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DNA interaction and antimicrobial screening of transition metal Complexes of 2-Aminophenol Schiff base

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ABSTRACT

Co(II), Ni(II), Cu(II) and Zn(II) metal complexes of a quadridentate Schiff base with a N_2O_2 donor set derived from condensation of benzene-1,3-dicarboxaldehyde (isophthaldehyde) with 2-aminophenol were synthesized and characterized by elemental microanalyses, molar conductance, magnetic susceptibility measurements and spectroscopic techniques *viz*, FT-IR, ^{1}H NMR, EPR, Mass and UV-Vis. The spectral data showed that, the ligand acts as a tetra dentate ligand and the bonding sites were deprotonated oxygen-O groups and azomethine (-C=N-) nitrogen atoms. Geometry of the metal complexes was examined and recognized as square planar. The interaction of the metal(II) complexes with calf-thymus DNA (CT-DNA) has been monitored by UV-Vis absorbance titration technique. The results indicated that all the complexes bind to CT-DNA through intercalation mode. The antimicrobial efficacy of these complexes reveals that they are more dynamic than free ligand. Among the metal complexes, Cu(II) complex exhibit higher efficacy against all the pathogens. Moreover, gel electrophoresis method indicates that these complexes cause effective cleavage of pBR 322 DNA in the presence of activators.

Keywords: Quadridentate Schiff base; Calf-thymus DNA; antimicrobial efficacy; Gel electrophoresis.

1. INTRODUCTION

In recent years, great attentions have been paid to the construction using flexible N-donor bridging ligands. Transition metal complexes of Schiff bases are extensively studied due to synthetic flexibility, selectivity and sensitivity toward a variety of metal atoms [1]. The use of ligands in generation of metal complex relatively spars; they found useful in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical, in medicine as antibiotics and anti-inflammatory agent, antiradical activities and biological activities [2,3]. Some complexes containing nitrogen and oxygen donor atoms in the complexes are effective as stereo specific catalysts for oxidation, reduction, hydrolysis, biological activity and other transformations of organic and inorganic chemistry [4,5]. Some research groups found that the Schiff base transition metal complexes of 2-aminophenol based Schiff bases have been the subject of extensive investigation because of their wide use in various fields. Metal complexes derived from 2-aminophenol can specially have a variety of application in analytical and biological areas [6,7]. Typical metal (II) complexes have square planar or square pyramidal geometries as important bioactive compounds *in vitro* and *in vivo* aroused an ever-increasing interest in these agents as potential drugs for therapeutic intervention in various diseases [8]. DNA is a significant cellular receptor, many chemicals bring to bear their antitumor effects by binding to DNA and by this means changes the replication of DNA and inhibits the growth of the tumour cells, which is the basis of designing new and more efficient antitumor drugs. During the last decade several transition metal complexes have been used as tools for understanding DNA structure, as agents for mediation of DNA cleavage or as chemotherapeutic agents. Metal complexes offer an opportunity to explore the effects of central metal atom, the ligands and the coordination geometries on the binding event. Moreover, their

activity depends on the mode and affinity of their binding ability to the DNA strands [9–11]. A number of metal chelates have been used as agents for mediation of strand scission of duplex DNA, probes of DNA structure in solution and chemotherapeutic agents [12,13]. Schiff base metal(II) complexes appear to be very promising agents for anticancer therapy having effective cytotoxic activities [14,15]. Copper is known essential metal. It is widely distributed in living cells and body. It is of interest to investigate DNA binding and cleavage activity of metal complexes of essential metals and thus attracted much attention. In the light of the above and in continuation of ongoing research on DNA binding and cleavage activities of transition metal complexes [16,17], herein we report the DNA binding and cleavage activities of metal complexes with synthesized Schiff base ligand derived from 2-aminophenol and isophthalaldehyde.

2. EXPERIMENTAL SECTION

2.1. Materials and methods

All chemicals and metal salts (Merck) were used as received. Commercial solvent (ethanol) was distilled and then used for the synthesis of ligand and its complexes. DNA was purchased from Bangalore Genei (India). The microanalyses (C, H, and N) were performed in Carlo Erba 1108 analyzer at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow, India. Molar conductivities in DMSO (10^{-3}M) at room temperature were measured by using Systronic model-304 digital conductivity meter. Magnetic susceptibility measurement of the complexes was carried out by Gouy balance using copper sulphate pentahydrate as the calibrant. Infrared spectra (4000–400 cm^{-1} KBr disc) of the samples were recorded on an IR Affinity-1 FT-IR Shimadzu spectrophotometer. NMR spectra were recorded in DMSO-d_6 on a Bruker Avance Dry 300 FT-NMR spectrometer, using TMS as the internal reference. EPR spectra were recorded on a Varian E 112 EPR spectrometer in DMSO solution both at room temperature (300 K) and liquid nitrogen temperature (77 K). The absorption spectra were recorded by using Shimadzu model UV-1800 spectrophotometer at room temperature. Cyclic Voltammetric (CV) experiments were achieved on a CHI 620C electrochemical analyzer in fresh distilled DMSO solution. Mass spectrometry experiments were performed on a JEOL-AccuTOF JMS-T100LC mass spectrometer equipped with a custom-made electro spray interface (ESI).

