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Research Article

Antimicrobial and DNA Cleavage activities of Macrocyclic Cu(II), Ni(II), Co(II), and Zn(II) Schiff base Complexes

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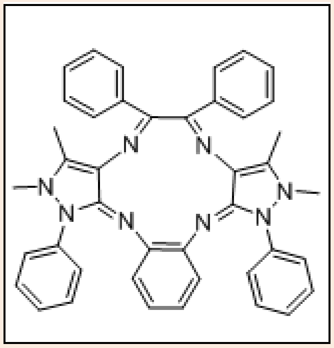
Abstract

A series of transition metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) have been synthesized from their corresponding metal chlorides using a macrocyclic Schiff base as a ligand which is derived from benzil, 4-aminoantipyrine and o-phenylenediamine. Elemental analyses, magnetic susceptibility, molar conductance, Mass, FT-IR, Far IR, UV-Vis, VSM,^{1}H NMR, ^{13}C NMR,SEM, EDX and ESR have been used to assign the chemical structure of synthesized transition metal complexes. The UV-Vis, magnetic susceptibility and ESR the spectral data reveal that all complexes are found to have octahedral geometry. Antimicrobial screening tests indicate that the metal complex of Cu(II) has good antimicrobial activity than the other metal complexes. The nuclease activity of the above metal complexes shows that Co(II)complex has cleave DNA effectively.



Keywords: macrocycliccomplex, benzil, 4-aminoantipyrine, o-phenylenediamine, antimicrobial activity

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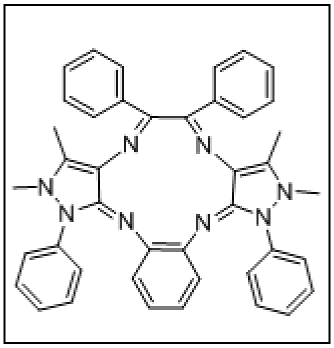
Introduction

Schiff base of 4-Aminoantipyrine and its metal complexes have varied applications in biological [1-3] clinical, analytical, corrosion science and pharmacological area[4-6] .Studies of new kinds of chemotherapeutic schiff bases and their functions are attracting the attention of biochemists[7-8]. Earlier reports on Schiff base and its metal complexes showed that some drugs have greatest activity as metal complexes when compared to the ligands [9-10] .Macrocylic compounds are used as ligands for the preparation of metal complexes of pharmacologically important[11-12]. Macrocylic compounds have been severed as ligands because they have potential donor sites in their structure. Especially nitrogen containing macrocylic have a strong tendency to form stable complexes with transition metal. Probe into the literature clearly reveal that much work has been carried out on metal complexes of macrocyclic ligand and are reported to posses antimicrobial and HIV activities[13-15]. In the present study novel tetra dentate Schiff base was prepared by reacting Benzil, 4-aminoantipyrine and o-phenylenediamine and it serves as a ligand it is capable of binding with the metals through nitrogen atom of two different types of azomethine group.

Experimental

Synthesis of Schiff base

To the methanolic solution of 4-aminoantipyrine (0.40 g 0.2 mmol),benzil (0.10 g, 0.10 mmol) was added and the reaction mixture was kept over magnetic stirrer and stirred well for 3 h. The light brown coloured solid separated out from the mixture was filtered and recrystallized from methanol and dried over vaccuo. The solid thus obtained is refluxed with o-phenylenediamine (0.04 g ,0.10 mmol) dissolved in 40 ml ethanol for 30 h.The brown coloured solid separated out was filtered off and dried over $CaCl_2$.



Scheme 1

Synthesis of Complexes

A solution of metal(II)chloride in methanol (0.2 mmol) was refluxed with methanolic solution of the Schiff base (0.2 mmol) for 6 h. The volume of the solution was then reduced to one third by keeping on a hot water bath. The solid complexes precipitated were filtered, washed thoroughly with methanol and dried in vaccuo.

