



RESEARCH ARTICLE

**Preparation, Spectral Characterization, Antimicrobial and DNA Cleavage Studies
of Macrocyclic Metal (II) Complexes**

Malathy Moorthy¹, Rajavel Rangappan*¹

Department of Chemistry, Periyar University, Periyar Palkalai Nagar, Salem-636 011, India.

Manuscript No: IJPRS/V3/I1/00076, Received On: 12/02/2014, Accepted On: 21/02/2014

ABSTRACT

Cobalt(II), Nickel(II), Copper(II) and Manganese(II) complexes having the general composition $[M(L)(X)]X$ (where $M=Co, Ni, Cu$ and Mn and $X=Cl$) have been synthesized from 2,6-pyridinedicarboxylic acid, 4-amino acetophenone and o-phenylene diamine. The structure of the complexes was characterized by elemental analysis, conductivity measurements, magnetic moment, IR, UV-Vis, electrochemical and ESR spectral studies. Molar conductance measurements revealed that the complexes are electrolytic in nature. The redox behavior of the complexes was explained by cyclic voltammetry. The IR spectra of all metal complexes indicate that the ligands coordinates to the metal ions through four azomethine nitrogen atoms and one pyridyl nitrogen atoms and also show the coordination of one chlorine atom. The magnetic moment values and electronic spectral data suggest an octahedral geometry for all the complexes. The Schiff base and their complexes have been screened for their *in-vitro* antibacterial and antifungal activities against different species of pathogenic bacteria and fungi. The DNA cleavage study was done by agarose gel electrophoresis technique on CT- DNA.

KEYWORDS

Antimicrobial, Macrocyclic complexes, 2,6-pyridinedicarboxylic acid, DNA cleavage

INTRODUCTION

The field of macrocyclic chemistry of metals is developing very fast because of its variety of applications and importance in the area of coordination chemistry. The macrocyclic Schiff bases have been widely studied due to their selective chelation to central metal ions depending on the number, type and position of their donor atoms, the ionic radius of metal ion and coordinating properties of counter ions. The development of the field of bioinorganic chemistry has been an important factor in supporting the growth of interest in complexes of macrocyclic compounds.

The current research dealing with metal complexes of heteronuclear Schiff bases has expanded enormously and includes diversified subjects comprising their various aspects in biocoordination and bioinorganic chemistry.¹ In recent years much interest is focused on the synthesis of macrocyclic complexes with potential medicinal application.

Metal coordination complexes have been widely studied for their antimicrobial and anticancer properties.² The stability of macrocyclic metal complexes depends upon a number of factors, including the number and types of donor atoms present in the ligand and their relative positions within the macrocyclic skeleton as well as the number and size of the chelate rings formed on complexation.³ Macrocyclic nickel complexes find use in DNA recognition and oxidation

*Address for Correspondence:

Dr. Rajavel. Rangappan

Department of Chemistry,
Periyar University, Periyar Palkalai Nagar,
Salem-636 011, India.

E-Mail Id: drrajavelpu@gmail.com

while the macrocyclic copper complexes find use in DNA binding and cleavage. Macrocyclic complexes are also well known for their antibacterial and antifungal activities.⁴ Copper complexes have been extensively studied because of their biologically accessible redox potential and relatively high affinity for nucleobases. Recent studies demonstrated that some multinuclear copper (II) complexes can efficiently promote DNA cleavage by selectively oxidizing deoxyribose (or) nucleobase moieties.⁵ When some kinds of metal complexes interacted with DNA, they could induce the breakage of DNA strands by appropriate methods. Thus, after the double DNA strands are broken, the replication ability of cancer gene is destroyed. The activity of the Schiff base ligands and their metal complexes usually increases as the concentration increases. Studies show that the antimicrobial activities of the metal complexes were increased when compared with the corresponding Schiff bases. In the present work, we have synthesized four macrocyclic Schiff base complexes from a macrocyclic ligand derived from 2,6-pyridinedicarboxylic acid, 4-amino acetophenone and o-phenylene diamine. The complexes have been characterized by Spectral, Antimicrobial and DNA cleavage studies.