2.2. Synthesis

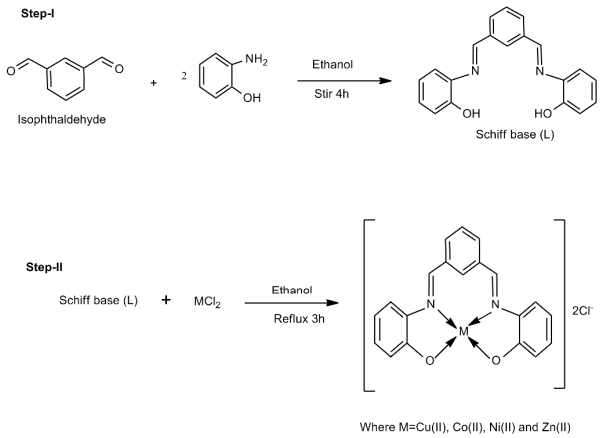
2.2.1. Synthesis of Schiff base ligand (L)

The Schiff base ligand synthesized by an ethanolic solution of 2-aminophenol (10 mmol) was mixed with an ethanolic solution of isophthaldehyde (5 mmol), 2 drops of acetic acid and magnetically stirred in a round bottom flask. The contents were refluxed over water bath for 3 h. The contents were then cooled and filtered. On evaporation of the solvent the Schiff base ligand was yielded as brownish yellow coloured solid. It was filtered and recrystallized from hot ethanol and dried under vacuum in a desiccator over anhydrous calcium chloride.

Yield: 74%; brownish yellow colour; Chemical Formula: $C_{20}H_{16}N_2O_2$ Anal.Calc. (%): C (75.9), H (5.1), and N (8.9); Found (%): C (75.6), H (4.8), and N (8.2); FT-IR (KBr) (cm^{-1}): 1599 (C=N) and 3426 (OH); ^{1}H NMR (DMSO-d_6) δppm: 6.9-8.2 (m,Ar-H), 5.25 (s,-OH), and 8.94 (s,HC=N); UV-Vis. in DMSO, cm^{-1} (transition): 37313 (π-π*) and 28735 (n-π*).

2.2.2. Synthesis of Schiff base metal (II) complexes (1-4)

Metal(II) complexes were prepared by using the following general procedure. Hot ethanolic solution (10 mL) of the Schiff base (1 mmol) and their corresponding metal chlorides (1 mmol) in ethanol (10 mL) were mixed thoroughly and the resulting solution was stirred and refluxed for *ca* 3 h. Then the mixture was reduced to one-third on a water bath and cooled. The solid product was filtered and then recrystallized from ethanol and dried in vacuum. Schematic route for the synthesis of Schiff base ligand (L) and its metal complexes (1-4) are exemplified in Scheme 1.



Scheme - 1: Schematic route for the synthesis of Schiff base ligands (L) and its metal complexes (1-4).

[CuL]Cl_2(1): Yield: 69 %; black colour; Anal.Calc.for $C_{20}H_{14}N_2O_2Cl_2Cu$ (%): C (53.5), H (3.5), N (6.2) and Cu (14.1); Found (%): C (53.1), H (3.1), N (6.2) and Cu (13.8); FT-IR (KBr) (cm^{-1}): 1611 (-C=N), 750 (C-O), 559 (M-N) and 456 (M-

O); Λ_m ($\Omega^{-1}mol^{-1}cm^2$) 85.6; μ_{eff} (BM) 1.92; UV-vis. in DMSO, cm^{-1} (transition): 23255 (d-d).

[CoL]Cl_2 (2): Yield: 64%; pink colour; Anal.Calc.for $C_{20}H_{14}N_2O_2Cl_2Co$ (%): C (54.1), H (3.2), N (6.3), and Co (13.3); Found (%): C (53.8), H (3.0), N (6.1), and Co (13.0); FT-IR (KBr) (cm^{-1}): 1583 (-C=N), 716 (C-O), 565 (M-N) and 466 (M-O); Λ_m ($\Omega^{-1}mol^{-1}cm^2$) 92.6; μ_{eff} (BM) 1.72; UV-vis. in DMSO, cm^{-1} (transition): 23809 (d-d).

[NiL]Cl_2 (3): Yield: 68%; green colour; Anal.Calc.for $C_{20}H_{14}N_2O_2Cl_2Ni$ (%): C (54.1), H (3.2), N (6.3), and Ni (13.2); Found (%): C (54.1), H (3.0), N (6.1), and Ni (13.0); FT-IR (KBr) (cm^{-1}) 1587 (-C=N), 720 (M-O), 559 (M-N) and 461 (M-O); Λ_m ($\Omega^{-1}mol^{-1}cm^2$) 98.5; μ_{eff} (BM) 1.82; UV-vis. in DMSO, cm^{-1} (transition): 23474 (d-d).