Analytical and physical measurements

All the reagents, such as benzil, 4-aminoantipyrine,o-phenylenediamine and various metal(II)chlorides were of Aldrich and Merck products. The solvents like methanol, DMSO and DMF were purified and dried by standard procedures[16-17].The micro analytical data of compounds were recorded at IISC Bangalore. The mass spectra of the ligands were recorded at IIT Madras. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker DPX - 300 spectrometer using MeOD as solvent and TMS as an internal standard at IIT Madras. Far IR spectra of the complexes were recorded on a Perkin –Elmer 783 spectrometer in the range of 400-0 cm^{-1}using KBr pellet at IISC, Bangalore. The UV-Visible spectra were recorded on a Schimadzu UV spectrometer in the range 200-700 nm at CLRI, Madras. The magnetic susceptibility of the complex was recorded by vibrating sample magnetometer (VSM) at IIT Madras. The effective magnetic moments were calculated using the formula μ_{eff}=2.228 $(x_M T)^{1/2}$, where X_M is the corrected molar susceptibility. The molar conductance was measured on ELICO CM–180 using DMSO as the solvent at room temperature. SEM-EDX for ligands and its complexes were recorded at IISC Bangalore. The antibacterial activity of Schiff base ligands and their metal complexes were studied against bacteria like *E.coli, S.aureus*, and fungus like *A.niger* and *A.flavas.*The DNA cleavage ability of the newly synthesized compound was examined by gel electrophoresis method.

Antimicrobial activity

The antimicrobial activity was determined for the investigated compounds by tests against the bacteria: *E.coli, S.aureus*, and fungus: *A.niger* and *A.flavas* by well diffusion method[18] using agar nutrient as the medium. The stock solution was prepared by dissolving (100 mg) of compounds in DMSO. In a typical procedure a well was made on the agar medium inoculated with micro organism. The well was filled with the test solution using micropipette and the plate was incubated 24 h for bacteria and 72 h for fungi at 35 °C. During this period the test solution diffused and the growth of the inoculated microorganisms was affected.

DNA cleavage activity

The DNA cleavage ability of the newly synthesized compound was examined by gel electrophoresis method. The electrophoresis of the samples were done according to the following procedure (Sam brook et al., 1989)[19].

The compounds were added separately to the DNA sample. The sample mixtures were incubated at 37 ºC for 2 h. 250 mg of agarose well dissolved in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) . When the gel attains ~55 ^{0}C, it was poured into the gel cassette fitted with comb. When the gel gets solidify the comb was removed and the gel was placed in the electrophoresis chamber flooded with TAE buffer 20 µl of DNA sample (mixed with bromophenol blue dye @ 1:1 ratio), was carefully loaded into the wells, along with standard DNA marker and the constant 50 V of electricity was passed for around 45 min. The gel was removed carefully and stained with ETBR solution (10 µg/ml) for 10-15 min and the bands were observed under UV transilluminator.

Results and Discussion

The complexes are soluble in methanol and DMSO.Conductivity measurement of all the complexes in 10^{-3} M DMSO solution indicated the non-electrolyte (below 2 S cm^2mol^{-1}). The analytical data of the reported complexes are as follows.

$Co(C_{42}H_{36}N_8)Cl_2$ (1).Yield: 60%; F.W: 782.60; Anal. Calcd: C,64.46; H,4.64; N,14.32; Co,7.53%.Found: C,64.40; H,4.60; N,14.31; Co,7.32%

$Ni(C_{42}H_{36}N_8)Cl_2$ (2) Yield :65%; F.W: 782.40; Anal. Calcd: C, 64.48; H, 4.64; N,14.32; Ni,14.32%.Found: C,64.40; H,4.60; N,14.31; Ni, 14.20%.

