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Synthesis, characterization, antimicrobial and DNA cleavage studies on some metal complexes incorporating 4- chlorobenzaldehyde and 4-aminoantipyrine

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ABSTRACT

Metal complexes of Schiff base (L) ligand, prepared via condensation of 4- chlorobenzaldehyde and 4-aminoantipyrine, are prepared. The ligand is characterized based on elemental analysis, mass, IR and NMR spectra. Metal complexes are reported and characterized based on elemental analyses, IR, electronic spectra, magnetic moment, molar conductance and cyclic voltammetry (CV). From the elemental analyses, 1:2 [M]:[ligand] complexes are prepared with the general formulae $[M(L_2)Cl_2]$ (M = Co(II), Ni(II), Cu(II), Zn(II) and Cd(II)). The IR results demonstrate that the co-ordination sites are the azomethine nitrogen and carbonyl oxygen atoms. The electronic spectral and magnetic measurement data indicate that the complexes exhibit octahedral geometry around the metal center. The in vitro biological screening effects of the synthesized compounds were tested against various microbial species and the results show that the metal complexes are more biological active than the ligand. The DNA cleavage activity of the ligand and its complexes were assayed on pUC18 DNA using gel electrophoresis. The result shows that the Co(II) and Cu(II) complexes have completely cleaved the DNA.

Keywords*:* Schiff's base, electronic spectra, antimicrobial, DNA, ligand

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INTRODUCTION

Schiff bases of azomethine nitrogen donor heterocyclic ligands are well known due to their wide range of applications in pharmaceutical and industrial fields and have been found to act as antibacterial, antifungal, anticancer and herbicidal agents [1-7]. Schiff base can accommodate different metal centers involving various coordination modes thereby allowing successful synthesis of homo and hetero metallic complexes with varied stereochemistry [8, 9]. This feature is employed for modeling active sites in biological systems. Results from these studies have also shown that complexation of metals to Schiff base ligands serves to improve the antimicrobial and anticancer activities of the ligands [10, 11]. Metal complexes of nitrogen–oxygen chelating agents derived from 4-aminoantipyrine Schiff bases have been studied extensively due to their pronounced applications in biological, clinical, analytical and pharmacological areas [12-16]. Y.X. Sun *et al* have studied the crystal structure of 4-(4-chlorobenzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one [17]. The present study deals with the synthesis, characterization and biological studies of the Schiff base derived from 4-chlorobenzaldehyde and 4-aminoantipyrine and its Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes.

EXPERIMENTAL SECTION

4-Aminoantipyrine and 4-chlorobenzaldehyde were obtained from Sigma. Metal(II) chlorides were purchased from Merck. All other chemicals used were of AnalaR grade. Solvents were purified and distilled before use. The metal content in the complexes was determined by EDTA titration [18]. Elemental analysis was obtained using a Perkin-Elmer elemental analyzer. Conductivity measurements were made on freshly prepared 10^{-3} M solutions in DMSO at room temperature with a coronation digital conductivity meter. The IR spectra were recorded in KBr pellet on a JASCO FT/IR-410 spectrometer in the range 4000-400 cm^{-1}. Electronic spectra were recorded on a Perkin Elmer Lambda-25 UV/VIS spectrometer. The room temperature magnetic measurements were carried out using Guoy balance and the diamagnetic corrections were made using Pascal's constant. Cyclic voltammetric measurements were carried out in a Bio-Analytical system (BAS) model CV-50W electrochemical analyzer. The three electrode cell comprised of a reference Ag/AgCl, auxiliary platinum and working glassy electrodes. Tetrabutylammonium perchlorate was used as supporting electrolyte.

Synthesis of Schiff base ligand

A 1:1 equimolar methanolic solution of 4-aminoantipyrine (5 mmol) and 4-chlorobenzaldehyde (5 mmol) were mixed and gently heated for 2 h with constant stirring. The characteristic dark yellow precipitate obtained by Schiff base condensation was filtered out and kept for crystallization, dissolving in methanol. Fine dark yellow crystals were obtained upon slow evaporation at room temperature. It was washed with alcohol, ether and dried in vacuum desiccator over anhydrous calcium chloride. (Yield: 85 %).