[ZnL]Cl_2 (4): Yield: 65%; pale yellow colour; Anal.Calc.for $C_{20}H_{14}N_2O_2Cl_2Zn$ (%): C (53.2), H (3.1), N (5.3), and Zn (14.5); Found (%) : C (53.0), H (3.0), N (5.1) and Zn (14.2); FT-IR (KBr) (cm^{-1}): 1583 (-C=N), 724 (M-O), 562 (M-N) and 470 (M-O); ^1H NMR (DMSO-d_6) δppm: 6.9-8.2 (m,Ar-H), and 8.9 (s,-HC=N); Λ_m ($\Omega^{-1}mol^{-1}cm^2$) 80.4; μ_{eff} (BM) diamagnetic; UV-vis. in DMSO, cm^{-1} (transition): 44444 (LMCT).

2.3. DNA Binding Interaction

The interaction between metal complexes and DNA was studied using electronic absorption, viscosity and electrochemical methods. Disodium salt of calf thymus (CT) DNA was stored at 4°C. All the experiments involving the interaction of the complexes with CT DNA were carried out in Tris–HCl buffer (50 mM Tris–HCl, pH 7.2) containing 5 % DMSO at room temperature. A solution of CT DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.89, indicating the CT DNA sufficiently free from protein [18]. The concentration of DNA was measured using its extinction coefficient at 260 nm (6600 $M^{-1}cm^{-1}$) after 1:100 dilutions. Stock solutions were stored at 4°C and used not more than 4 days. Doubly distilled water was used to prepare solutions. Concentrated stock solutions of the complexes were prepared by dissolving the complexes in DMSO and diluting properly with the corresponding buffer to the required concentration for all the experiments.

2.3.1Electronic Absorption Titrations

Absorption titration experiments were performed by maintaining a constant concentration of the complexes (30 µL), but varying the CT DNA concentration (0–180 µL) in buffer. After each addition of CT DNA to the complexes, the absorption readings were noted. The data were then fitted to the following equation (1) to obtain the intrinsic binding constant K_b values for interaction of the complexes with DNA.

$$[DNA] / (\varepsilon_a - \varepsilon_f) = [DNA] /(\varepsilon_b - \varepsilon_f) + 1/ [K_b((\varepsilon_b - \varepsilon_f)] \quad \text{---------- (1)}$$

The above equation, [DNA] denotes the concentration of DNA, absorption coefficients ε_a, ε_f and ε_b correspond to A_{obs}/[complex], free complex′s extinction coefficient and the extinction coefficient of the complex in the totally bound form, respectively. From the equation (Eq. (1)), slope ($1/(\varepsilon_b-\varepsilon_f)$ and intercept ($1/[K_b(\varepsilon_b - \varepsilon_f)]$) were found out[19]. Finally by comparing the identified slope and intercept, K_b was calculated.

2.3.2. Cyclic Voltammetric Studies

Cyclic voltammetry studies were performed on a CHI 620C electrochemical analyzer with three electrode system of glassy carbon as the working electrode, a platinum wire as auxiliary electrode, Ag/AgCl as the reference electrode and 50 mM NaCl, 5 mM Tris buffer (pH 7.2). Solutions were deoxygenated by purging with N_2 for 30 min prior to measurements [20].

2.4. Gel Electrophoresis Experiment

Interaction between the ligand and the complexes with pBR 322 plasmid DNA was studied by agarose gel electrophoresis. DNA cleavage activity of these compounds were monitored by using the reaction mixture containing 1 µg of plasmid DNA, 250 µg of sample, 2 µL of DMSO (1%) and 5 µL of hydrogen peroxide (40 mmol). The reaction mixture was incubated at 37 ºC for 3 h. After incubation, 2 µL of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol) was poured to a platform fixed with a comb to form slots and the electrophoresis was performed at 50 V for 1 h in TBE (Tris-Borate EDTA) buffer using 1% agarose gel stained with ethidium bromide (1 µg) and the bands were photographed.

2.5. Antimicrobial Screening

The *in vitro* antimicrobial screening of L and its complexes had been carried out against certain human sensitive pathogenic Gram-positive bacteria (*S.aureus* and *B.subtilis*) and Gram-negative bacteria (*S.typhi* and *E. coli*) and fungi (*A.niger, C.lunata, R.bataticola* and *C.albicans)* using broth micro dilution method [21]. The nutrient agar and dextrose agar were served as the medium for the growth of bacteria and fungi, Streptomycin and Nystatin were chosen as standards for antibacterial and antifungal activity, respectively. The samples were incubated at 37°C for 24 h (bacteria) and 48 h (fungi), respectively. The results were recorded in terms of MIC (Minimum Inhibitory Concentration). MIC is the lowest concentration of an antimicrobial

compound that arrests the growth of a microorganism.

3. RESULTS AND DISCUSSION

The synthesized ligand and its metal complexes were characterized by various physicochemical techniques and synthesized compounds were found to be stable in air and moisture. These complexes are soluble freely in DMF and DMSO. All the analytical data with respect to these complexes are agreed to the proposed general formula $[ML]Cl_2$. The magnetic susceptibility data deduces the monomeric nature of these complexes. In addition, the higher molar conductance values of these metal complexes indicate their electrolytic properties.