MATERIALS AND METHOD

All the reagents and solvents used for the synthesis were of analar grade and were used without further purification. All the solvents were distilled and used according to the Literature method⁶. The C, H and N analysis were performed by using Carlo-Eraba 1106 instrument. Electronic absorption spectra were recorded on Perkin Elmer Lambda 25 UV-Vis spectrometer between 200-800 nm by using DMSO solvent. Magnetic susceptibility measurements of the complexes were carried out by Guoy method using $(\text{Hg}[\text{Co}(\text{SCN})_4])$ as the calibrant. The IR spectra of the ligands and the complexes were recorded in KBr-pellets on a range between $400\text{-}4000\text{cm}^{-1}$. The molar conductivity measurements were carried out by using Elico-CM Conductivity Bridge with 10^{-3} M DMF as solvent. The EPR spectrum was

recorded on an E-112 ESR spectrometer with X-band frequency (9.5 GHz). Electrochemical studies were recorded in EG &G PAR model 174A polarographic analyser using three-electrode configuration in DMSO solution. 0.1 M tetrabutylammonium perchlorate was used as supporting electrolyte, platinum as working and counter electrode and their metal complexes were carried out by disc diffusion method at different concentrations.

Synthesis

Synthesis of Macrocyclic Ligand (L)

Macrocyclic ligand (L) was prepared by the drop wise addition of an ethanolic solution of 4-amino acetophenone (2.7034 g, 0.02 mmol) to an ethanolic solution of 2,6-pyridinedicarboxylic acid (1.6712 g, 0.01 mmol) with constant stirring. After the addition was completed an ethanolic solution of o-phenylene diamine (1.0814 g, 0.01 mmol) was added to the above solution. The above contents were stirred well and refluxed for 2hrs. The compound formed was filtered, washed with ethanol and dried in a desiccator. Synthetic route of the ligand is given in the Figure 1.

Synthesis of Macrocyclic Complexes

Macrocyclic Co (II), Ni(II), Cu(II) and Mn(II) complexes were prepared by the drop wise addition of an hot ethanolic solution (0.01 mol) of metal chlorides to an hot ethanolic solution of (0.01 mol) the ligand prepared with constant stirring. The mixture was kept under reflux for 3hrs. The solid complexes formed were filtered off, washed with ethanol and dried in a desiccator.

DNA Cleavage Studies

CT DNA cleavage study was carried out by using gel electrophoresis. The reaction mixture containing supercoiled $40\mu\text{M}$ CT DNA, $50\mu\text{M}$ metal complexes and $50\mu\text{M}$ H_2O_2 in Tris- HCl buffer (pH 7.2) was incubated at 37°C for 2 hrs. After incubation, the samples were electrophoresed for 2 hrs at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH-7.2).

