synthesis of Complex

The Tc (0.8890gm, 2 mmol) was dissolved in hot 20 ml (70% ethanol), introduced dropwise an aq. solution of M (II) salts (M = Cd & Mo) (2mmol) under stirring condition in a 50 ml R.B flask. The combination was refluxed for two hrs to homogenize it and to this well stirred ethanolic solution of Sal (0.2ml, 2mmol) was added to the reaction mixture. After refluxing for eight hrs, pH was adjusted at 7-8 by adding ammonia solution. The mixture was left overnight to room temperature until the precipitation occurs. The obtained precipitates were filtered off, wash with aqueous ethanol then recrystallized from ethanol which results in the formation of pure and pale orange amorphous form of Cadmium complex and dried in a desiccator over anhydrous $CaCl_2$. The percentage yield of the metal complexes was of about 60-70 %.

The similar route has been employed for the preparation of the Molybdenum Complex (Mo-TcSal) of the mixed ligand (Figure 1).

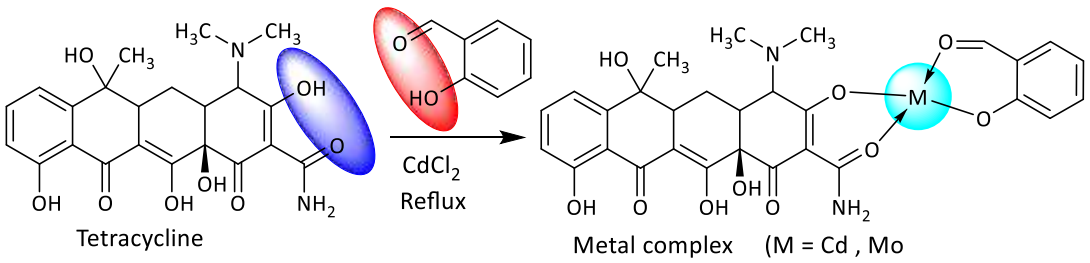

Figure 1: Proposed equation of reaction for the preparation of Cadmium complex of the mixed ligand.

Conductivity

The specific conductivity of ligand and the metal complexes of Cadmium and Molybdenum (II) decrease on decreasing the concentration (Table S1). On decreasing the concentration, the conductivity also decreases because of the number of ions per unit volume carrying the current decreases on dilution[9,10]. The greater conductivity was seen in the metal complexes in comparison to the ligand (Table S1) which also indicates the complex formation between

the ligands and the definite metal ions which could signify the greater tendency of formation of ions (complexion) in the metal complex [11]. The specific conductivity of metal complex follows the charge density order since the specific conductivity increases with the metal complexation[12].

Surface Tension measurement

Surface tension is called as one of the crucial factors for measuring the wettability of a solution of a fluid which can spread over or stick to a solid surface[13]. The surface tension is a principle quantity which signifies the property of assembly and segregation dynamics of surface-active materials and plays an essential role in emulsion formation hence surface tension measures mechanism of kinetic diffusion [14].

The surface tension of ligand and the metal complexes of Cadmium and Molybdenum (II) increase on decreasing the concentration (Table S1). Hence, this study shows that surface tension decreases as we increase the concentration of the solution. This occurs due to larger interaction between molecules of liquid than molecules present in air or in non-polar solvents[15].

The surface tension of the metal complex often decreases as molecules become adsorbed[16] in comparison with ligand (Table S1). Because there is a greater probability of adsorbed molecules interacting as the surface coverage increases, the enthalpy of adsorption depends on the amount of surface coverage.

FT-IR spectroscopy

Characteristics frequencies of Infra-Red absorption band of metal complexes were introduced in Table 2 & Figures S1 & S2. These bands signify the nature of functional groups attached to the metal ions in the metal complexes. The complexes which show the bands within the range of 3431-3396 cm^{-1} attributes to υ(O-H) or υ(N-H). The shoulder peaks lie at 2935 cm^{-1} and 2925 cm^{-1} corresponds to υ (CH) Methyl. Similarly, peaks at 1600-1800 cm^{-1} indicate the υ(C=O). The peaks at 1515-1580 cm^{-1} correspond tou (C=C) aromatic proton. The IR absorption frequency at 1457 cm^{-1} signifies the υ(C=N) Amide. The appearance of absorptions in the regions 1125-1140 cm^{-1} denotes υ(C-O). One of the essential characteristics absorption frequencies of metal complexes denoting their synthesis is the vibrational frequency of υ(M-N) &υ(M-O) bond which lies from the region between 440-500 cm^{-1} and a medium intensity band at a region of 590-605 cm^{-1}.