$Cu(C_{42}H_{36}N_8)Cl_2$(3) Yield :68%; F.W: 787.20; Anal. Calcd: C, 64.08; H,4.61; N, 14.23;Cu, 8.07 %.Found:C, 64.02; H, 4.57; N, 14.23; Cu, 8.11%

$Zn(C_{42}H_{36}N_8)Cl_2$(4).Yield :70%; F.W: 789.10; Anal. Calcd: .C, 63 93; H, 4.60; N, 14.20; Zn,8.29 %Found: C, 63.87; H, 4.56; N, 14.19; Zn,8.20%

IR spectra

A pair of bands corresponding to $\nu(NH_2)$ appeared at 3210-3435 cm^{-1} [20] in the IR spectrum of o-phenylenediamine is found absent in the IR spectra of all the metal complexes . Furthermore no strong absorption band was observed in the spectra of all complexes near 1700 cm^{-1}, indicating the absence of the >C=O group of benzil and thus confirming the condensation of oxygen atom the carbonyl group of benzil and the nitrogen atom of amino group of the o-phenylenediamine [21-22]. A strong absorption band in the region 1614 -1620 cm^{-1} [23] may be assigned to ν(C=N) [24-25] These are the strong evidence for the formation of the macrocyclic frame [26].The lower values of ν(C=N) are due to drift of lone pair electron density of the azomethine nitrogen towards the central metal atom [27].The bands in the region 1508-1518 cm^{-1} indicates the presences of the ν(C=C) aromatic phenyl groups. The C-N stretching vibrations of the complexes occur in the region 1217-1230 cm^{-1}.

From the Far IR spectrum of each complex, it has been revealed there bands at 450 – 460 cm^{-1} are due to **ν** (M-N) [28-29]. Cleary shows the coordination of the azomethine nitrogen to the central metal atom [30].The bands appeared at 310 – 343 cm^{-1} in all complexes have been assigned to **ν** (M-Cl) [31]. These two frequencies support the formation of M–N and M–Cl bonds.

Table 1 Infrared spectral data ν / cm^{-1}, of the divalent of Co(II), Ni(II), Cu(II) and Zn(II)complexes derived from benzil, 4-aminoantipyrine and o-phenylenediamine

Compounds	**Complex**	**ν (C=N)**	**ν (C=C)**	**ν (C –N)**	**ν (M-N)**	**ν (M–Cl)**
1	$C_{42}H_{36}N_8$	1645	1591	1230	–	–
2	$[Co(C_{42}H_{36}N_8)Cl_2]$	1614	1508	1228	450	321
3	$[Ni(C_{42}H_{36}N_8)Cl_2]$	1616	1509	1228	460	343
4	$[Cu(C_{42}H_{36}N_8)Cl_2]$	1616	1510	1230	440	310
5	$[Zn(C_{42}H_{36}N_8)Cl_2]$	1620	1518	1217	455	318

^1H NMR Spectra

The spectrum of the ligand was recorded in MeOD showed the following signals.

δ: 2.75 (S ,6H) $(=C-CH_3)_2$, δ: 3.33 (S ,6H) $(N-CH_3)_2$, δ: 7.34 – 7.98 (M ,24) [32-33] aromatic proton

^{13}C NMR Spectra

δ:140.4 (-C=N), δ:128.96 aromatic carbon, δ:48.13 & 35.94 $(N-CH_3)$, $(C-CH_3)$ group present in the ring [34].