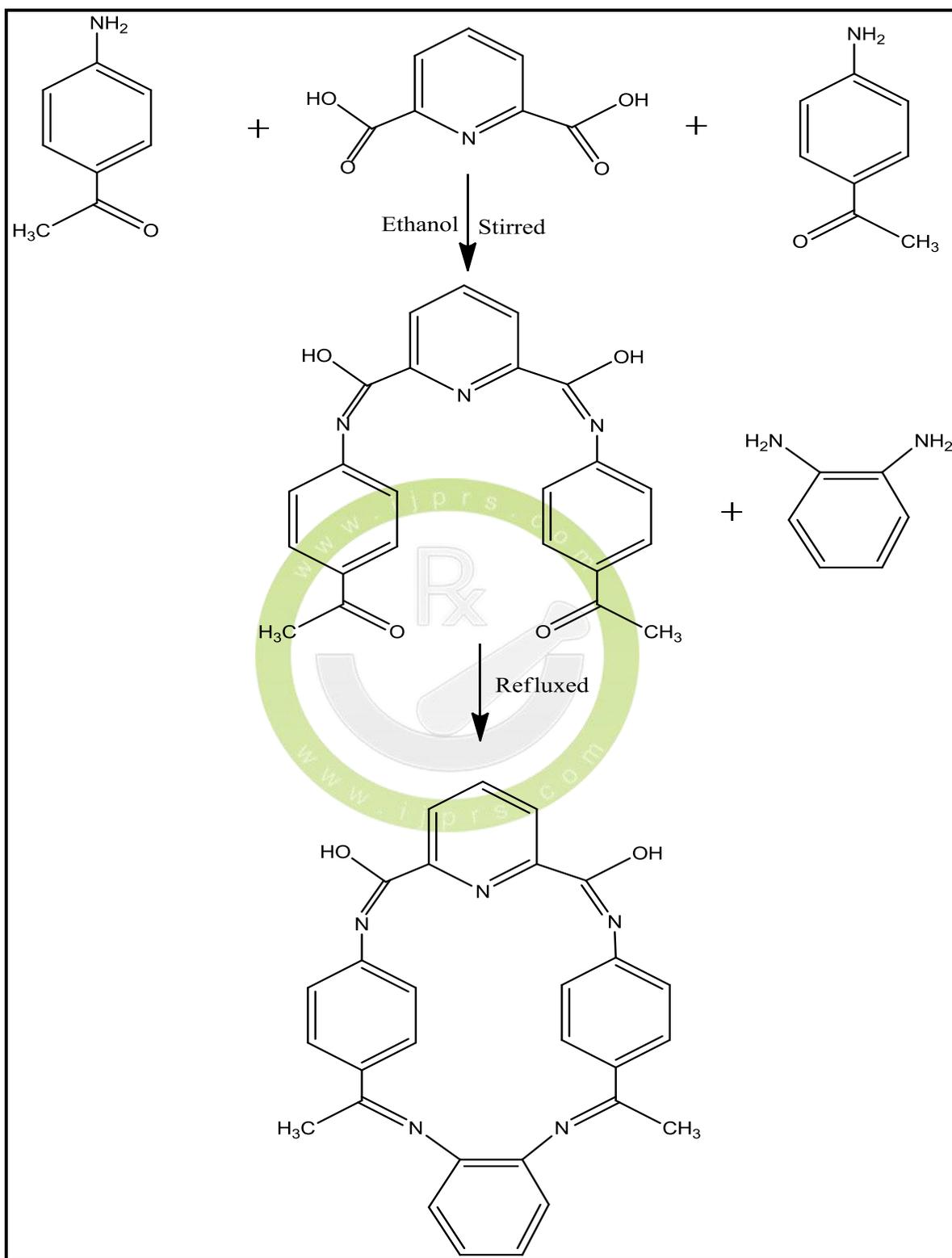


Figure 1: Synthesis of the Macrocyclic Ligand

After electrophoresis, the gel was stained using $1 \mu\text{g cm}^{-3}$ ethidium bromide (EB) and photographed under UV light at 360nm.^{7,8}

Antimicrobial Assay

The *in vitro* antibacterial activity of the macrocyclic Schiff base ligand and its metal complexes were tested against the Gram +ve bacterial species such as *Staphylococcus aureus* and *Bacillus subtilis* and Gram -ve bacterial species such as *Escherichia coli* and *Klebsiella pneumoniae*. The antifungal activities were tested against the fungi *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* (yeast). The screening was carried out at room temperature by the disc diffusion method using the standard procedure.^{9,10}

Streptomycin was used as the standard drug for bacterial screening and *Amphotericin-B* was used as the standard drug for fungal screening. Nutrient agar medium was employed as culture media for bacterial screening and Sabouraud dextrose agar medium as culture media for fungal screening. The disc of whatmann No.1 filter paper having the diameter 5mm were soaked into the solution of the complexes dissolved in DMSO at different concentrations ($50 \mu\text{g mL}^{-1}$ and $75 \mu\text{g mL}^{-1}$). After drying at room temperature, the discs were then placed on the inoculated plates containing respective medium. The plates were then incubated at 37°C for 24-48 hrs and 27°C for bacteria and fungus respectively. After the period of incubation, the antibacterial activities were recorded by measuring the diameter (mm) of the inhibitory zone and the antifungal activities were recorded as % of inhibition. The following relation was used to calculate the fungal growth inhibition:

$$\text{Fungal growth inhibition (\%)} = (A-B) \times 100 / A$$

Where, A is the diameter of fungal colony in control plate and B is the diameter of fungal colony in test plate.

The antibacterial and antifungal activities shown by the macrocyclic ligand and their complexes were compared with the standard drugs.

RESULTS AND DISCUSSION

All the complexes were synthesized with very good yield (>80%) by Schiff base condensation of 2,6-pyridinedicarboxylic acid with 2 equivalents of 4-amino acetophenone and one equivalent of o-phenylene diamine. The general composition for all the complexes was found to be $[\text{M}(\text{L})(\text{X})]\text{X}$ (where M=Co,Ni,Cu and Mn and X=Cl). The analytical data of the ligand and the complexes were given in table 1. The analytical data for all the complexes indicates 1:1 metal-ligand stoichiometry and are in good agreement with the proposed molecular formula. All the complexes were stable in air and are soluble in DMF and DMSO. The test for anion was positive directly without decomposing the chelates, indicating their presence outside the coordination sphere.

Molar Conductance

Molar conductance (Λ_m) values of the macrocyclic complexes were determined using 10^{-3} M solutions in DMF. The observed molar conductance values are listed in table 2. The molar conductance values of the macrocyclic complexes indicate their 1:1 electrolytic nature.

