

## Synthesis, Characterization and Biological Screening of Unsymmetrical 11-Membered Tetraaza Macrocyclic Complexes containing Urea

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**Abstract:** Unsymmetrical macrocyclic complexes of Mn<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, Cu<sup>II</sup> and Zn<sup>II</sup> have been synthesized by the template process using bis(benzil)ethylenediamine as precursor. The precursor and the metal complexes were characterized by elemental analysis, magnetic susceptibility, conductance measurement, IR, UV- Vis spectra, <sup>1</sup>HNMR, <sup>13</sup>C-NMR, EPR and antimicrobial assay. Macrocyclic complexes were prepared by the interaction of Bis(benzil)ethylenediamine with transition metal chlorides and urea. Based on analytical, spectral and magnetic moments, an octahedral geometry around the metal ion is suggested for the complexes. The shifts of the  $\nu(\text{C}=\text{N})$  (azomethine) stretches have been monitored in order to find out the donor sites of the ligands. Antimicrobial activities of the ligand and the complexes were screened against one gram positive and four gram negative bacteria. The activity data show that the metal complexes are more potent than the parent ligand.

**Key words:** Urea, Bis(benzil)ethylenediamine, Mn<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, Cu<sup>II</sup> and Zn<sup>II</sup>.

### 1. INTRODUCTION:

Coordination chemistry of macrocyclic ligands is a fascinating area which has attracted the attention of many inorganic chemists. The intense interest in synthetic macrocycles and their metal complexes depends on the fact that they mimic naturally occurring macrocyclic molecules in their structural and functional features due to rich chemical properties(1-4). Tetraazamacrocycles and their metal complexes have been studied extensively in connection with their resemblance to naturally occurring porphyrins. In this paper, we report the synthesis and characterization of new bivalent transition metal complexes from the reaction of bis (benzil) ethylenediamine with urea which show the marked effect of any build-up of ligand strain on the coordination geometry of the central metal. The possible biological application of these complexes was determined through antibacterial sensitivity assay.

## 2. EXPERIMENTAL:

### 2.1. Materials and methods:

All the chemicals used for the preparation of the ligands were of BDH quality, AR grade. The microelemental analysis was carried out using CHN-Rapid Analyzer. Conductivity was measured on a Toshniwals conductivity bridge using dip type platinised platinum electrode. Molecular weights were determined by the Rast camphor method. The magnetic susceptibilities were measured out by using guoy balance. Proton NMR spectra were recorded on an EM 300-30MHz NMR spectrometer in DMSO. IR spectra (KBr) of the samples were recorded on a shimadze FTIR-8400s spectro-photometer. The electronic spectra (chloroform) were recorded on the Lamda 35 spectrometer.

### 2.2. Synthesis of $Mn^{2+}$ , $Co^{2+}$ , $Ni^{2+}$ , $Cu^{2+}$ and $Zn^{2+}$ complexes of Ligand (L) with urea

The chlorides of  $Mn^{2+}$ ,  $Co^{2+}$ ,  $Ni^{2+}$ ,  $Cu^{2+}$  and  $Zn^{2+}$  ions (4.3mmol) in methanol is refluxed with methanolic solution (25ml) containing bis(benzil)ethylenediamine (L) (2g, 4.3 mmol) and Urea (0.2582g, 4.3mmol) in 1:1:1 mole ratio for 5-6 hours on a heating mantle. The solutions are cooled to room temperature and transferred to evaporating dish and set aside for few hours. The solid complexes separated out are filtered washed several times with hot water, then with cold methanol, dried in vacuum over anhydrous calcium chloride and recrystallised using methanol + benzene mixture (1/1).

### 2.3. Antimicrobial assay:

Anti microbial properties of the synthesized metal complexes have been evaluated as per the method described earlier(5). The test organisms were isolated from the clinical samples from patients attending Government Hospital, Kanchipuram. Tamilnadu, India. The organisms were isolated in nutrient agar medium and selectively cultured at 37°C for 24 hrs. The bacterial strains were identified by biochemical and standard antibiogram tests as per the directions from Bergy's manual for determinative bacteriology. Circular disc of 6 mm diameter were made from the whatman no 1 filter paper. Discs were impregnated with equal volume (50 µl) of each metal complexes dissolved in DMSO at a concentration of 100 µg/ml. The discs were aseptically placed over plates of Muller Hinton agar (MHA, Difco) seeded with each of test pathogens, and the inoculum was adjusted to 0.5 Mc Farland turbidometry. The plates were incubated in an upright position at 37°C for 24 hours and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 12 mm were considered as having low antibacterial activity. Diameters between 12 and 16 mm were considered moderately active, and these with > 16mm were considered highly active. The clinical strains were also tested for their sensitivity against the standard antibiotics, ciprofloxacin (5 mcg) and nalidixic acid (10 mcg) by the disc diffusion method. The solvent control (only DMSO) was also maintained throughout the experiment.