Table 1: Elemental microanalysis, electronic absorption and physical measurement data

Complex	Empirical Formula	Mol. Wt.	Colour	M. Pt. (°C)	Calculated (found)%				Uv./Vis. Peak (nm)	Assignment
					C	H	N	O		
Cd-TcSal	$C_{29}H_{28}CdN_2O_{10}$	676.95	Pale Orange	239.5	51.450 (51.102)	4.170 (5.706)	4.140 (4.256)	23.630 (14.251)	280 369 768	π→π* n→π* LMCT
Mo-TcSal	$C_{29}H_{28}MoN_2O_{10}$	660.50	Pale Yellow	228.5	52.730 (42.789)	4.270 (4.635)	4.240 (4.686)	24.220 (17.003)	297 371 447	π→π* n→π* LMCT

Table 2: FT-IR spectral data of Cd & Mo- TcSal metal complexes

Complex	υ(O-H) or υ(N-H)	υ (CH) Methyl	υ(C=O)	υ(C=C) Aromatic	υ(C=N) Amide	υ(C-O)	υ(M-O)	υ(M-N)
Cd-TcSal	3431	2935	1770	1518	1457	1128	596	499
Mo-TcSal	3396	2925	1618	1576	1457	1133	602	440

^{1}H & ^{13}C-NMR spectral analysis

The ^{1}H-NMR spectrum of metal complexes was recorded in DMSO-d_6 solvent and offers about the precious facts about the structure, dynamic reaction state, proton environment and coordination sites of the metal complexes molecule. The ^{1}H-NMR spectra of metal complexes were shown in Figures S3-S4 & Table S2. The signals at (δ= 3.415 ppm) revealing to (CH_2) and singlet signal peak appeared at (δ= 2.5 ppm) is assigned to DMSO-d_6 solvent in the region between (δ= 2.509-2.517 ppm) and peaks in the location between (δ= 1.495-1.896 ppm) signify the peak for methyl proton. Similarly, the peaks in the region of (δ= 2.682-2.963 ppm) represent [N-(CH_3)$_2$]. The aromatic proton lies in the location of (δ= 6.904-7.539 ppm). Similarly, primary amide proton lies in the vicinity between (δ= 7.645 -7.660 ppm).

^{13}C-NMR spectrums of metal complexes were done in DMSO-d_6 which display chemical shifts at (199.513) ppm indicate signal for phenyl ketone. Aromatic carbon confirmed a signal in the range between (111-131) ppm. The chemical shift for the carbon of the carbonyl group (-CO-) appears between (172.484-157.742) ppm. Also, the chemical shift at 40 ppm is due to DMSO-d_6. The measured value of all the carbon atoms with their chemical shifts is introduced in Figure S5-S8 & Table S3.

ESI-Mass spectral analysis

ESI- Mass spectrum of Cd (II) & Mo (II) metal complexes shows a peak at (M/Z=676.95) and (M/Z= 661) amu of the parents' ion which indicates proposed formula for the complexes. The value of molecular mass along with other

spectral data are necessary for the generation of the structure of the organic compounds. The rest peak of the mass spectrum is the fragment peaks. The successive decrease peak of the target compounds gives the series of the peak of various fragments. Their intensities provide stability of fragments ions. The mass spectrum of metal complexes is shown in Figures S9-S10**.**

Electronic Absorption Spectroscopy

UV/Visible spectrum of the metal complex (Cd-Tc/Sal) had been done on DMSO solution within a wavelength range between 250-800 nm. The electronic spectrum shows excessive-high intense absorption peaks at 280, 369 and 768 nm which signify (π→ π*)&(n→ π*) transitions. The spectral data was presented in Figure S11**.** Together with their electronic transition and suggested geometries. Electronic emission spectra of Cd (II) did not exhibit any d-d transition because its d^{10} orbital is filled but exhibit absorption bands due to metal-ligand charge transfer (MLCT) indicating the d^{10} system. Similarly Molybdenum complex shows absorption bands at 297,371,447 nm.

TGA/DTA Study

The thermogravimetric study provides useful information about the thermal and kinetic stability of the compounds which was carried by running the analysis in a nitrogen atmosphere at room temperature to 860 °C with a linear rate of heating at 10 °C/min. One important purpose of TGA records signifies that the associated molecules of water inside complexes help the elemental analysis. Correlation of thermal events with continuous heating of the chemicals is useful to withdraw information about kinetic parameters and the mass loss during the decomposition. The thermal records which include thermal decomposition, percentage mass loss, and thermodynamic and kinetic parameters of every decomposition steps have been derived and covered in Table 3 & 4. Results indicate good agreement with theoretical formula supported through micro- elemental analysis.