Infrared Spectra

In order to study the binding mode of the Schiff base to the metal in the complexes, the IR spectrum of the free ligand was compared with the spectra of the complexes. IR spectral data for the ligand and their complexes are summarized in table 3. The strong bands appeared at 1646 cm^{-1} in the macrocyclic ligand may be assigned to the azomethine $\nu(\text{C}=\text{N})$ vibrations.¹¹ In the IR spectra of the Co(II),Ni(II),Cu(II) and Mn(II) complexes the absence of the bands in the region at $\sim 3400 \text{ cm}^{-1}$ and at $\sim 1700 \text{ cm}^{-1}$, the characteristic frequency of the free $-\text{NH}_2$ group and free carbonyl group respectively, suggests that the complete condensation had occurred.^{12,13} The shifting of $\nu(\text{C}=\text{N})$ to a lower side, i.e 1615-1620 and the appearance of the new band in the region 470-490 due to the $\nu(\text{M} \leftarrow \text{N})$ stretching, in the IR Spectra of the complexes provides strong

Table: 1 Elemental Analysis Data of Ligand and its Metal Complexes

Compound	Molecular Weight	Found(calc.)%				
		C	H	N	Cl	M
C ₂₉ H ₂₃ N ₅ O ₂	473	73.43 (73.57)	4.55 (4.86)	14.4 (14.8)	-	-
[Co(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	603	57.68 (57.73)	3.79 (3.82)	11.57 (11.61)	9.69 (9.76)	11.72 (11.76)
[Ni(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	602	57.61 (57.75)	3.77 (3.82)	11.58 (11.62)	9.67 (9.74)	11.71 (11.77)
[Cu(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	607	57.31 (57.29)	3.68 (3.79)	11.47 (11.52)	10.42 (10.46)	11.63 (11.67)
[Mn(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	599	58.07 (58.11)	3.79 (3.84)	11.72 (11.69)	9.14 (9.17)	11.79 (11.84)

Table: 2 Molar Conductances and CV Data of Ligands and Their Complexes

Compound	Molar conductance Λ ($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)	CV data (V)		
		E _{pa}	E _{pc}	ΔE_p
C ₂₉ H ₂₃ N ₅ O ₂	-	-	-	-
[Co(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	76	-0.62	-1.26	0.64
[Ni(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	83	-0.583 -1.592	-0.618 -1.496	0.035 -0.096
[Cu(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	88	-0.58	-0.62 -1.35 -1.58	-0.04
[Mn(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	85	0.864 -0.685	-0.654 -1.601	0.214 0.916

Table: 3 Infrared Spectral Data of Ligands and Their Complexes

Compounds	$\nu(\text{C}=\text{N})$ cm^{-1}	Pyridine ring cm^{-1}			$\nu(\text{M}-\text{N})$ cm^{-1}	$\nu(\text{M}-\text{Cl})$ cm^{-1}	Carboxylic -OH (3000-2500 cm^{-1})
		1512	595	482			
C ₂₉ H ₂₃ N ₅ O ₂	1646	1512	595	482	-	-	2850
[Co(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	1615	1520	600	490	488	325	2847
[Ni(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	1609	1524	618	506	482	320	2844
[Cu(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	1598	1528	621	516	476	318	2841
[Mn(C ₂₉ H ₂₃ N ₅ O ₂)] Cl	1605	1532	632	521	470	328	2852

evidence for the coordination of azomethine nitrogen.¹³⁻¹⁵ The bands at 1572, 595 and 482 due to the pyridine-ring-stretching, in-plane-ring-bending and out-of-plane-ring-bending respectively are shifted to higher region in the IR spectra of the complexes also indicates that the nitrogen of pyridine ring is involved in coordination. Therefore, it is clear from the IR spectra that $-NH_2$ groups of the two 4-aminoacetophenone have condensed with the carbony groups of 2,6-Pyridinedicarboxylic acid to give rise to an N_5 arrangement of 4 azomethine and one Pyridine nitrogen atoms equally suitable for coordination. The appearance of new band in the region 310-330 in the complexes are assigned to $\nu(M-Cl)$ stretching vibrations¹⁶. This shows the coordination of one chlorine atom to the metal ions.

Electronic Spectra and Magnetic Susceptibility

The electronic absorption spectra provides information about the ligand arrangement in transition metal complexes, and also useful to distinguish the geometry of the complexes. Electronic spectral data of the ligand and its complexes were given in the table 4. In the Electronic spectrum of the ligand two absorption bands at 242 and 330 nm were observed. The first band corresponds to the $\pi \rightarrow \pi^*$ transition and the second band corresponds to the $n \rightarrow \pi^*$ transition. Complexation of the ligands with the metal ions results in a slight shift of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions in all the macrocyclic complexes. The electronic spectra of Co(II) macrocyclic complex exhibit three absorption bands at 996, 658 and 494nm respectively. These various bands can be assigned to ${}^4T_{1g(F)} \rightarrow {}^4T_{2g(F)}$, ${}^4T_{1g(F)} \rightarrow {}^4A_{2g(F)}$ and ${}^4T_{1g(F)} \rightarrow {}^4T_{1g(P)}$ transitions respectively, consistent with the octahedral geometry around the cobalt(II)ion.¹⁶⁻¹⁹ In addition the band at 418nm is assigned to L \rightarrow M charge transfer. The octahedral geometry of the Co(II) complex is further supported by the magnetic moment value of 4.86 B.M.¹⁷⁻¹⁹ Electronic spectra of nickel complex show the d-d transition bands at 937,594 and 398 nm respectively. These three