## 3. RESULTS AND DISCUSSION:

### 3.1. Molar conductance measurements

The molar conductivity values of  $10^{-3}$  M solutions in acetonitrile are shown in Table 1.2.

The values of the complexes,  $[Mn(C_{31}H_{24}N_4O)Cl_2]$ ,  $[Co(C_{31}H_{24}N_4O)Cl_2]$ ,  $[Ni(C_{31}H_{24}N_4O)Cl_2]$ ,  $[Cu(C_{31}H_{24}N_4O)Cl_2]$  and  $[Zn(C_{31}H_{24}N_4O)Cl_2]$  are found to be 39.44, 32.59, 31.15, 35.90 and 32.29  $mhocm^2mol^{-1}$  respectively. These values suggest that these complexes are non-electrolytic in nature.

**Table 1.1. Physical and analytical data of the complexes (16-20)**

Complex	Empirical Formula	Colour	Mol. Wt.	M.Pt. (°C)	Yield (%)
$[Mn(C_{31}H_{24}N_4O)Cl_2]$	$C_{31}H_{24}MnN_4OCl_2$	Dark pink	594	109	57
$[Co(C_{31}H_{24}N_4O)Cl_2]$	$C_{31}H_{24}CoN_4OCl_2$	Green	598	112	61
$[Ni(C_{31}H_{24}N_4O)Cl_2]$	$C_{31}H_{24}NiN_4OCl_2$	Dark blue	598	111	51
$[Cu(C_{31}H_{24}N_4O)Cl_2]$	$C_{31}H_{24}CuN_4OCl_2$	Pale green	603	107	63
$[Zn(C_{31}H_{24}N_4O)Cl_2]$	$C_{31}H_{24}ZnN_4OCl_2$	colourless	605	123	57

From the molecular weight determination, elemental analysis, magnetic and conductance value, the predicted compositions of the complexes are shown below:  $[\text{Mn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  (**16**),  $[\text{Co}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  (**17**),  $[\text{Ni}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  (**18**),  $[\text{Cu}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  (**19**) and  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  (**20**).

**Table 1.2. Elemental analysis, magnetic moment and molar conductance of the complexes (16-20)**

Ligand/Complex	Elemental Analysis % Found (Calculated)				$\mu_{\text{eff}}$ BM	Molar Conductance ( $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ )
	C	H	N	M		
$\text{C}_{31}\text{H}_{24}\text{MnN}_4\text{OCl}_2$	62.57 (62.62)	3.97 (4.04)	9.35 (9.42)	9.18 (9.24)	5.86	39.44
$\text{C}_{31}\text{H}_{24}\text{CoN}_4\text{OCl}_2$	62.15 (62.20)	3.93 (4.01)	9.31 (9.36)	9.79 (9.85)	4.91	32.59
$\text{C}_{31}\text{H}_{24}\text{NiN}_4\text{OCl}_2$	62.17 (62.20)	3.92 (4.01)	9.30 (9.36)	9.76 (9.81)	3.27	31.15
$\text{C}_{31}\text{H}_{24}\text{CuN}_4\text{OCl}_2$	61.62 (61.69)	3.95 (3.98)	9.20 (9.28)	10.46 (10.53)	1.90	35.90
$\text{C}_{31}\text{H}_{24}\text{ZnN}_4\text{OCl}_2$	61.41 (61.49)	3.89 (3.96)	9.22 (9.26)	10.79 (10.85)	Dia	32.29

### 3.2. Infrared spectral studies

The IR spectral results are given in Table 1.3. The spectrum of the ligand (Fig 1.1) shows band corresponding to C=O groups. But the spectra of the complexes don't show any stretching and bending which correspond to stretching and bending of the C=O group(6). However some changes are observed in C=N vibrations which show that the coordination occurs through this site(7). A sharp band of high intensity occurring at  $1667\text{ cm}^{-1}$ , characteristic of  $\nu(\text{C}=\text{N})$  absorption in the ligand is shifted to lower frequencies  $1632\text{--}1646\text{ cm}^{-1}$ , in the transition metal ion complexes. Thus it is concluded that a tetradentate macrocycle is formed having coordination through azomethine nitrogens.