The thermogram of the cadmium complex has been shown that decomposition takes place in three steps within the temperature range of 246.89 - 634.32 °C. In the first decomposition stage, weight loss of 14.96% (3.1143 mg) in the temperature range 246.89-286.67 °C. The second and third stages occur with % weight loss of 11.7239 % (11.7239 mg) and 1.9447 % (1.9447 mg) within the temperature range of 341.54-398.11 °C and 580.03-634.32 °C. In every case, decomposition starts by loss of coordinated or crystallized water molecules of the complexes only or other components of ligand moiety which confirms the composition of metal complexes. This suggests that cadmium complex is thermally stable. Similarly, the thermogram of Molybdenum complex was decomposed at three ranges in the temperature range of 197.38-805.15 °C. In the first decomposition stage, weight loss of 16.899 % (1.6899 mg) take place within the temperature range of 197.38-260.48 °C. The second and third decomposition stages occur with % mass loss of 1.6459 % (16.459mg) and 1.4743% (1.4743 mg) in the temperature range of 721.21-742.98 °C and 789.75-805.15 °C which are presented in Figure 2 & Figure S12. The residue formed was metal oxide such as CdO & MoO.

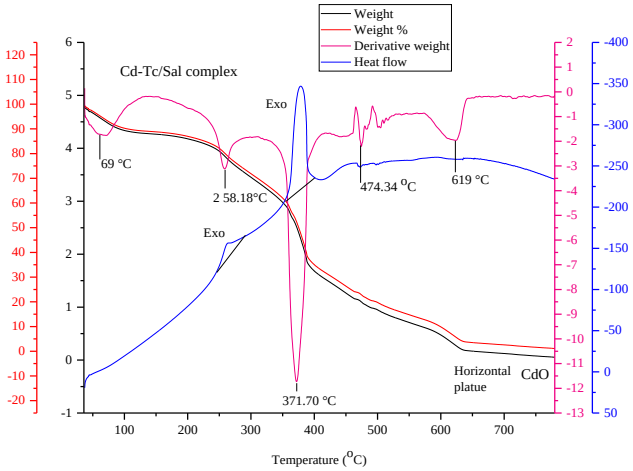

Figure 2: Thermogram of Cd-TcSal complex

Scanning Electron Microscopy Study

The microstructures, as well as morphological properties of metal complexes, were examined through SEM (Scanning electron microscopy). A high beam of energy of an electron of SEM produces a different variety of signals to the solid surface which results in the generation the image of shape, size, strength, ductility and the arrangement of atoms in an object. SEM is also an important tool in forensic science, metallurgy, gemology and in medicinal science [17]. The SEM micrographs of Cadmium and Molybdenum complex shown in Figure 3. From the SEM micrograph, it signifies the formation of successful nanosized particle. The cadmium complex shows that the particles are extremely agglomerated in nature due to induced crystal growth by itself [18].

Table 3: Kinetics and thermodynamic parameter of Cadmium and Molybdenum metal complexes

Complexes	r	A(s^{-1})	Tmax (K)	E* (kj/mol)	Δs* (j/kmol)	ΔH* (kj/mol)	ΔG* (kj/mol)
Cd-complex	-0.9955	$2.05x10^{17}$	531.18	184.920	120.970	18.505	116.248
	-0.9986	$4.01x10^{21}$	644.85	263.260	162.240	257.901	153.281
	-0.9988	$1.72x10^{24}$	892.81	421.170	209.930	413.751	226.324
Mo-complex	-0.9979	$7.55x10^{9}$	496.22	102.526	-60.040	98.401	128.196
	-0.9992	$7.16x10^{61}$	1007.15	1196.867	929.110	1188.494	252.745
	-0.9963	$1.59x10^{104}$	1070.54	2100.898	216.17	2091.997	186.058

Table 4: Thermal decomposition data of Cadmium and Molybdenum complexes

Complexes	TG range (°C)				DTA		
	Δm% (cal.)	Ti	Tf	T_{DTG}	Mass loss	T_{dta}	Peak
Cd-complex	14.96	246.89	286.67	258.18	-3.1143	246.89	Exo
	11.7239	341.54	398.11	371.70	-11.7239	-	Exo
	1.9447	580.03	634.32	619.81	-1.9447	634.11	
Mo- Complex	16.899	197.38	260.48	223.22	-1.6899	197.38	
	1.6459	721.21	742.98	734.15	-16.459	-	
	1.4743	789.75	805.15	797.54	-1.4743	805.15	