transitions are assigned to the ${}^3A_{2g(F)} \rightarrow {}^3T_{2g(F)}$, ${}^3A_{2g(F)} \rightarrow {}^3T_{1g(F)}$ and ${}^3A_{2g(F)} \rightarrow {}^3T_{1g(P)}$ transitions. This transition suggests octahedral geometry of the nickel complex.²⁰⁻²² The new band observed at 418nm was assigned to the L \rightarrow M charge transfer band. The magnetic moment value at 3.12 B.M. further confirms the octahedral geometry for Ni(II) complex²³. Manganese (II) complex exhibit three bands at 591, 402 and 480 nm indicates the ${}^6A_{1g} \rightarrow {}^4T_{1g}$, ${}^6A_{1g} \rightarrow {}^4E_g$ and ${}^6A_{1g} \rightarrow {}^4T_{2g}$ transitions respectively, which indicates octahedral geometry.²²⁻²⁴ Macrocyclic Cu(II) complex shows a single broad band at 580 nm. This band may arise due to ${}^2T_{2g} \rightarrow {}^2E_g$ transition, which denotes an octahedral geometry. The band at 380nm was assigned to the L \rightarrow M charge transfer band. Octahedral geometry of Cu(II) complex was further confirmed by its magnetic moment value at 1.78 B.M.^{23,25}

ESR Spectra

ESR spectra of the copper(II) complexes gives g_{\parallel} value 2.33 and g_{\perp} value 2.13. The ratio $g_{\parallel} > g_{\perp} > 2.0023$ calculated for Cu(II) complex suggest that the unpaired electron is localized in dx^2-y^2 orbital, indicates that the complex is axially elongated octahedral geometry.²⁶⁻²⁸ The g-values are related by the expression $G = (g_{\parallel} - 2) / (g_{\perp} - 2)$, if $G > 4$, the exchange interaction is negligible, whereas when $G < 4$, a considerable exchange interaction is indicated in the solid complex. For the Cu(II) complex reported in this paper, the 'G' value was found to be 2.5385 which is less than 4 and indicate the exchange interaction in the solid Cu(II) complex.

Electrochemical Studies

CV data of the ligand and its complexes are given in the table 2. Macrocyclic Mn(II) complex exhibit two well-defined quasireversible one-electron cyclic responses at $E_{pc} = -0.654$ V with a corresponding oxidation peak at $E_{pa} = 0.864$ V and at $E_{pc} = -1.601$ V with a corresponding oxidation peak at $E_{pa} = -0.685$ respectively. The two ΔE_p values 0.214 and 0.916 suggest the reversible nature of the redox process¹⁷.

Table: 4 Magnetic Susceptibility and Electronic Spectral Data of Ligand and Metal Complexes

Compounds	Electronic spectra λ_{\max} (nm)					μ_{eff} (B.M)
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L→M	d-d transition	Assignments	
$\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2$	242	330	-	-	-	-
$[\text{Co}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	238	326	418	996 658 494	${}^4\text{T}_{1g(\text{F})} \rightarrow {}^4\text{T}_{2g(\text{F})}$ ${}^4\text{T}_{1g(\text{F})} \rightarrow {}^4\text{A}_{2g(\text{F})}$ ${}^4\text{T}_{1g(\text{F})} \rightarrow {}^4\text{T}_{1g(\text{P})}$	4.86
$[\text{Ni}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	240	328	418	937 594 398	${}^3\text{A}_{2g(\text{F})} \rightarrow {}^3\text{T}_{2g(\text{F})}$ ${}^3\text{A}_{2g(\text{F})} \rightarrow {}^3\text{T}_{1g(\text{F})}$ ${}^3\text{A}_{2g(\text{F})} \rightarrow {}^3\text{T}_{1g(\text{P})}$	3.12
$[\text{Cu}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	236	325	378	580	${}^2\text{T}_{2g} \rightarrow {}^2\text{E}_g$	1.78
$[\text{Mn}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	235	328	412	591 402 480	${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$ ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g$ ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}$	5.90