Synthesis of metal Schiff base complexes

Metal chloride (1 mmol) was dissolved in MeOH and the solution was filtered and added dropwise into methanolic solution of Schiff base ligand (2 mmol). The above mixture was magnetically stirred and refluxed for 6 h. The metal complexes obtained were filtered, washed with MeOH and dried *in vacuo* (Yield: 60-70 %).

In vitro antimicrobial activity

Antibacterial and antifungal activities of the ligand and its complexes were tested *in vitro* against the bacterial species *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*; fungal species, *Aspergillus niger, Aspergillus flavus* and *Candida albicans* by the disc diffusion method [19]. Amikacin was used as the standard antibacterial agent whereas Nystatin was used as the standard antifungal agent. The test organisms were grown on nutrient agar medium in petri plates. The compounds were prepared in DMF and soaked in filter paper disc of 5 mm diameter and 1 mm thickness. The discs were placed on the previously seeded plates and incubated at 37 ^{o}C and the diameter of inhibition zone around each disc was measured after 24 h for bacterial and 72 h for fungal species.

DNA cleavage analysis

The compounds were added separately to the pUC18 DNA sample. The samples mixtures were incubated at 37ºC for 2 h. The electrophoresis of the samples was done according to the following procedure. Weigh 300mg of agarose and dissolve it in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) by boiling. When the gel attains ~55^{0}C, pour it into the gel cassette fitted with comb. Let the gel to solidify. Carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer. Load DNA sample (mixed with bromophenol blue dye @ 1:1 ratio), carefully into the wells, along with standard DNA marker and pass the constant 100 V of electricity till the dye front reaches the end of gel. Remove the gel and carefully stain with ETBR solution (10 µg/ml) for 10-15 min and observe the bands under UV transilluminator.

RESULTS AND DISCUSSION

Characterization of Schiff base ligand

The results of elemental analysis (Table 1) of the Schiff base ligand are in good agreement with those calculated for the suggested formula. The Schiff base is soluble in all common organic solvents. The DART mass spectrum of the ligand shows a well-defined molecular ion peak at m/z = 325.32 (Relative Intensity = 14%), which coincides with formula weight of the Schiff base. In the ^{1}H-NMR spectrum (Fig.1a), the signal for azomethine proton (-CH=N-) in the ligand appears as a singlet at 9.99 ppm. The multiplet signals obtained in the δ 7.0-8.0 ppm range are due to the aromatic protons of Schiff base ligand. The signal for pyrazolone ring carbon attached methyl protons (-CH_3) appear

as a singlet at δ 2.48 ppm while pyrazolone ring nitrogen attached methyl protons (>N-CH_3) appear as a singlet at δ 3.16 ppm. In the ^{13}C-NMR spectrum (Fig.1b), the azomethine carbon signal has appeared at 155 ppm. The pyrazolone ring carbon attached methyl carbon (-CH_3) and pyrazolone ring nitrogen attached methyl carbon (>N-CH_3) peaks have been observed in the expected range at 9 and 35 ppm. The aromatic carbon signals are seen at 106-157 ppm range depending on their electronic environment. The IR spectrum of the ligand displays a sharp band at 1602 which can be assigned to >C=N stretching frequency. Further, the Schiff base ligand exhibits a band at 1655 cm^{-1} due to υ(C=O). The electronic spectrum of the ligand shows a broad band at 335 nm, due to $\pi - \pi^*$ transition of the azomethine (>C=N) chromophore. In addition, the other intense absorption band at higher energy, 220-300 nm, is due to the $\pi - \pi^*$ transition of the benzene ring and other characteristic transition of the Schiff base.