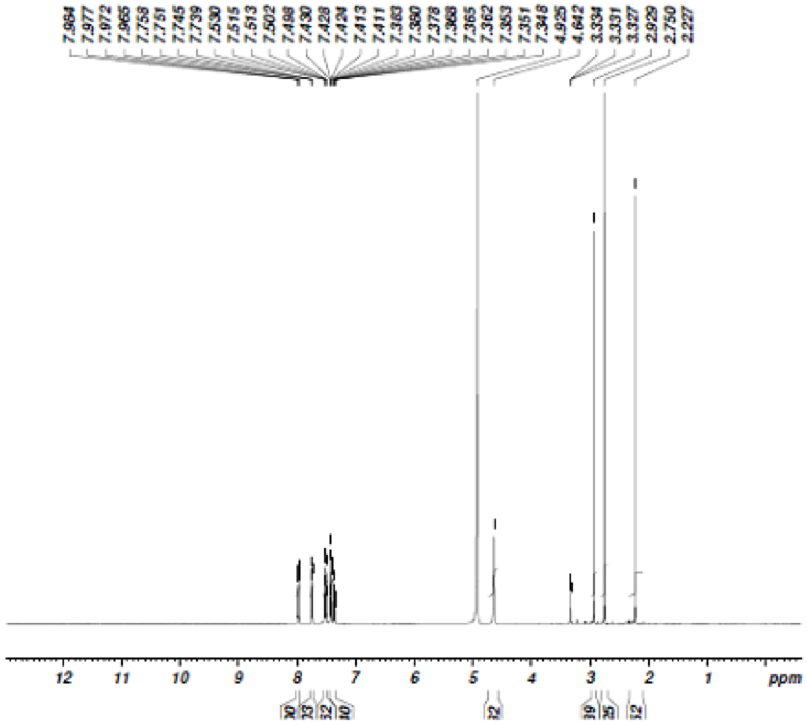


Figure 1 ^{1}H NMR (a) Spectra of the ligand

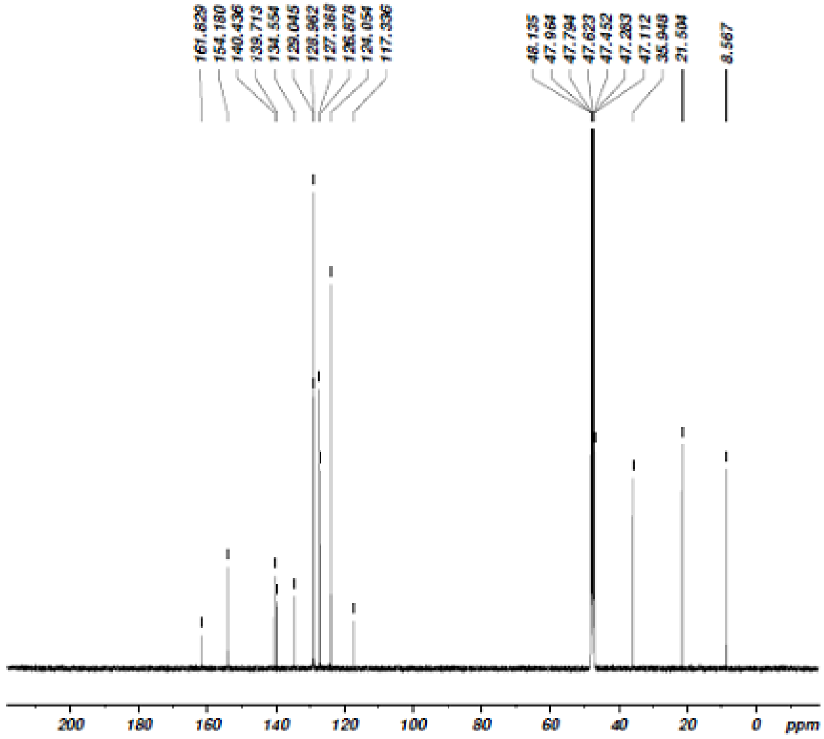


Figure 2 ^{13}C NMR (b) Spectra of the ligand

Electronic spectra

UV-Vis spectra of complexes were revealed using 10^{-3} M methanolic solution of the complex.

Cobalt complex.

The solution spectra of Co(II) complexes exhibit three bands at 11264 cm^{-1},15123 cm^{-1} and 23980 cm^{-1} and are assigned to $^4A_2g(F) \rightarrow {}^4T_1g(P)$, $^4A_2g(F) \rightarrow {}^4T_1g(F)$, and $^4A_2g(F) \rightarrow {}^4T_2g(F)$ transition respectively. These spectral data suggest that the complex have octahedral geometry. [34].

*Nickel complex***.**

The solution spectra of Ni(II) complexes exhibit only one band at 22624 $cm^{-1}$is assigned to$^3A_2g(F) \rightarrow {}^3T_{1g}(P)$ transition which favours an octahedral geometry.