The presence of a band in the range of  $1601\text{--}1605\text{ cm}^{-1}$  is attributed to the  $\nu(\text{C}=\text{O})$  group of amide. The strong as well as sharp band in the range of  $1146\text{--}1168\text{ cm}^{-1}$  is due to the  $\nu(\text{C}-\text{N})$  vibration. The medium intensity absorption band in the range of  $3300\text{--}3304\text{ cm}^{-1}$  is attributed to  $\nu(\text{C}-\text{H})$  vibration. The band characteristic of the benzil moiety appeared in the range of  $1440\text{--}1443\text{ cm}^{-1}$  ( $\nu_{\text{asym}}\text{ C}_6\text{H}_5$ ) and  $1317\text{--}1340\text{ cm}^{-1}$  ( $\nu_{\text{sym}}\text{ C}_6\text{H}_5$ ). In the far IR spectra of all the complexes, additional bands are observed in the range of  $419\text{--}430\text{ cm}^{-1}$  is assigned to  $\nu(\text{M}-\text{N})$  vibrations which substantiate the participation of azomethine nitrogen in coordination. The infrared spectrum of  $[\text{Co}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  is depicted in Fig. 1.2. And also, bands observed in the region  $325\text{ cm}^{-1}$  which is assigned to  $\gamma\text{ M}-\text{Cl}$  vibrations which substantiate the participation of chloride in coordination (Fig. 1.3).

**Fig. 1.3. Characteristic IR bands ( $\text{cm}^{-1}$ ) of the ligand and its complexes (17, 18 & 19)**

Ligand/Complex	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{O})$ of amide	$\nu(\text{C}-\text{H})$	$\nu(\text{C}-\text{N})$	$\nu_{\text{s}}\text{ C}_6\text{H}_5$	$\nu_{\text{a}}\text{ C}_6\text{H}_5$	$\gamma\text{ M}-\text{N}$	$\gamma\text{ M}-\text{Cl}$
$\text{L}_1(\text{C}_{30}\text{H}_{24}\text{N}_2\text{O}_2)$	1772	1667	-	3317	1169	1312	1439	-	-
$[\text{Co}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$	-	1644	1601	3303	1146	1339	1440	424	325
$[\text{Ni}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$	-	1632	1605	3300	1148	1340	1440	430	-
$[\text{Cu}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$	-	1646	1605	3304	1168	1317	1443	419	-