Cobalt (II) complex exhibits one quasi reversible transfer process with a reduction peak at $E_{\text{pc}} = -1.26$ V with a corresponding oxidation peak at $E_{\text{pa}} = -0.62$ V. The peak separation (ΔE_{p}) of this couple is 0.64 V.^{16,23} Nickel (II) complex exhibits two quasireversible one electron process. It exhibits two cathodic peaks (E_{pc}) at -0.618 V and -1.496 due to the reduction of the $\text{Ni}(\text{II}) \rightarrow \text{Ni}(\text{I}) \rightarrow \text{Ni}(\text{0})$ respectively. The anodic peak (E_{pa}) at -0.583 v corresponds to the oxidation behavior of the ligand moiety and at -1.592 V corresponds to the oxidation of $\text{Ni}(\text{I}) \rightarrow \text{Ni}(\text{II})$.²⁹ A cyclic voltammogram of Cu(II) complex displays a reduction peak at $E_{\text{pc}} = -0.62$ V with a corresponding oxidation peak at $E_{\text{pa}} = -0.58$ V. The peak separation of this couple (ΔE_{p}) is 0.8 V and increased with scan rate. Cu(II) complex also exhibits 2 irreversible peaks in the reduction side at -1.35 and 1.58 V assigned for

the reactions $\text{Cu}(\text{II}) \rightarrow \text{Cu}(\text{I})$; and $\text{Cu}(\text{I}) \rightarrow \text{Cu}(\text{0})$ respectively.^{23,30}

DNA Cleavage Study

Gel electrophoresis experiments were conducted at 37°C using synthesized compounds at low concentration in the presence of H_2O_2 as an oxidant Figure 2. The cleavage is inhibited by free radical scavengers implying that hydroxyls radical (or) peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metallocomplexes bound hydroxyl radical (or) a peroxy species generated from the co-reactant H_2O_2 .³¹ The gel electrophoresis results indicate that at low concentration the complexes were able to cleave the CT-DNA, as compared to the control DNA. The different DNA cleavage efficiency of the complexes was due to the different binding affinity of the metal complexes to DNA. Among the macrocyclic complexes,

Cu(II) complex was found to be highly active in cleaving CT DNA in present of H_2O_2 , due to the increased production of hydroxyl radical (OH^\cdot) by cuprous ions similar to well-known Fenton reaction.^{7,8,32}

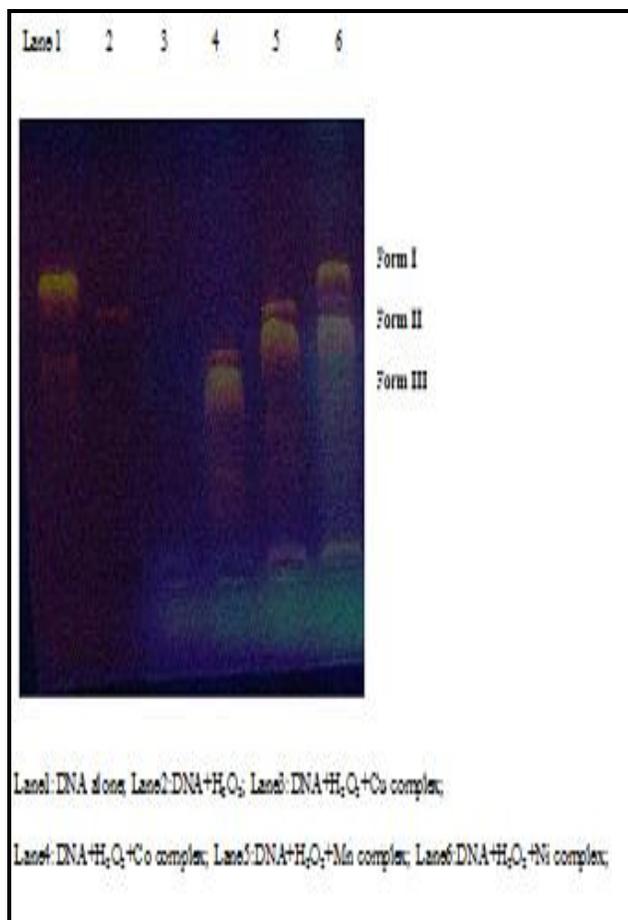


Figure 2: DNA Cleavage Studies of Macrocyclic Metal Complexes

Antimicrobial Assay

The ligand and their metal complexes have been tested for their antimicrobial activity against the bacterial species *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* and fungi such as *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* (yeast), which were evaluated by disc diffusion methods. The *in vitro* antimicrobial activities of the ligand and their complexes are given in table 5 & 6 and in Figures 3 & 4. The concentrations used to study the antibacterial and antifungal activities were $50\mu\text{g mL}^{-1}$ and $75\mu\text{g mL}^{-1}$. The

zones of inhibition formed were measured with calipers. The antibacterial activities of the compounds are represented by size of the diameter in mm and the antifungal activities are represented by percentage growth of inhibition.