*Copper complex***.**

The solution spectra of Cu(II) complexes exhibit only one band at 23148 $cm^{-1}$and is assigned to the $^3A_2g \rightarrow {}^3T_2g$ transition which shows that complex has distorted octahedral configuration around the Cu(II) ion [35].

Magnetic susceptibility measurement

Vibrating scanning magnetization study was carried out for the entire complex at room temperature. The results of the study are shown in figure 2

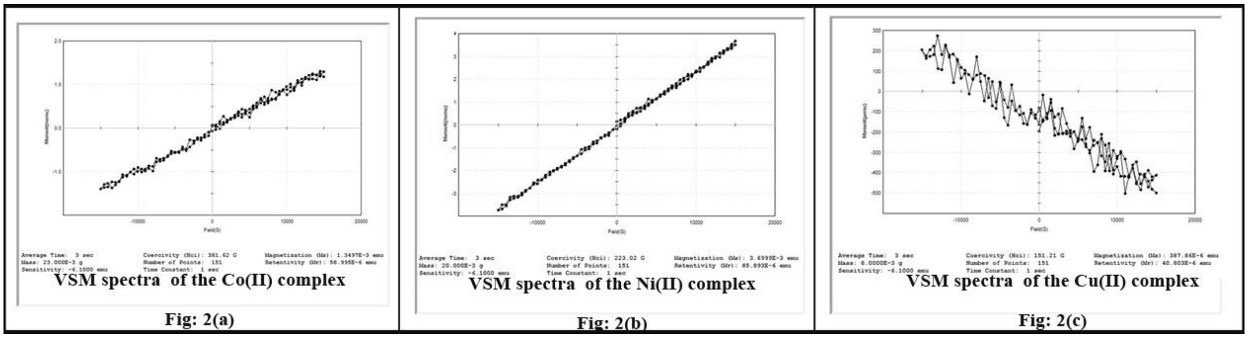
*Cobalt complex***.** The magnetic moments of the Co(II) complexes was found in the range 1.99 BM. The lower value magnetic moments of complex is not pure octahedral complex, suggesting flattening towards planer arrangement [36-39].

*Nickel complex***.** The magnetic moment of Ni(II) complexes was found in the range 3.55 BM. The magnetic moments value range 3.14-3.68 BM has been reported earlier for octahedral complex [40].This value slightly higher than the spin only value of 2.83 BM probably due to slight distortion from the pure octahedral to D_4h symmetry.

*Copper complex***.** The magnetic moments of the Cu(II) complexes was found in the range 1.81 BM.indicate one electron paramagnetism. This value is slightly higher than the spin only value of 1.77 BM. The slightly higher value of the magnetic moment indicates that complex are monomeric in nature and there is no metal-metal interaction along axial position in the complex and have distorted octahedral environment [41-43].

Table 2 Magnetic moment values of the Schiff base metal (II) complexes

Complex	Magnetic momentum (BM)
$[Co(C_{42}H_{36}N_8)Cl_2]$	1.99
$[Ni(C_{42}H_{36}N_8)Cl_2]$	3.55
$[Cu(C_{42}H_{36}N_8)Cl_2]$	1.81



VSM spectra of the Co(II) complex

Fig: 2(a)

VSM spectra of the Ni(II) complex

Fig: 2(b)