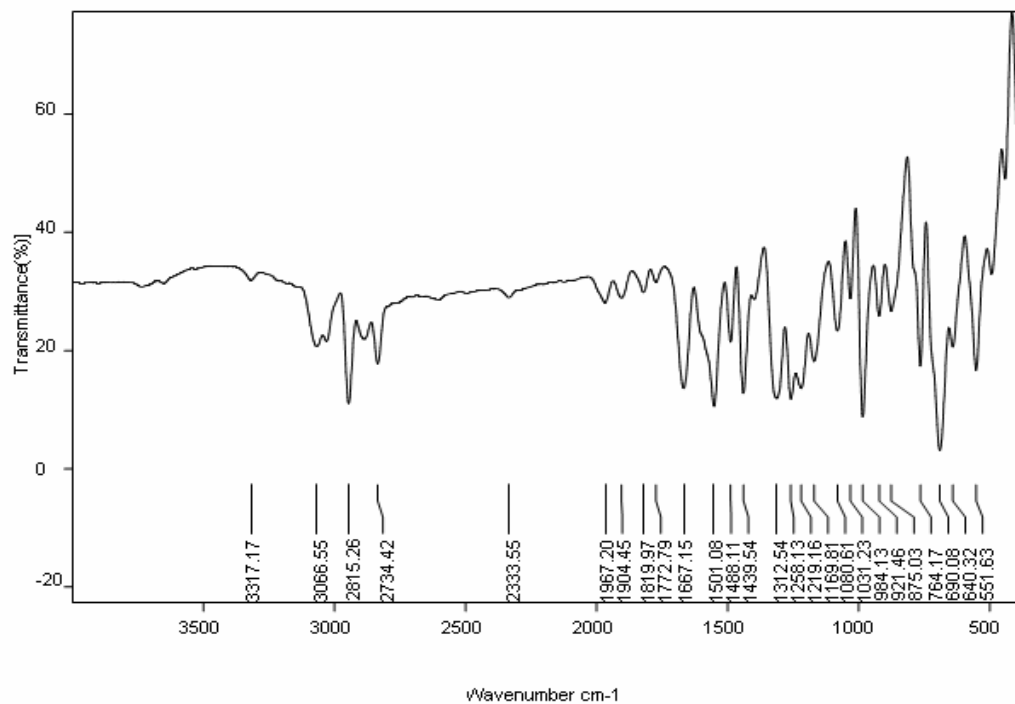


Fig. 1.1. FT-IR spectrum of ligand

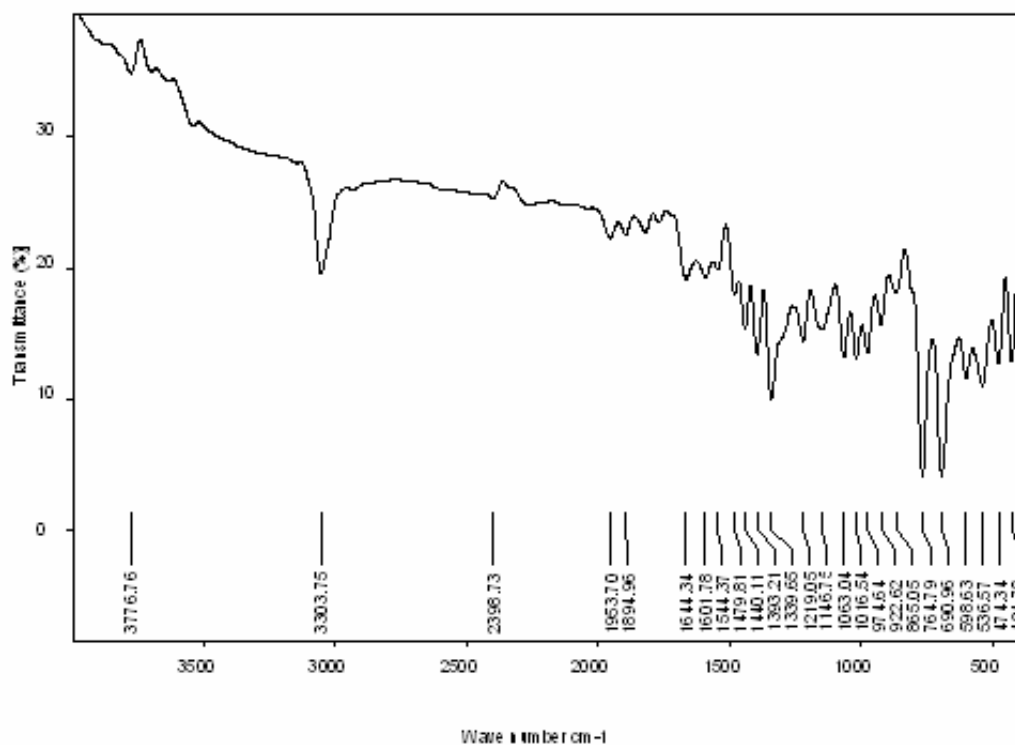


Fig. 1.2. FT-IR spectrum of  $[Co(C_{31}H_{24}N_4O)Cl_2]$  complex

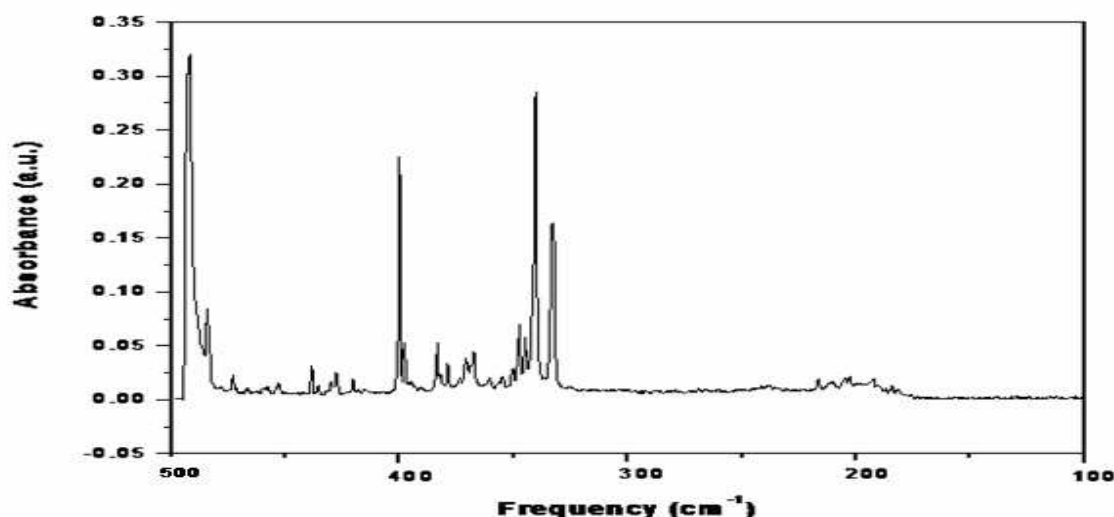


Fig. 1.3. Far-IR spectrum of  $[\text{Co}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex

### 3.3. Magnetic moment and Electronic spectral analysis

The magnetic moment data (Table 1.2) and electronic spectral data have been found to be helpful for determining the geometry of the complexes.

The magnetic moment at room temperature of cobalt (II) complex is 4.91 B.M. which lies in the range 4.82-5.05 B.M. This was indicative of a high-spin octahedral geometry. The electronic spectrum of this complex displays a band at 516nm ( $19380\text{cm}^{-1}$ ) which is assigned to the transition  ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$  ( $\nu_3$ ). This is considered as a characteristic band for cobalt(II) in octahedral environment.