This result indicates that the tested compounds were found to inhibit the growth of the bacteria and fungi to a considerable degree. The antibacterial and antifungal activities of the Schiff base ligand and their metal complexes arise due to the presence of hydroxyl groups and azomethine groups. The activity of the ligand and their complexes increases as the concentration increases. The macrocyclic metal complexes are found to be more active than that of the ligand, due to the chelation of the ligand with metal ions. Such increased activity of the metal chelates can be explained on the basis of chelation theory.^{33,34} Chelation makes the Schiff base complexes to act as more powerful and potent bacteriostatic agents, thus inhibits the growth of bacteria and fungi more than that of the parent Schiff bases. The metal complexes restrict the growth of the organisms by disturbing the respiration process of the cell^{29,35} and thus block the synthesis of proteins. The variation in the activity of the metal complexes against the organisms depends on the impermeability of the cells of the microbes. The complexes may involve in the formation of hydrogen bond through azomethine group with the active centers of the cell, resulting in interference with the normal cell process. It is well known that chelation is not the only criterion for antimicrobial activity. Some important factors such as the nature of the ligand, the concentration, hydrophilicity, lipophilicity and the presence of co-ligands have a considerable influence on the antimicrobial activity, which shows that metal chelates significantly increases the antimicrobial activities of the complexes. The microbial activity of the macrocyclic complexes synthesized follows the order⁷: $\text{Cu(II)} > \text{Mn(II)} > \text{Co(II)} > \text{Ni(II)} > \text{L}$. Thus the microbial activity of all the complexes is higher than that of the free ligand.

Table 5: *In Vitro* Antimicrobial Activity of Ligand and Its Metal Complexes

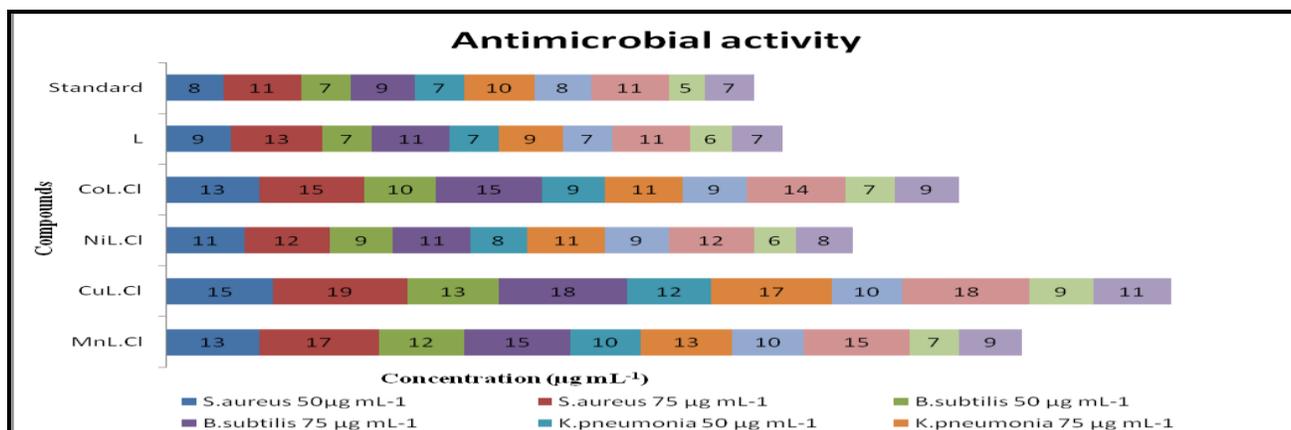
Compounds	Diameter of growth of inhibition zone (mm)									
	a		b		c		d		e	
	Concentration ($\mu\text{g mL}^{-1}$)									
	50	75	50	75	50	75	50	75	50	75
Standard	8 ^f	11 ^f	7 ^f	9 ^f	7 ^f	10 ^f	8 ^f	11 ^f	5 ^g	7 ^g
$\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2$	9	13	7	11	7	9	7	11	6	7
$[\text{Co}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	13	15	10	15	9	11	9	14	7	9
$[\text{Ni}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	11	12	9	11	8	11	9	12	6	8
$[\text{Cu}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	15	19	13	18	12	17	10	18	9	11
$[\text{Mn}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	13	17	12	15	10	13	10	15	7	9

a- *Staphylococcus aureus*, b-*Bacillus subtilis*, c- *Klebsiella pneumonia*, d-*Escherichia coli*, e-*Candida albicans*, f- *Streptomycin*, g- *Amphotericin-B*,