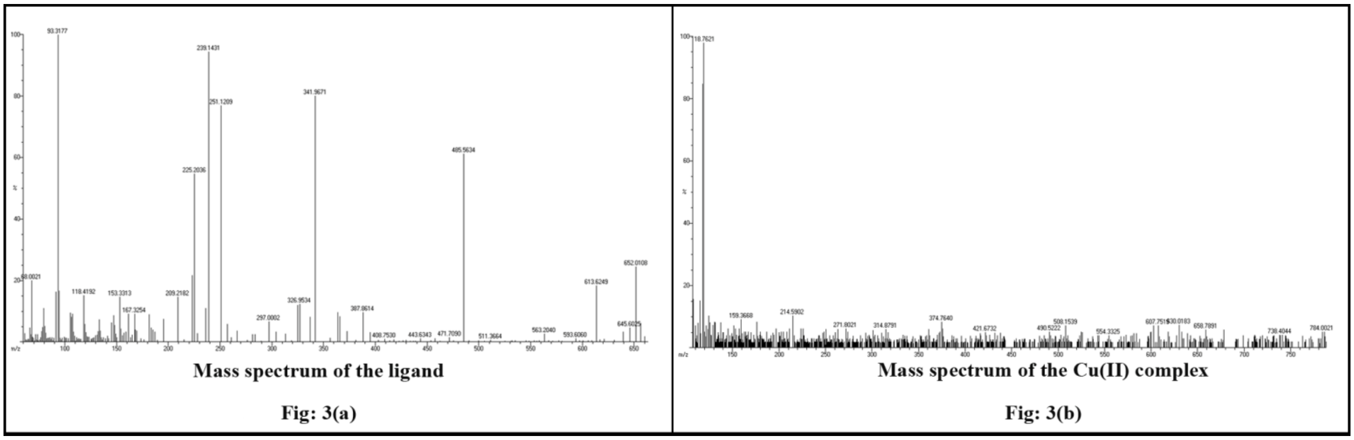
VSM spectra of the Cu(II) complex

Fig: 2(c)

Figure 2 VSM spectra of the metal complex

Mass Spectra

The ESI mass spectra of the ligand and its Cu(II) complex $[Cu(L)Cl_2]$ recorded at room temperature were used to compare their stoichiometry composition [44]. The Schiff base showed a molecular ion peak at m/z 652 which was also supported by the ‛nitrogen rule’ since the compound possess eight nitrogen atoms. The Cu(II) complex shows the molecular ion peak at m/z 784 confirms the stoichiometry of metal chelates as ML type [45].



Mass spectrum of the ligand

Fig: 3(a)

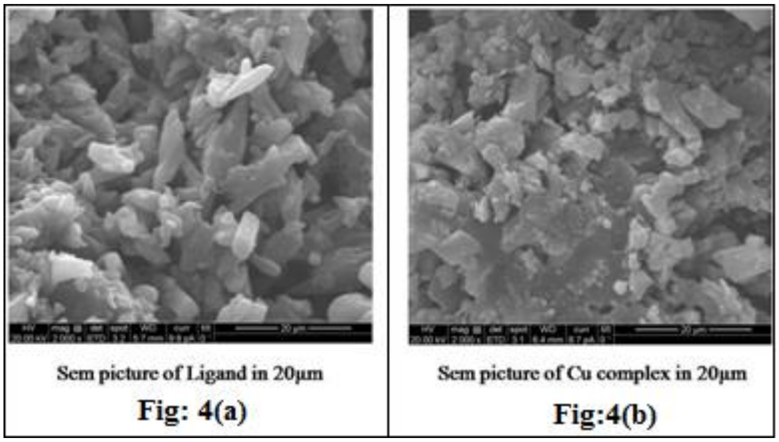
Mass spectrum of the Cu(II) complex

Fig: 3(b)

Figure 3 Mass spectra of the ligand (a) and its Cu(II)complex (b)

SEM- EDX analysis

The surface morphology of ligand and Cu(II)complex has been examined using scanning electron microscope at a magnification of 2000 X. The distinct morphology of ligand and Cu(II) complex can be observed. The size of the flakes is greater in the free ligand than the Cu(II) complex



Sem picture of Ligand in 20µm

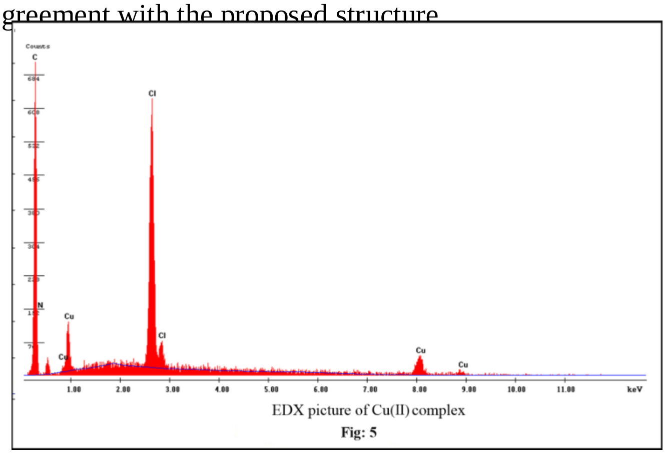
Fig: 4(a)