3.1. Infrared Spectra

The comparative analysis of IR spectra of the free ligand and newly synthesized corresponding complexes provided information on the coordination fashion of this ligand to the involved metal ions. The spectra of the ligand (L) and its complex (1) are recorded in the region 400-4000 cm^{-1} and shown in figure 1 and 2. The FT-IR spectrum of parent Schiff base ligand show some main characteristic features, the first one is the appearance of the intense band at 1599 cm^{-1}, which is assigned to ν(C=N) (azomethine moiety), indicating the formation of the Schiff base [22], while the second feature is the appearance of

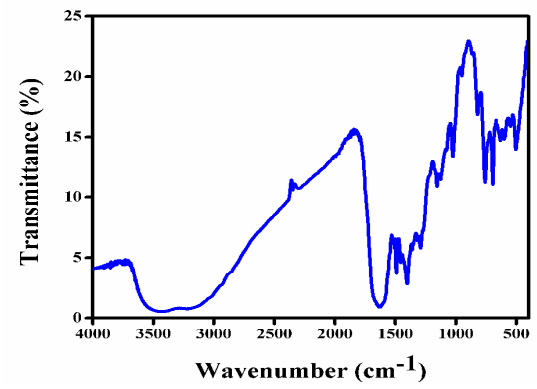


Figure - 1: FT-IR spectrum of L.

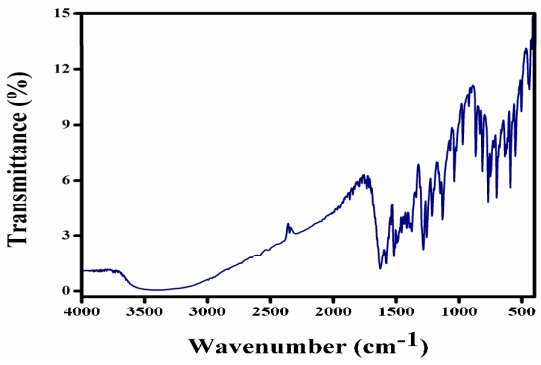


Figure – 2: FT-IR spectrum of complex (1).

medium intense band at 3426 cm^{-1}, which is attributed to ν(OH). On comparison to the parent Schiff base ligand, upon complex formation, the ν(C=N) band is shifted towards lower frequencies in the spectra of metal complexes (1600-1611 cm^{-1}) indicating the involvement of the azomethine nitrogen in coordination with metal ion [23]. Furthermore, the disappearance of the ν(OH) upon complexation was an indicative of deprotonation prior to coordination through the oxygen atom in all the complexes. Moreover the shift ν(C–O) band at 750 cm^{-1} to 716 cm^{-1} in these complexes confirm the participation of oxygen on chelation [24]. Conclusive evidence of the bonding is also shown by the observation that new bands in the spectra of all metal complexes appearing in the low frequency regions at 452-466 cm^{-1} and 559-565 cm^{-1} which are characteristic to the ν(M-O) and ν(M-N) [25]. Thus, the FT-IR spectral data provide strong evidences for the complexation of the tetra dentate Schiff bases with ONNO sequence.

3.2 Magnetic moments and electronic spectra

The geometry of the metal complexes could be deduced from the absorption spectra and magnetic data of the metal complexes. Electronic spectra of Schiff base ligand (**L**) and its complex (1) are recorded (Figure 3 and 4) at room temperature in DMSO medium in the range of 200 – 1100 nm. The free ligand exhibits two intense bands in 45454 cm^{-1} and 37313 cm^{-1} region due to π → π* and n → π* transitions, respectively. These transitions are shifted to either up field or down field frequencies due to the coordination of the ligand with metal ions. The complex (1) shows d-d bands appeared at 23255 cm^{-1} region that strongly favours the square planar geometry around the metal ion, which are assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition, strongly supported the square planar geometry around the Cu(II) ion [26]. It was further supported by the magnetic susceptibility value (1.78 BM). Alongside, d-d band at 23809 cm^{-1} is detected in Co(II) complex, with respect to $^1A_{1g} \rightarrow {}^1B_{1g}$ transition, which indicates the square planar geometry. The magnetic moment value (2.75 BM) of this complex for the square planar geometry, revealed that the four unpaired electrons with the low spin four coordinated square planar environment observed around the Co(II) ion [27]. Ni(II) complex shown d-d band at 23474 cm^{-1} corresponds to $^1A_{1g} \rightarrow {}^1B_{1g}$ transition. The square planar geometry was established by the observed diamagnetism of Ni(II) complex. Similarly, the Zn(II) complex is also being noticed to exhibit diamagnetism, which has not shown any d–d transition in the visible region. Altogether, based on absorption spectra, magnetism and elemental analysis, the

stoichiometry of this complex concluded to reveal tetra coordinate, which could be square planar geometry.