Table 6: *In vitro* Antifungal Activity of Ligands and Its Metal Complexes

Compounds	Fungal growth of inhibition (%)			
	<i>A.niger</i>		<i>A.flavus</i>	
	Concentration ($\mu\text{g mL}^{-1}$)			
	50	75	50	75
Standard	33	35	27	30
$\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2$	32	37	30	33
$[\text{Co}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	42	45	35	40
$[\text{Ni}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	39	43	33	40
$[\text{Cu}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	47	57	43	48
$[\text{Mn}(\text{C}_{29}\text{H}_{23}\text{N}_5\text{O}_2)] \text{Cl}$	41	47	37	45

h-*Amphotericin-B*

Figure 3: *In Vitro* Antimicrobial Activity of Macrocyclic Ligand and Metal Complexes

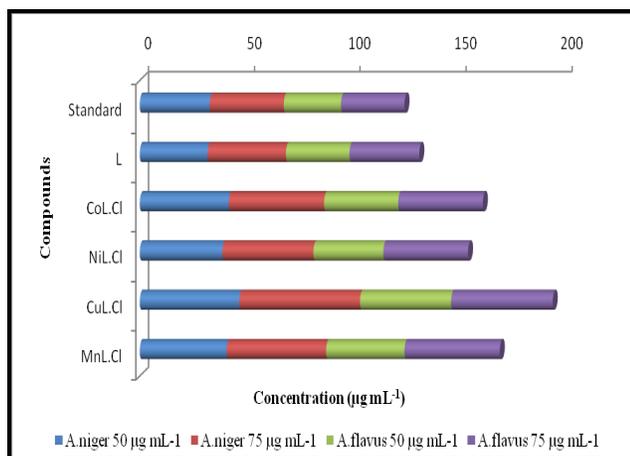


Figure 4: *In Vitro* Fungal Activities of Macrocylic Ligand and Metal Complexes

CONCLUSION

All the macrocylic complexes were prepared with very good yield. The analytical data for all the complexes indicates 1:1 metal-ligand stoichiometry and are in good agreement with the proposed molecular formula. The test for anion was positive directly without decomposing the chelates, indicating their presence outside the coordination sphere. The Molar conductance value of all the macrocylic complexes indicates their electrolytic nature. The IR spectral data clearly indicates the coordination through four azomethine nitrogen atoms, one pyridine nitrogen atom and one chlorine atom. The magnetic moment values and electronic spectral data of suggest an octahedral geometry for all the macrocylic mononuclear complexes. Electrochemical studies show the redox behavior of the Co(II), Ni(II), Cu(II) and Mn(II) ions in the complex. The ESR spectra of Cu(II) complex coincide with the related systems and confirms octahedral geometry. The Antibacterial and antifungal activities of the macrocylic complexes were found to be more than that of the ligand and the standard. The gel electrophoresis studies of the macrocylic complexes clearly indicate that the copper complex effectively cleaves CT DNA in present of H₂O₂ than the other complexes, due to the increased production of hydroxyl radical (OH[•]). Based on these studies the suggested structure

for the synthesized macrocylic complexes can be assigned as follows (Figure 5).

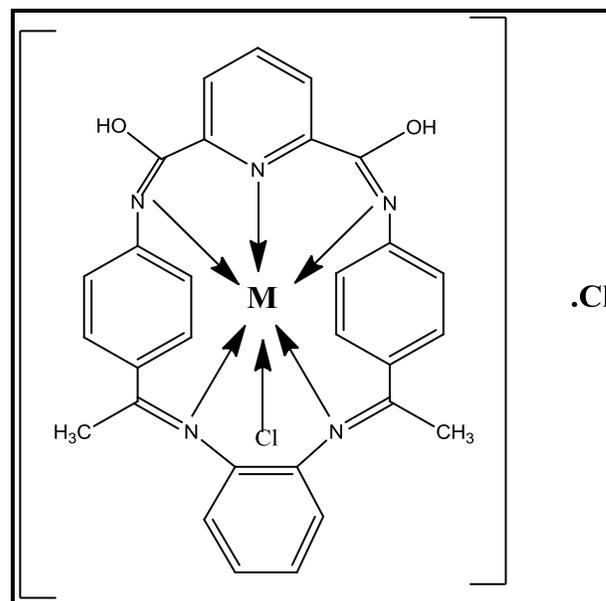


Figure 5: Structure of the Complexes

Where, M= Co(II), Ni(II), Cu(II) and Mn(II)

ACKNOWLEDGEMENT

The Supervisor and HOD, Department of Chemistry, Periyar University, Salem, Tamilnadu, India, are acknowledged for providing the research facilities and for their encouragement and support.

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