Table 1. Analytical and physical data of the Schiff ligand and its complexes

Compound	Empirical formula	Colour	Elemental analysis Found (calcd) %				Λc (Ohm^{-1} cm^2mol^{-1})	λ_{max} (nm)	μ_{eff} (B.M)
			C	H	N	M			
L	$C_{18}H_{16}N_3OCl$	Dark Yellow	65.87 (66.36)	4.65 (4.95)	12.45 (12.90)	-	-	220, 250, 335	-
$[CoL_2Cl_2]$	$CoC_{36}H_{32}N_6O_2Cl_4$	Brown	55.21 (55.33)	4.01 (4.13)	10.43 (10.75)	7.63 (7.54)	05	685, 512	5.08
$[NiL_2Cl_2]$	$NiC_{36}H_{32}N_6O_2Cl_4$	Light green	54.93 (55.01)	4.03 (4.13)	10.42 (10.76)	7.68 (7.51)	09	~1100, 696, 383	3.02
$[CuL_2Cl_2]$	$CuC_{36}H_{32}N_6O_2Cl_4$	Deep Green	55.39 (55.01)	4.68 (4.10)	10.56 (10.69)	8.74 (8.08)	05	846, 424	1.82
$[ZnL_2Cl_2]$	$ZnC_{36}H_{32}N_6O_2Cl_4$	Light Yellow	54.73 (54.88)	4.48 (4.09)	10.56 (10.67)	8.01 (8.30)	07	220, 250, 326	Dia
$[CdL_2Cl_2]$	$CdC_{36}H_{32}N_6O_2Cl_4$	Light Yellow	51.65 (51.79)	3.72 (3.86)	10.39 (10.07)	13.25 (13.46)	12	220, 250, 330	Dia

Table 2. IR spectral data of the Schiff base ligand and its complexes (cm^{-1})

Compoun	υ_{azo}.(C=N)	υ(C=O)	υ_{ring}(C-N)	υ(M-O)	υ(M-N)
L	1602	1655	1231	-	-
$[CoL_2Cl_2]$	1594	1648	1217	510	430
$[NiL_2Cl_2]$	1590	1644	1220	516	430
$[CuL_2Cl_2]$	1591	1645	1223	520	438
$[ZnL_2Cl_2]$	1596	1650	1221	522	436
$[CdL_2Cl_2]$	1594	1646	1224	521	434

Table 3. Antimicrobial activity results of the Schiff base ligand and its complexes

Compound	Inhibition zone (μg/mL)						
	Bacteria species				Fungi species		
	S.aures	*E.coli*	*P. aeruginosa*	*B. subtilis*	*C. albicans*	*A. niger*	*A. flavus*
L	11.5	10.5	5.7	11.5	6.9	5.4	8.9
$[CoL_2Cl_2]$	14.3	15.2	10.5	11.5	12.5	16.9	10.2
$[NiL_2Cl_2]$	12.0	10.5	6.5	10.5	10.5	9.8	9.8
$[CuL_2Cl_2]$	12.5	16.5	16.2	17.5	11.5	18.5	15.4
$[ZnL_2Cl_2]$	12.3	10.2	12.5	8.7	10.2	7.2	9.5
$[CdL_2Cl_2]$	11.5	12.4	7.3	9.8	8.2	7.5	9.0
*Amikacin**	19.2	20.5	20.5	18.5	-	-	-
*Nystain**	-	-	-	-	19.5	20.0	19.5