The electronic spectrum of nickel (II) chloride complex exhibits main d-d band at 386nm ( $25907\text{cm}^{-1}$ ), corresponding to  ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$  transition. This is considered as a characteristic band for nickel(II) in octahedral environment. The magnetic susceptibility values of Ni(II) complex is found to be 3.27 B.M. which is very close to the value for their octahedral environment.

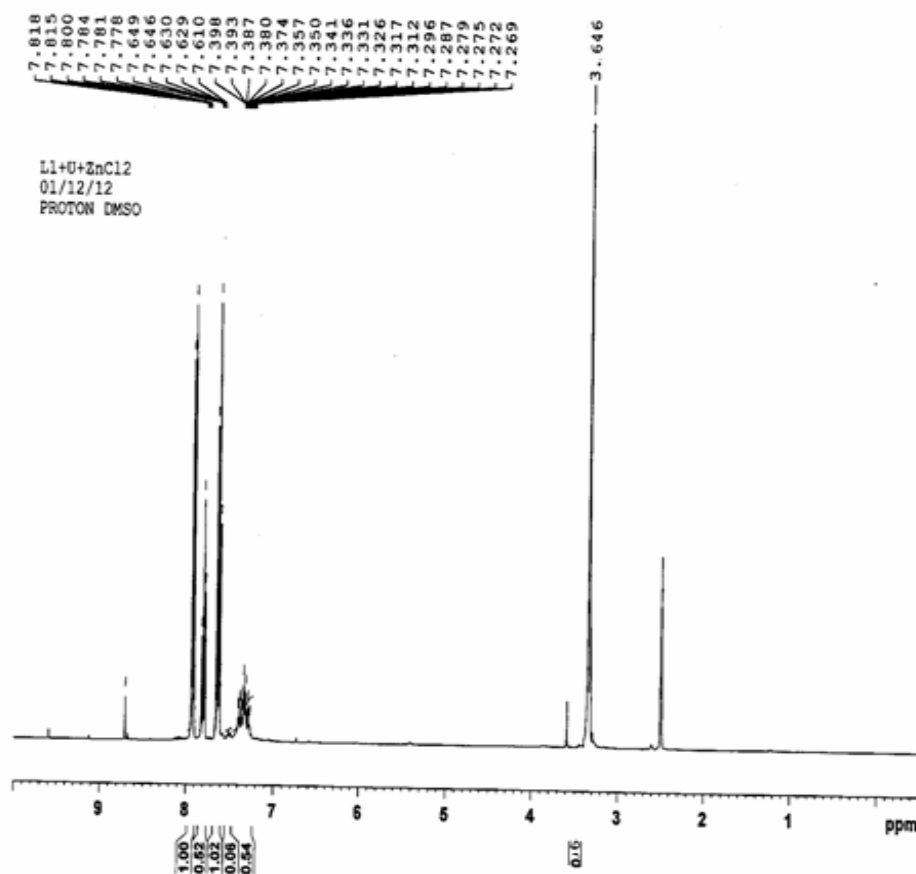
The observed electronic spectrum of copper(II) chloride complex displays a band at 695nm ( $14388\text{cm}^{-1}$ ) in the visible region is due to  ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$  transition, which is in good agreement with a distorted octahedral geometry for copper(II) ion(8). The magnetic susceptibility values for this complex are found to be 1.90B.M. which are further in conformity for distorted octahedral geometry.