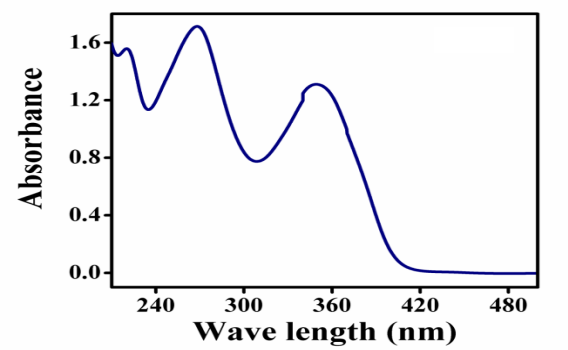


Figure - 3: UV-Vis. spectrum of L 3.3 ^{1}H NMR Spectra.

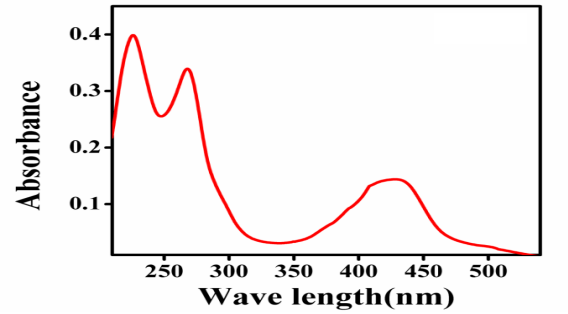


Figure - 4: UV-Vis. spectrum of complex (1)

3.3. ^{1}HNMR spectra

The NMR spectrum is exploiting to determine the identity of prepared ligand and its diamagnetic metal complexes. The ^{1}H NMR spectra of free Schiff base ligand (L) and its diamagnetic Zn(II) complex are recorded in d_6-DMSO solution using tetra methyl silane (TMS) as internal standard. The free ligand (Figure 5) showed a sharp singlet signal at 9.8 ppm, assigned to the azomethine hydrogen atom. The ^{1}H NMR spectrum of L shows peaks at 7.2-8.3 ppm (m) and 5.3 ppm (s) agreed to the phenyl multiplet and (-OH) of 2-aminophenol moiety. The azomethine proton (-CH=N) signal in the spectrum of the zinc(II) complex is shifted up field at 8.7 ppm (s), compared to the free ligand, suggesting shielding of azomethine group due to the coordination with metal ion. Further on complexation the absence of -OH signals in the spectrum of Zn(II) complex suggests that deprotonated phenolate-O group was involved in the chelation. There is no appreciable change noticed with remaining signals of the complex.

3.4. EPR spectra

To get further information about the geometry, metal-ligand bonding and to determine the magnetic interactions of the metal complex [28], the EPR spectra of Cu(II) complex were recorded both at room temperature and liquid nitrogen temperature in DMSO. The spin-Hamiltonian parameters of the Cu(II) complex are summarized in table 1. The EPR spectrum of the Cu(II) complex (Figure 6) exhibits axially symmetric g-tensor parameters with $g_{II} > g_{\perp} > 2.003$ indicating that the copper site has a d_{x2-y2} ground state characteristic of a square planar geometry [29]. From the values of g factors, the geometric parameter G, representing a measure of exchange interaction between Cu(II) centres in polycrystalline compound can be determined by using the formula:

$$G = (g_{II} - 2) / (g_{\perp} - 2)$$

As reported earlier, if G > 4.0, the local tetragonal axes are aligned parallel or only slightly misaligned. If G < 4.0, significant exchange coupling is present and the misaligned is

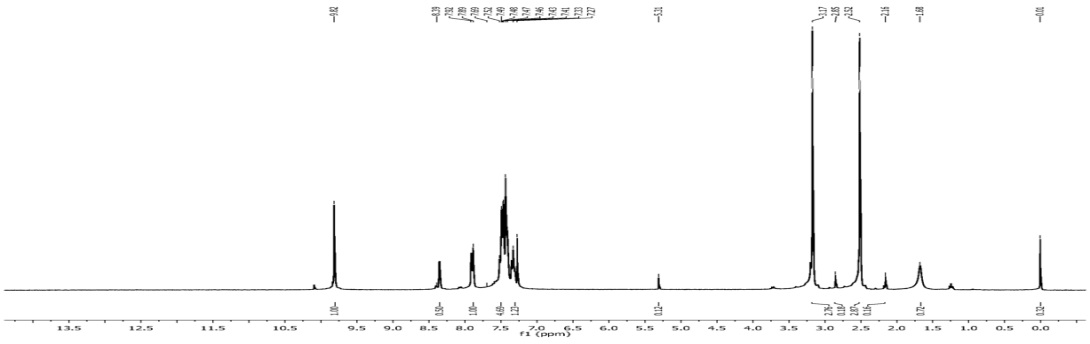


Figure - 5: ^{1}H NMR spectrum of L.