**Standard*

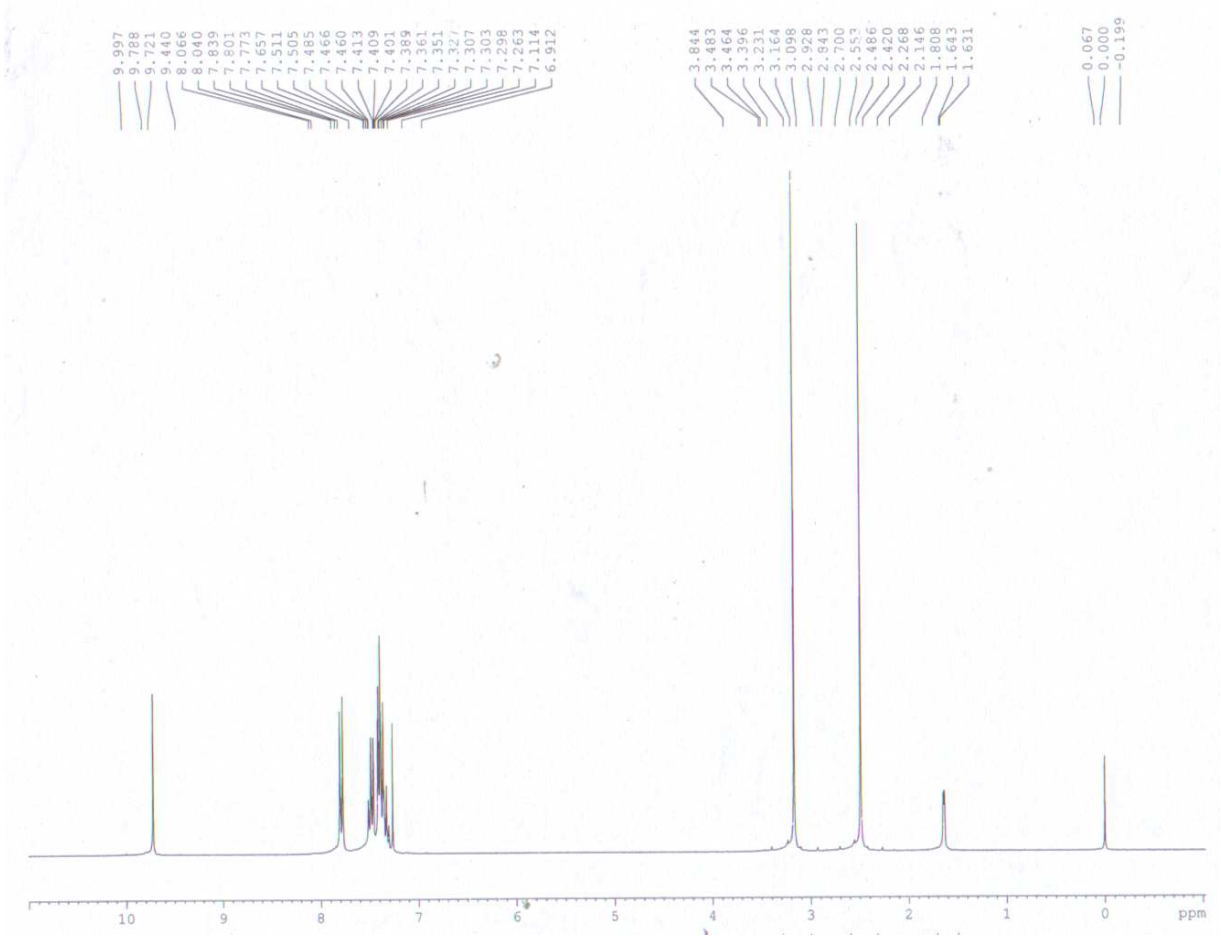
Characterization of metal Schiff base complexes

The analytical data and physical properties of the metal Schiff base complexes are listed in Table 1. The Schiff base complexes are soluble in CH_3CN, DMF and DMSO and insoluble in other common organic solvents. The analytical data indicate that the metal to ligand ratio is 1:2 for all the complex systems. The low conductivity values of the metal complexes (Table 1) suggest their non-electrolytic nature [20]. The DART mass spectrum of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes shows a peak at m/z 781.87 (10 %), 781.07 (8 %), 786.50 (11 %), 788.07 (14 %) and 834.77 (12 %) respectively, corresponding to their molecular weight. The mass spectrum of all the complexes indicates that the complexes are monomeric confirming the metal to ligand ratio to be 1:2 in the complexes.