### 3.4. NMR spectral analysis

A comparison of the spectra of the ligand with the spectra of the corresponding complexes show the absence of  $-\text{NH}_2$  signals, indicating deprotonation of  $-\text{NH}_2$  group of diamine, which suggests that there may be coordination of metal ion through nitrogen atom as evinced by IR spectra also. A singlet observed at  $\delta$  3.65 ppm in the complex may be assigned to methylene protons adjacent to the nitrogen atoms. The multiplet of aromatic protons was observed at  $\delta$  7.27 – 7.82 ppm in the spectra of the precursor and the metal complex of the macrocycles (Table 1.3). The characteristic signals in  ${}^1\text{H}$  NMR spectra of  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex are given in Fig. 1.4.

**Table 1.3.**  $^1\text{H}$  NMR Spectral Data ( $\delta$ , ppm) of the Precursor and  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex

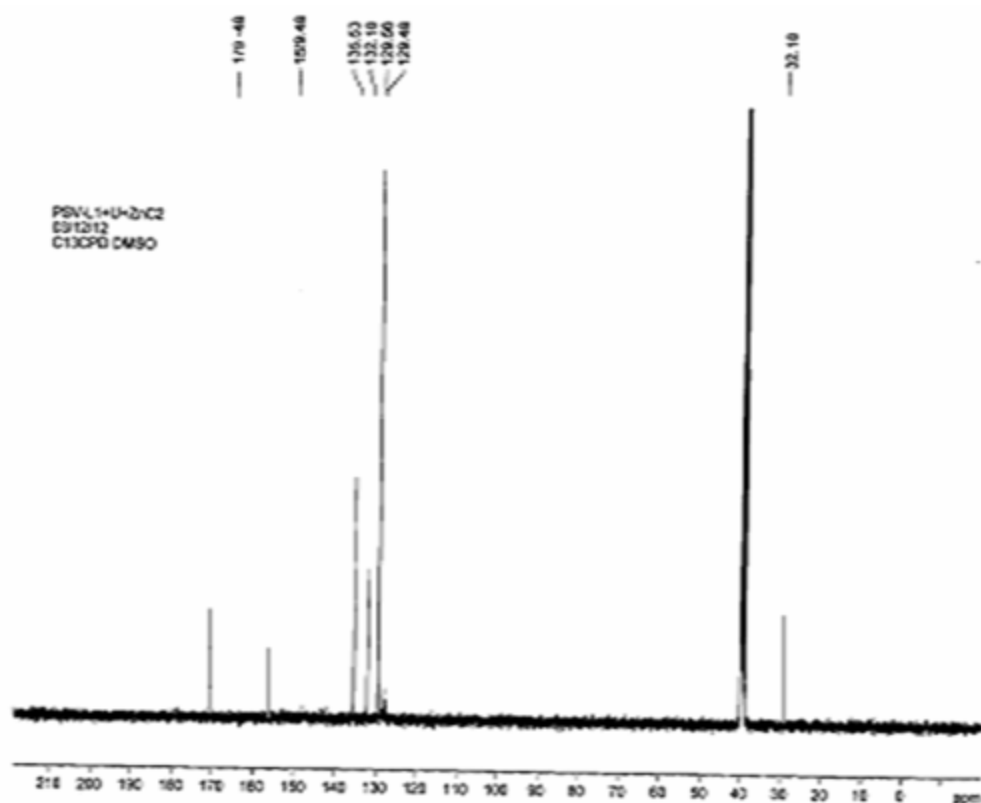
Compound	-NCH <sub>2</sub> (s)	Aromatic Protons		
		2	3	4
L(C <sub>30</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> )	3.58	7.80 (d)	7.40 (dd)	7.60 (d)
$[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$	3.65	7.82 (d)	7.27 (dd)	7.63 (d)