Table - 1: The spin Hamiltonian parameters of the Cu(II) complex (1) in DMSO solution at LNT

Complex	g-tensor			$A \times 10^{-4}$ (cm^{-1})			G
	g_{II}	$g_{\perp}$	g_{iso}	A_{II}	$A_{\perp}$	A_{iso}	
1	2.28	2.08	2.15	158	88	112	4.8

appreciable. The observed value for the exchange interaction parameter for Cu(II) complex is 4.8 G which suggests that the local tetragonal axes are aligned parallel or slightly misaligned and the unpaired electron is present in the d_{x2-y2} orbital. The G value for Cu(II) complex is greater than four (4.8) suggests the absence of exchange coupling between Cu(II) centers in the solid state [30].

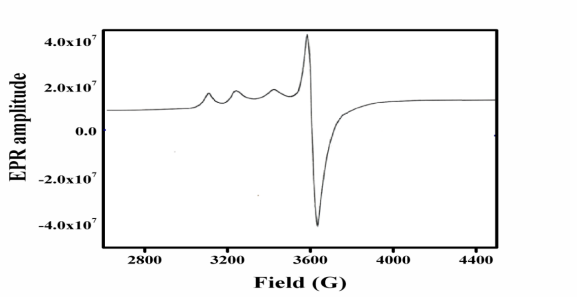


Figure - 6: EPR spectrum of complex (1) at LNT

3.5. Mass Spectra

The ESI-mass spectra of synthesized Schiff base ligand (L) and the metal complexes were recorded at room temperature. The obtained molecular ion peaks confirm the proposed formulae for the synthesized compounds. The mass spectrum of the (L) is depicted in figure7. The mass spectrum of L shows the molecular ion peak at m/z 316 corresponding to $[C_{20}H_{16}N_2O_2]^+$. Also, the spectrum exhibits the fragments at m/z 284, 121 and 105 corresponding to $[C_{20}H_{16}N_2]^+$,$[C_7H_7NO]^+$and $[C_7H_7N]^+$ respectively. The mass spectrum of complex (1) shows molecular ion peaks at m/z 448, which is equivalent to its molecular weight. The fragmentation of $[CuL]Cl_2$ gives elimination of chlorine followed by demetallation of copper ion and the m/z value is 316, corresponding to the fragment ion $[C_{20}H_{16}N_2O_2]^+$. Moreover, the spectrum exhibits the fragments at m/z 284, 121 and 105 corresponding to $[C_{20}H_{16}N_2]^+$,$[C_7H_7NO]^+$and $[C_7H_7N]^+$ respectively. The m/z of all the fragments of ligand and complexes confirm the stoichiometry of the complexes as $[ML]Cl_2$. The observed peaks are in good agreement with their formulae as expressed from micro analytical data. Thus, the mass spectral data emphasize the conclusion drawn from the analytical and conductance values.

3.6. DNA Binding studies

3.6.1. Absorption titration

Absorption titration can observe the interaction of metal complexes with DNA. In general, complex binds to DNA through intercalation binding, usually the results obtained in hypochromism and red shift (bathochromism), due to the strong stacking interaction among the aromatic chromophore of the complex and the base pairs of DNA. The absorption spectra of the complexes (1) and (3) in the absence and presence of CT DNA are given in figure 8. The absorption spectra of copper, cobalt, nickel and zinc complexes showed an intensive absorption bands at 424, 344, 340 and 282 nm in 5 mM Tris-HCl, 50 mM NaCl, at pH 7.2 buffer solutions respectively. On increasing the concentration of CT DNA resulted in the minor bathochromic shift in the range ~ 0.8-2.0 nm and significant hypochromicity lying in the range ~12.2-22.5 % of all the complexes indicating appreciable intercalation binding of the complexes to the CT DNA [31].

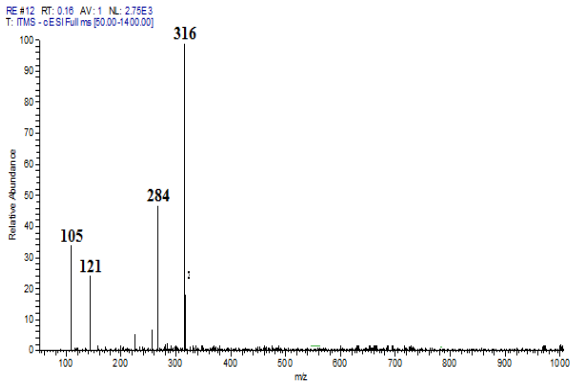


Figure - 7: ESI-Mass spectrum of L.

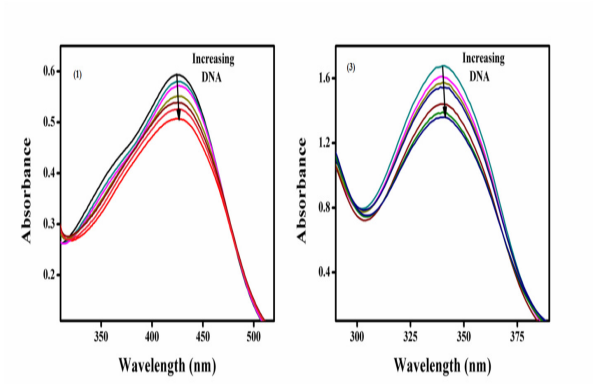


Figure - 8: Absorption spectra of complexes (1) and (3) in buffer pH =7.2 at 25⁰C in presence of increasing amount of DNA. Arrow indicates the changes in absorbance upon increasing the DNA concentration.