IR spectra

The important IR spectral data are given in Table 2. On complexation the band at 1602 cm^{-1} for the azomethine group in the free ligand was shifted to lower frequency in the range ~1594-1590, indicating the coordination of the azomethine nitrogen atom to the metal ion. The sharp band at 1655 cm^{-1} for υ(C=O) group of the free ligand has been shifted to ~1650-1644 cm^{-1} in the complex indicating the linkage between the metal ion and carbonyl oxygen atom. Further, the spectrum of all the metal complexes show new bands in the 521–510 cm^{-1} and 438–430 cm^{-1} regions, which may probably be due to the formation of M-O and M-N bonds, respectively [21].

Fig. 1a. ^{1}H-NMR spectra of Schiff base ligand



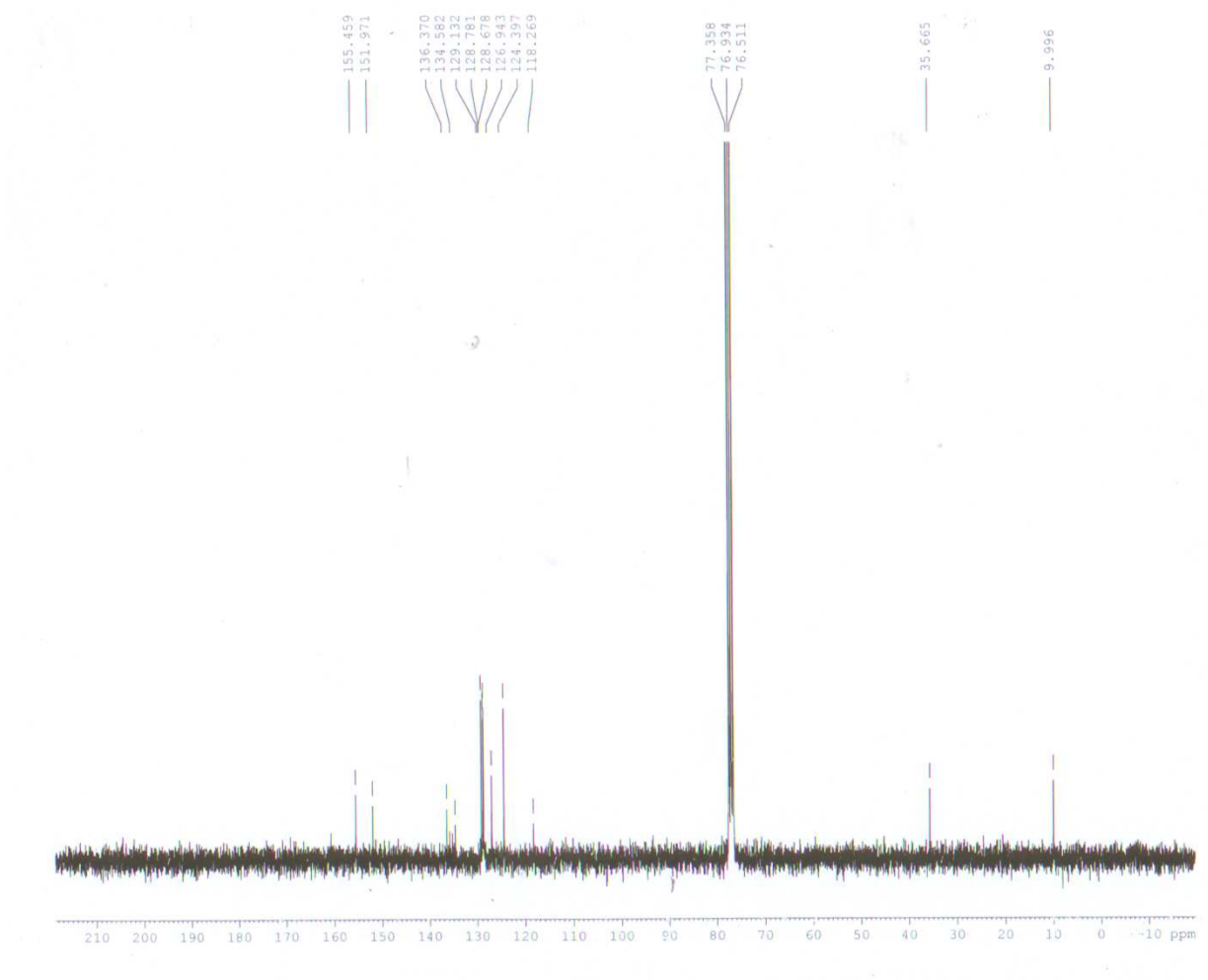
Electronic spectra

The electronic spectrum of Co(II) complex exhibits transitions at 685 and 512 nm respectively. The three possible d–d bands of high spin Co(II) octahedral complexes are $^4T_{1g}$ (F) → $^4T_{2g}$ (F), $^4T_{1g}$ (F) → $^4A_{2g}$ (F)) and $^4T_{1g}$ (F) → $^4T_{1g}$(P). The last transition will be highest in energy and may appear under the envelope of ligand-centered transitions [22]. Thus the two transitions of the present Co(II) complex can be assigned to $^4T_{1g}$ (F) → $^4T_{2g}$ (F) and $^4T_{1g}$ (F) → $^4A_{2g}$ (F)) respectively, which demonstrates its octahedral. The electronic spectrum of the Ni(II) complex