Sem picture of Cu complex in 20µm

Fig:4(b)

Figure 4 SEM images of the ligand (a) and its Cu(II) complex

EDX analysis is used to calculate the percentage level of various elements present in the metal complex. The results by energy dispersive x-ray analysis (EDX) indicated that there are no contamination .The percentages of elements present in the complex are in agreement with the proposed structure.



EDX picture of Cu(II) complex

Fig: 5

Figure 5 EDX of the Cu(II) complex

ESR spectra

The ESR spectra of Cu(II) complexes were recorded at room temperature (300k). The four well resolved peaks in the low filed region corresponding to $g_{\parallel}$ (2.260) and $g_{\perp}$ (2.065).The trend $g_{\parallel}$ (2.260) $>g_{\perp}$ (2.065) $>g_e$ (2.0023) observed for copper complex suggests that the unpaired electron is localized in dx^2-y^2 orbital for Cu(II) ion [46-47]. If G>4.0, the local axes are aligned parallel or only slightly misaligned. If G<4.0 significant exchange coupling is present and the misalignment is appreciable. The G-values (4.11) calculated using $G = (g_{\parallel} - 2.0023) / (g_{\perp} - 2.0023)$ indicates that the local tetragonal axes are aligned paralled or slightly misaligned and that the unpaired or present in the dx^2-y^2 orbital. The spin orbit coupling constant , λ value (-650 cm-1) calculated using relations $g_{av} = 1/3(g_{\parallel} + 2\, g_{\perp})$ and $g_{av} = 2(1 - 2\lambda/10Dq)$ is less than the free Cu(II) ion (-832cm-1) which also supports covalent character of M-L bond in complex[47]. The covalency parameter $\propto^2$ is calculated using the following equation.

$\propto^2$ (Cu) =–$(A_{\parallel}/0.036) + (g_{\parallel} - 2.0023) + 3/7(g_{\perp} - 2.0023) + 0.04$. If the value of =0.5, it indicates complete covalent bonding, while the value of $\propto^2$=1.0 suggest complete ionic bonding .The observed value of $\propto^2$ (0.90) of the complex is less than unity, which indicates that the complex has some covalent character in the ligand environment [49].

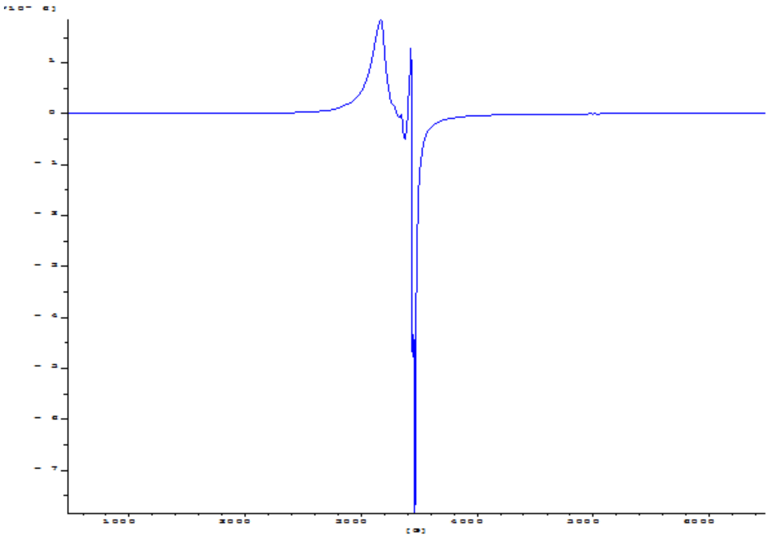
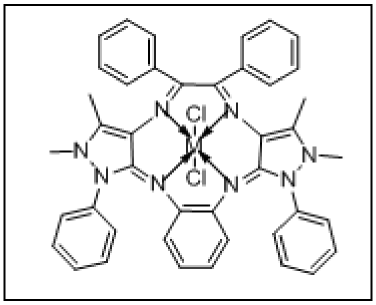