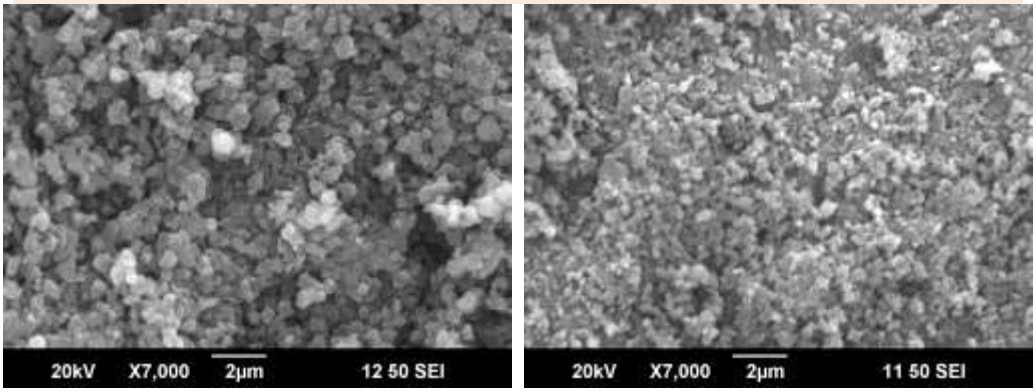

Figure 3: SEM micrograph of i) Cd-TcSal ii) Mo-TcSal

Similarly Molybdenum complex has the shape of nanoparticles which has almost spherical of some aggregation complex[19]. Careful examination of the single crystal honestly suggests the nanoscale measurement of the single crystal of the complexes.

Molecular Modeling Study

The proposed structure of metal complexes was carried out with 3D modeling Via the Cs Chem 3D program. Correct stereochemistry was once finished via manipulation and Change to gain low energy molecular geometry. Metal complexes, Potential energy will be the sum of all the energy of following types: $E = E_{str} + E_{ang} + E_{tor} + E_{vdw} + E_{oop} + E_{ele}$ where E's represents energy value for various interaction. The subscripts signify the bond stretching, angle bending, deformation angle, Van der Waals interactions, out of plane bending, simple bending, and electronic interaction respectively. The total steric energy for cadmium complex is 916.0934 Kcal/mol and has the geometry of Trig Planar and presented in Figure S13.

Antibacterial Sensitivity Study

The metal complexes were carried out the antibacterial sensitivity which was screened in vitro of their inhibitory activity against some clinical pathogenic bacterias like *S. aureus, E. coli, P. aeruginosa* through the use of Kirby Bauer paper disc diffusion technique. For this test, the complex was prepared by dissolving in DMSO solvent. Zone of inhibition signifies mean of three readings which were presented in the Figures S14-15 & Table 5 that displays the effect of prepared complexes on different bacterial pathogens. From the antimicrobial study, all-metal complexes show activity towards tested pathogens while ligand does not show any effects. The complexes show the greater activity in comparison to ligand results in the complex formation where the ligand is bonded with the metal ions. Hence increase in the concentration of metal complexes increase the inhibition zone of bacterial growth[20]. From the reported data, it is clear that Cadmium and Molybdenum metal complex shows better antibacterial activity at higher concentration and considerable activity at a lower concentration. The *E.coli* of Molybdenum complex found much less active. Similarly, *S.aureus* of Cadmium complex suggests better results. So all the pathogens had been found susceptible towards the synthesized derivatives of two Tetracycline (Figure S16).

Table 5: Antibacterial growth data of Cadmium and Molybdenum metal complexes.

Complexes	The diameter of zone of inhibition in (mm)								
	S. aureus			*P. aeruginosa*			*E. coli*		
Concentration (µg/µl)	25	12.5	6.25	25	12.5	6.25	25	12.5	6.25
Cd-Tc/Sal complex	28	24	22	17	16	15	22	21	19
Mo-Tc/Sal complex	20	18	16	12	11	10	11	10	9
Amk (30mcg/disc)	27			21			23		
Tc	31			31			31		
DMSO	0			0			0		

CONCLUSION

In this paper, the metal complex of Cd (II) & Mo (II) was successfully synthesized via by using Tetracycline as primary ligand and Salicylaldehyde as the secondary ligand. The synthesized complexes had been analyzed by way of micro-elemental analysis, FT-IR, (^{1}H & C^{13})-NMR, UV/Vis, SEM, TGA /DTA. Molecular modeling suggests coordination behavior of metal ions with the ligand and as a result, received results are very much close to the experimental data. The color change has seen all through the chemical procedure truly indicates deprotonation for the duration of formation of the complex. The evidence for the purity of the complex was validated through the melting point. The anti-microbial tests had been carried under Kirby-Bauer paper disc diffusion technique by using clinical bacterial pathogens like *S.aureus, E. coli, P.aeruginosa* which suggests gorgeous antibacterial activity and comparable sensitivity test of ligand, as well as metal complexes truly, shows that they exhibit strong activity against *S.aureus.*

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