shows three bands in the region ~1100, 696 and 383 nm attributable to $^3A_2g(F) \rightarrow {}^3T_2g(F)$, $^3A_2g(F) \rightarrow {}^3T_1g(F)$ and $^3A_2g(F) \rightarrow {}^3T_1g(P)$ transitions respectively, suggesting an octahedral geometry around the Ni(II) [22]. The Cu(II) complexes in their spectra display a broad band centered at 846 nm and a weak shoulder at 424 nm region due to $^2E_g \rightarrow {}^2A_1$ g and $^2E_g \rightarrow {}^2B_1g$ transitions, respectively, indicating the complex to have distorted octahedral geometry [22].

Magnetic measurements

The Co(II) complex has a magnetic moment of 5.08 B.M (Table 1), which is in agreement with the reported value for octahedral Co(II) complexes [23, 24]. The present Ni(II) complex shows magnetic moment value of 3.02 within the range of 2.9–3.3 BM [23, 24] suggesting octahedral environment. The Cu(II) complex shows magnetic moment value 1.82 BM, higher than the spin-only value 1.73 BM expected for one unpaired electron, monomeric and consistent with a distorted octahedral geometry [23, 24]. The complexes of Zn(II) and Cd(II) are diamagnetic and according to the empirical formulae of these complexes, and octahedral geometry is proposed. Based on the above results, one can deduce the probable structures of the complexes as shown in Fig. 2.

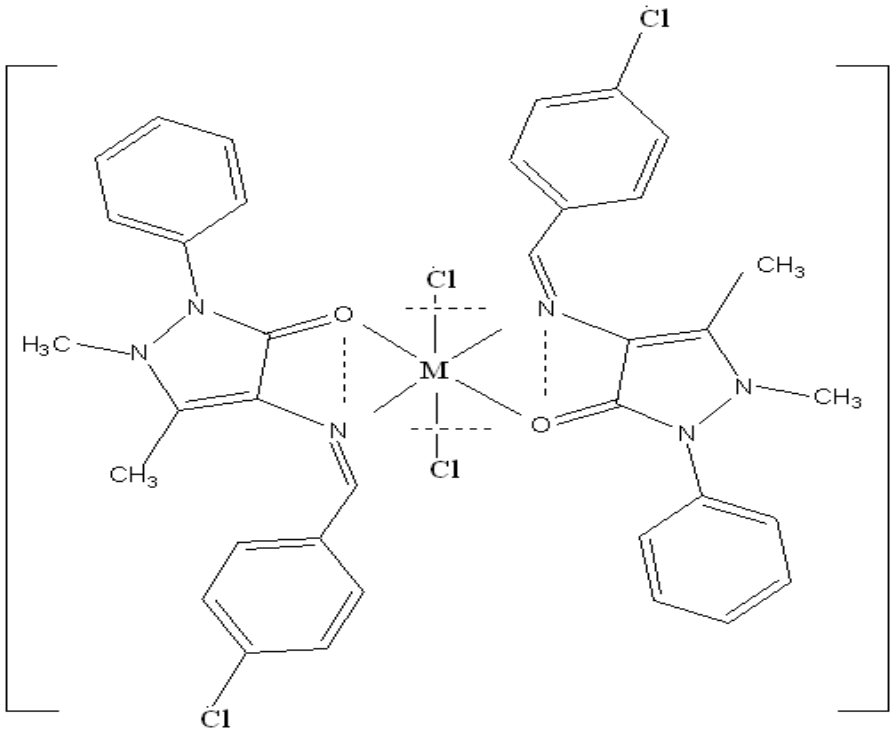
Fig. 1b. ^{13}C-NMR spectra of Schiff base ligand