Figure 6 ESR spectra of Cu(II)complex

Based on the above physical methods and spectral analysis the structure of the complex is proposed as follows [50] Scheme**: 2**



Where M = Co(II), Ni(II),Cu(II)orZn(II) and X = Cl^-

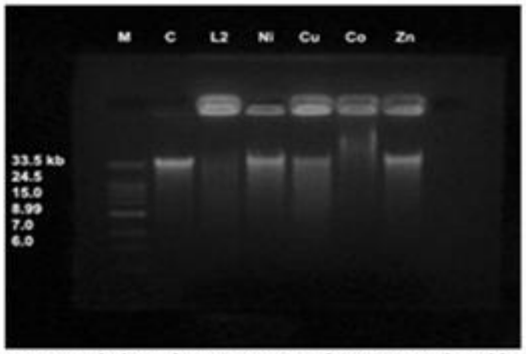
Antibacterial activity

In vitro antimicrobial activity was studied metal [Co(II),Ni(II), Cu(II) and Zn (II)] chloride solutions,DMSO solvent,free ligand and DMSO solution of metal complex. The test was studied against the bacteria: *E.coli, S.Aureus*, and fungi*: A.Niger and A.Flavas*. From the result of the antimicrobial, it has been revealed that the metal complexes are found as higher activity than the free ligand. This is explained by chelation theory [51] the polarity of the metal ion will be reduced to greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. This causes increase in delocalization of the π-electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocks the metal binding sites in the enzymes of micro organisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms [52].

Plate 1: Anti bacterial activity Plate 2:Anti Fungal activity

DNA cleavage activity

It is evident from gel electrophoresis analysis that because of the difference in molecular weight between the control and the treated DNA samples, the ligand and its metal complexes have acted on DNA. Compared to control DNA, the difference in the migration of the lanes of Schiff base ligand (L) and its metal (II) complexes is shown clearly in the present gel electrophoresis (Figure 6). It has been shown that ligand and its [Co(L) Cl_2] complex completely cleaves the super coil. On the other hand, there is only partial cleavage of super coil by [Ni(L)Cl_2], [Cu (L) Cl_2] and [Zn (L) Cl_2] complexes [53]. From the studies it is evident that only the control DNA does not show any apparent cleavage but the ligand and its metal complexes show the apparent cleavage. From the finding of this study, we can come to the conclusion that all the newly synthesize compound are good inhibitors of pathogenic micro organisms .which is evident from the observation on the DNA cleavage.



Agarose gel electrphoretic pattern of CT DNA induced by H_2O_2 and metal complexes M:DNA alone(lane 1),C: Control DNA (lane 2), Ligand (lane 3), [NiL]Cl_2 (lane 4), [CuL]Cl_2 (lane 5), [CoL]Cl_2 (lane 6), [ZnL]Cl_2 (lane 7)

Figure 7

Conclusion

The electron analyses, magnetic susceptibility, molar conductance, Mass,FT-IR,FAR IR,UV-Vis, ^{1}H NMR , ^{13}C NMR, ESR and SEM,EDX suggests that all the synthesized transition metal complex have octahedral geometry except Cu(II) complex which is distorted octahedral and it is clear that the synthesized Schiff base behaves as a tetra dentate ligand which coordinates through its four azomethine nitrogen .The synthesized Cu(II) complexes have good antimicrobial activity than other metal complexes [54]. The interaction of these complexes with CT–DNA was investigated by gel electrophoresis. From the observation, interestingly the DNA band was found to shift up in molecular weight by treating with sample Co(II) complex though significant DNA cleavage was found.

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