**Fig. 1.4**  $^1\text{H}$  NMR spectrum of  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex

The  $^{13}\text{C}$  NMR spectrum of  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex is shown in Fig 1.5. The spectral data of the precursor and the metal complex of the macrocycle (Table 1.4) also support the formation of this complex and the formation of the macrocyclic ring structure. In the spectra, there is no peak corresponding to keto group. But a peak at  $\delta$  170.40 ppm corresponds to keto group of an amide. Appearance of two peaks at  $\delta$  159.48 ppm and  $\delta$  32.10 ppm corresponds to  $>\text{C}=\text{N}$  and  $-\text{CH}$  carbons. Appearance of multiplet peaks in the range  $\delta$  127 – 132 ppm corresponds to Aromatic carbons.

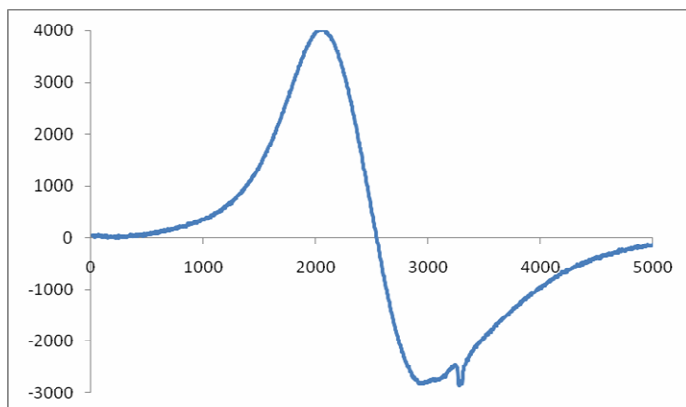
**Table 1.4.**  $^{13}\text{C}$  NMR Spectral Data ( $\delta$ , ppm) of the Precursor and  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex

Compound	Chemical Shift Values				
	>C=O	>C=N	-CH	C=O of amide	Aromatic Carbons
$\text{L}(\text{C}_{30}\text{H}_{24}\text{N}_2\text{O}_2)$	177.07	159.26	32.21	-	$\text{C}_1$ , 128.61; $\text{C}_2$ , 127.20; $\text{C}_3$ , 126.77; $\text{C}_4$ , 126.12
$[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$	-	159.48	32.10	170.40	$\text{C}_1$ , 132.20; $\text{C}_2$ , 129.55 $\text{C}_3$ , 127.59; $\text{C}_4$ , 129.36

**Fig. 1.5.**  $^{13}\text{C}$  NMR spectrum of  $[\text{Zn}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex

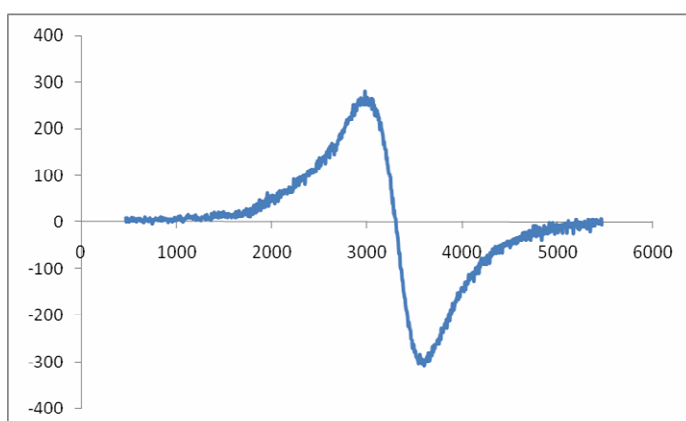
### 3.5. EPR spectral analysis

The EPR spectrum of  $[\text{Cu}(\text{C}_{31}\text{H}_{24}\text{N}_4\text{O})\text{Cl}_2]$  complex is recorded at X-band at room temperature. This is shown in Fig. 1.6. The computation made by Piesach and Blumberg's method suggests the presence of two  $g$  values. The geometry around the  $\text{Cu}^{\text{II}}$  ions is distorted octahedral geometry with  $g_{\parallel} = 2.228$  and  $g_{\perp} = 2.078$ . The axial symmetry parameter  $G$  is found to be 3.00 which suggest the absence of exchange interaction among magnetically equivalent ions in the unit cell. The  $g_{\parallel}$  is a function for representing covalence. The  $g_{\parallel}$  value is found to be less than 2.3, indicating covalent nature of the complex. The trend observed for the complex  $g_{\parallel} (2.228) > g_{\perp} (2.078) > g_e (2.0023)$ , suggesting the unpaired electron to be in the  $d_{x^2-y^2}$  orbital and the complex is octahedral(9).