Cyclic volumetric studies

The cyclic voltammogram of the Co(II) complex shows a well defined redox process corresponding to the formation of the quasi-reversible Co(II)/Co(I) couple. The cathodic peak at 1.425 V versus Ag/AgCl and the associated anodic peak at 0.593 V corresponds to the Co(II)/Co(I) couple. The peak to peak separation (ΔEp) is 0.832 mV indicating quasi-reversible one electron transfer process. The redox property of Ni(II) complex displayed an irreversible cathodic peak at –1.124 V, corresponding to the reduction of Ni(II)/Ni(I). The irreversibility of the Ni(II)/Ni(I) was checked by varying the scan rates with peak potentials. The cyclic voltammogram of the Cu(II) complex displayed two reduction couples at 0.823 V and 1.348 V versus Ag/AgCl with the corresponding anodic wave for the first reduction (0.384 V) and without the corresponding anodic wave for the second reduction on the reverse scan. The former has a peak separation value of 0.439 V indicating quasi-reversible character for the one electron transfer reaction of metal-based Cu(II)/Cu(I) couples and the later one can be assigned to ligand characteristic irreversible

reduction process. The cyclic voltammogram of the Zn(II) and Cd(II) complexes didn't show any characteristic peak potential indicating that the complexes stabilize the ligand in +2 oxidation state.



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

Fig. 2. Proposed structure of Schiff base complexes

Biological studies

In vitro antimicrobial activity

The *in vitro* biological screening results are given in Table 3. The standard error for the experiment is ± 0.001 cm and the experiment is repeated three times under similar conditions. DMF is used as negative control and Amikacin is used as positive standard for antibacterial and Nystatin for antifungal activities.

From the results, it has been observed that the metal complexes showed better activity than the free ligand under identical experimental condition. The enhanced activity of the complexes can be explained on the basis of Overtone's concept [25] and Tweedy's Chelation theory [26]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials makes which liposolubility is an important factor, which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the permeation of the complexes into lipid membranes and blocking of 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 the proteins that restricts further growth of the organism and as a result microorganisms die. On comparing the biological activity of the Schiff base and its metal complexes with the standard, it is seen that the biological activity follows the order; Cu(II)>Co(II)>Ni(II)>Zn(II)>Cd(II)>L.

DNA cleavage studies

Gel electrophoresis experiments using pUC18 DNA were performed with ligand and its metal complexes in the presence of H_2O_2 as an oxidant. From Fig. 3, it is evident that the Co(II) and Cu(II) complexes cleave DNA

completely whereas the Cd(II), Ni(II), Zn(II) as well as the free ligand have exhibit no significant cleavage activity in the presence of H_2O_2. The general oxidative mechanisms proposed account for DNA cleavage by hydroxyl radicals via abstraction of a hydrogen from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed. The capacity of metal complexes to activate dioxygen, or its reduced form hydrogen peroxide, will lead to the functionalization of an inert C-H bond of DNA to a C-O bond. DNA oxidation by metal complexes occurs by C-H bond activation at the deoxyriboses [27, 28].