The intrinsic binding constant values (K_b) for the complexes (1), (2), (3), and (4) were found to be 3.2×10^4 M^{-1}, 1.2×10^4 M^{-1}, 1.8×10^4 M^{-1} and 2.3×10^4 M^{-1} respectively. The binding constant (K_b) values of these complexes are compared to those observed for typical intercalators, ethidium bromide and $[Ru(bpy)_2(dppz)]^{2+}$ whose binding constants were in the order of 1.4×10^6 and $>10^6$ M^{-1} [32,33]. In order to compare quantitatively the binding strength of the complexes with CT DNA, the intrinsic binding constants (K_b) are obtained

Table2. Electronic absorption parameters for the interaction of DNA with synthesized complexes.

Complex	λ max		Δλ(nm)	[a]H%	$K_b \times 10^4 (M^{-1})$
	Free	Bound			
1	424	426.0	2.0	22.5	3.2
2	344	344.8	0.8	12.2	1.2
3	340	341.0	1.0	15.6	1.8
4	282	282.4	1.4	18.5	2.3

$^a H\% = [(A_{free} - A_{bound}) / A_{free}] \times 100\%$

Table - 3: Electrochemical parameters for the interaction of DNA with synthesized metal complexes.

Complex	$E_{1/2}$(V)[a]		[b]ΔEp(V)		Ip_a/Ip_c
	Free	Bound	Free	Bound	
1	-0.623	-0.325	2.354	3.565	0.95
2	-0.245	0.451	1.025	2.328	0.88
3	-0.452	0.036	1.356	2.348	0.82
4	-0.112	0.124	1.254	1.645	0.92

Data from cyclic voltammetric measurements: $^a E_{1/2}$ is calculated as the average of anodic (E_{Pa}) and cathodic (E_{pc}) peak potentials; $E_{1/2}{}^a = E_{Pa} + E_{pc} / 2$; $^b \Delta Ep = E_{pa} - E_{pc}$

by monitoring the changes in the absorbance for the complexes with increasing concentration of DNA. K_b is obtained from the ratio of slope to the intercept from the plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ *versus* [DNA]. The K_b values are shown in table 2.

3.7. Cyclic voltammetry

Electrochemistry has been growing helpful in elucidating the basic chemistry of biological systems. The cyclic voltammograms of complexes (1) and (2) in the absence and in presence of varying amount of DNA are shown in figure 9. The incremental addition of CT DNA to the complex causes shift in the potential of peak in cyclic voltammogram. This result shows that complex stabilizes the duplex (GC pairs) by intercalating way. The ip_c/ip_a ratios of these four redox couples of the complexes (1-4) are 0.95, 0.88, 0.82 and 0.92 respectively which indicate that the reaction of the complex on the glassy carbon electrode surface is quasi-reversible redox process. The incremental addition of CT DNA to the complex caused a shift in the peak potential in cyclic voltammogram.

This result revealed that complex stabilized the duplex (GC pairs) by intercalating way. Electrochemical parameters for the interaction of DNA with metal (II) complexes (1-4) are shown in table 3. Both the cathodic and anodic peak showed positive or negative shift indicating the intercalation of complex to DNA of base pairs. The incremental addition of CT DNA to the complex caused a positive shift in potential and a decrease in the current intensity. From these data, it is understood that the entire synthesized complexes interact with DNA through intercalating way [34].

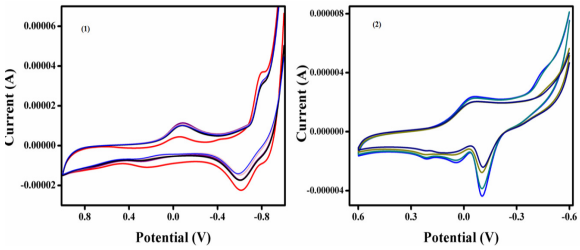


Figure - 9: Cyclic voltammograms of complexes (1) and (2) in buffer (pH = 7.2) at 25[0]C in presence of increasing amount of DNA.

3.8. Antimicrobial activity

Biological activity of the ligand and a series of its metal complexes (1-4) were screened for anti-bacterial activity against Gram-positive bacteria (*S.aureus and B.subtilis*), Gram-negative bacteria (*S.typhi and E. coli*) and anti-fungal activity against the fungi (*A.niger, C.lunata, R.bataticola and C.albicans*) using broth micro dilution method [35]. The antimicrobial results of Schiff base and its metal complexes are systematized in tables 4 and 5. The remarkable activity of Schiff base ligand may arise from the presence of azomethine group which may be one of the reasons for impart in elucidating the mechanism of transformation reaction in biological systems [36]. The results indicate that the complexes show more activity and the ligand has less activity against the same microorganisms under identical experimental conditions.