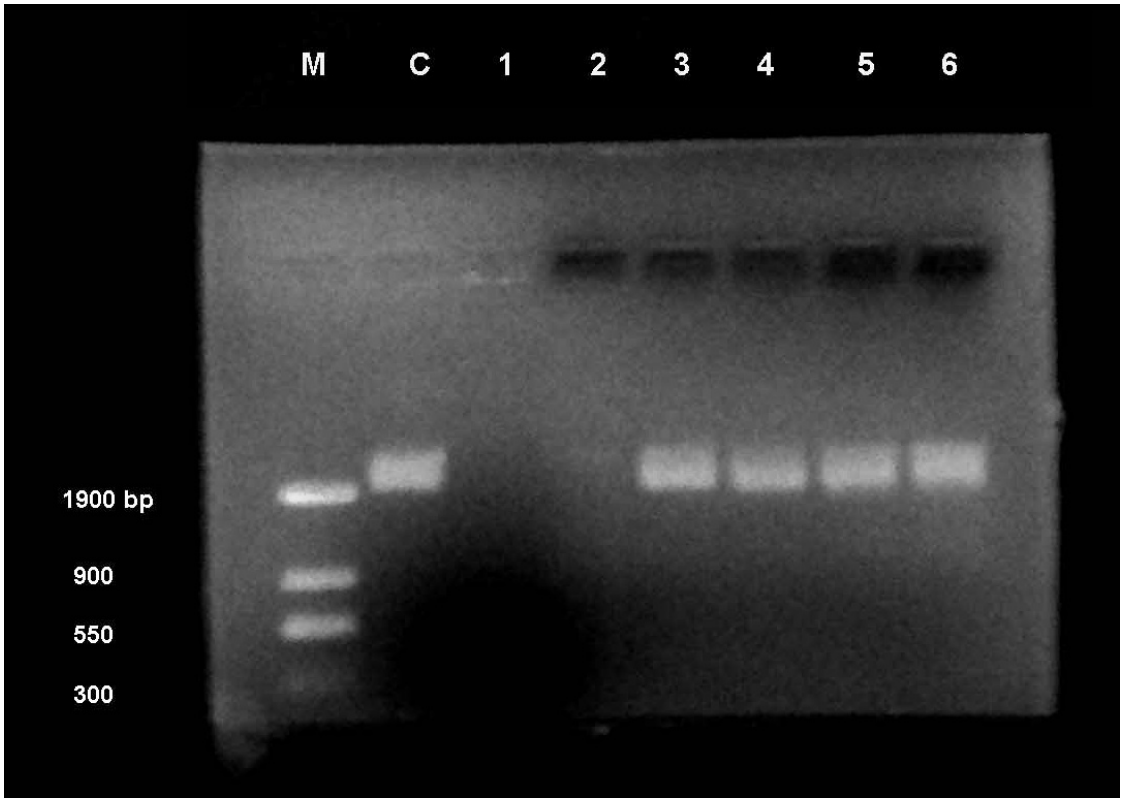


Fig.3. DNA cleavage studies of Schiff base ligand and its complexes
M- Marker, C- Control pUC 18 DNA (untreated sample), 1- [CuL_2Cl_2] + DNA + H_2O_2, 2- [CoL_2Cl_2] + DNA + H_2O_2, 3- [CdL_2Cl_2] + DNA + H_2O_2, 4- [NiL_2Cl_2] + DNA + H_2O_2, 5-[ZnL_2Cl_2] + DNA + H_2O_2, 6- ligand + DNA + H_2O_2

CONCLUSION

Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes with the Schiff base ligand derived from 4-chlorobenzaldehyde and 4-aminoantipyrine were synthesized and characterized by various physico-chemical methods. The analyses confirmed the composition and structures of the newly obtained complex combinations. The coordination of the Schiff base to the metal atom was found to be through the azomethine nitrogen, and the carbonyl oxygen atoms. The geometry of the complexes is assigned as octahedral. The Cu(II) complex shows better activity against most of the microbial species compared to that of ligand and other complexes. The DNA cleavage studies show that the Cu(II) and Co(II) complexes have completely cleaved the DNA.

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