Table - 4: Minimum inhibitory concentration (MIC) in µg/ml of Schiff base ligand (L) and their metal complexes (1-4) using Streptomycin (positive control) against different bacterial strains (µg/mL)

Compound	Minimum inhibitory concentration (MIC) (× 10^4 µM)			
	S.aureus	*B.subtilis*	*E.coli*	*S.typhi*
L	15.6	16.2	19.4	18.6
1	3.1	3.5	6.6	5.6
2	5.2	5.5	6.5	6.8
3	4.3	4.1	5.5	6.1
4	3.5	4.2	5.2	4.8
Streptomycin	2.5	2.1	2.8	3.5

Table - 5: Minimum inhibitory concentration (MIC) in µg/ml of Schiff base ligand (L) and their metal complexes (1-4) using Nystatin (positive control) against different fungal strains (µg/mL).

Compound	Minimum inhibitory concentration (MIC) (× 10^4 µM)			
	A.niger	*C.lunata*	*R.bataticola*	*C.albicans*
L	18.9	19.2	20.5	21.6
1	4.1	4.6	5.3	5.1
2	4.5	5.1	5.5	6.2
3	5.1	4.6	6.3	6.8
4	4.1	4.5	5.2	5.5
Nystatin	3.5	3.8	4.2	4.8

As previously mentioned, the metal complexes show improved antimicrobial activity than their corresponding ligand. According to the Overtone's concept of cell permeability, the lipid membrane surrounding the cell favours the passage of only lipid-soluble materials and therefore, liposolubility is an important factor which controls the antimicrobial activity. On chelation, polarity of the metal ion is reduced to a greater extent due the overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. The increased lipophilicity enhances the penetration of the complexes into the lipid membranes and blocks the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism [37].

3.9. DNA cleavage efficacy

The ability of the complexes to cleave pBR 322 DNA has been studied by using gel electrophoresis technique. DNA cleavage is controlled by relaxation of super coiled circular form of pBR 322 DNA into nicked circular form and linear form. In continuation, it is also reporting here the cleavage of pBR 322 DNA with complexes (1-4). Their cleavage efficacy can be identified by using the gel electrophoresis diagram as shown in figure 10. The aptitude of the complexes in affecting DNA cleavage has been investigated by gel electrophoresis using super coiled pBR 322 DNA in 5 mM Tris-HCl /50mM NaCl buffer solution (pH 7.2). When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the SC form will relax to produce a slow-moving open circular form-II (OC) [38,39].

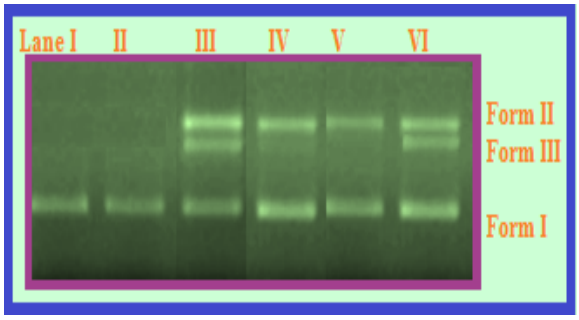


Figure - 10: Gel electrophoresis pattern showing cleavage of pBR 322 DNA treated with metal complexes. Lane I; DNA control; Lane II: DNA + L+ H_2O_2; Lane III: DNA + $[CuL]Cl_2$ + H_2O_2; Lane IV: DNA + $[CoL]Cl_2$ + H_2O_2; Lane V: DNA + $[NiL]Cl_2$ + H_2O_2 and Lane VI: DNA + $[ZnL]Cl_2$ + H_2O_2.

As seen from figure 10 there is no cleavage observed in the case of control (DNA itself). However, all the metal (II) complexes

reveal some good DNA cleaving properties. In detail, Cu (II) and Zn (II) complexes have shown complete DNA cleavage as evidenced by diminishing of lanes intensity while Co (II) and Ni (II) complexes have shown partial DNA cleavage. On comparison, Cu (II) complex stands out the best DNA cleaving agent than other complexes. The DNA cleavage of the metal (II) complexes may also depend on the intercalative binding of the DNA molecules to the metal (II) complexes. The consequence of this DNA cleavage indicates the important role in the isolation of metal ions from the complexes. It may also be concluded that compound which cleaves the DNA may also inhibit the growth of the pathogenic organism by cleaving the genome

4. CONCLUSION

A series of novel Schiff base ligand (L) metal complexes (1-4) have been synthesized and characterized by spectral and analytical data. Interactions of metal complexes with CT DNA were studied by UV absorption titration and cyclic voltammetry studies. All the experimental evidences indicate that these complexes can strongly bind to CT DNA *via* an intercalation mode. Antimicrobial screening shows that the complexes exhibit good biological activity against different organisms. The gel electrophoresis assay demonstrated that all the metal complexes promote the cleavage ability of the pBR 322 plasmid DNA. Results obtained from the present work would be very useful in understanding the interactions of the metal complexes with DNA and helpful in the development of their potential applications in biological and pharmaceutical fields in future.

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