**Fig. 1.6. EPR spectrum of [Cu(C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>O)Cl<sub>2</sub>] complex**

The EPR spectra of Mn(II) complex is recorded at room temperature as polycrystalline sample. The polycrystalline sample gives one broad isotropic signal centered at approximately, the free electron g-value (2.0023). The observed g-value is in the range 2.03-2.04. The broadening of the spectra is probably due to spin relaxation(10). The EPR spectra [Mn(C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>O)Cl<sub>2</sub>] complex is shown in Fig. 1.7.



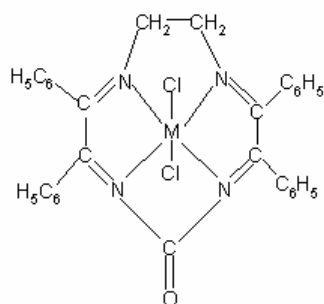
**Fig. 1.7. EPR spectrum of [Mn(C<sub>31</sub>H<sub>24</sub>N<sub>4</sub>O)Cl<sub>2</sub>] complex**

### 3.6. Structure of the complexes (16-20)

- 1) The elemental analyses correspond to the metal: ligand stoichiometry of 1:1.
- 2) The conductance measurement shows that the metal (II) chloride macrocyclic complexes are non-electrolytes.
- 3) The magnetic moments,  $\mu_{\text{eff}}$  of the macrocyclic complexes reveal that they are all of high spin type with paramagnetic nature (except zinc) and hence Mn, Co, Ni, Cu and Zn are in the +2 oxidation state.
- 4) The electronic spectral data are in conformity with the transitions for octahedral metal (II) chloride complexes.
- 5) The infrared spectral data show that the ligand coordinates to the metal ion through the four imines nitrogen atoms of the macrocyclic ring.
- 6) The far infrared spectral data confirm the presence of  $\gamma$ M-N and  $\gamma$  M-Cl bonds and proving the 'N' coordination with metals.
- 7) The structures of complexes are assigned on the basis of electronic spectra, magnetic moments and analytical data.

Thus from the above observations, the following structure is tentatively proposed for the complexes (Fig. 1.8).





M = Mn<sup>II</sup>, Co<sup>II</sup>, Ni<sup>II</sup>, Cu<sup>II</sup> and Zn<sup>II</sup>

**Figure 1.8.** Proposed structure of the complexes

### 3.7. Antibacterial Activity

In the present study, the newly synthesized metal complexes and the ligand were screened for their biological activities through *in vitro* analysis against five gram negative bacteria includes *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *E. coli*. All the strains were being cultured at 37 °C and stored at 4 °C. The complex was quantitatively analyzed by disc diffusion assay and minimum inhibitory assay. DMSO was used as solvent control. The Zone of inhibition values and the minimum inhibitory concentrations of the ligand and the complexes are summarized in Tables 1.5, 1.6 and exhibited in Figures 1.9 and 1.10.

The antibacterial activity values indicate that the complexes possess moderate growth inhibition potency compared to those of their parent ligand. The antibacterial activity of the metal complexes was increases with increase in concentration of the complexes.

**Table 1.5. Zone of inhibition (in mm) of ligand and macrocyclic complexes**

Ligand/Complex	Zone of inhibition in mm				
	<i>Vibrio cholerae</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>E.coli</i>
Ligand	--	--	--	--	--
[Mn(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] (16)	--	--	--	--	--
[Co(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] (17)	16	8	10	8	9
[Ni(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] (18)	7	--	--	--	--
[Cu(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] (19)	12	6	7	6	7
[Zn(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] (20)	--	--	--	--	--

The complex 17 showed highest zone of inhibition activity against *V. cholera* (16mm) and moderate antimicrobial activity against *Shigella flexneri* (8mm), *Pseudomonas aeruginosa* (10mm), *Staphylococcus aureus* (8mm) and *E. coli* (9mm). This complex is found to be active against all microbes comparing other complexes. Among all the five Schiff bases complexes, the complex 17 exhibited MIC value of 2.5 mg/ml concentration after 24 hrs incubation at 35 °C ± 2 against *Vibrio cholerae* species.

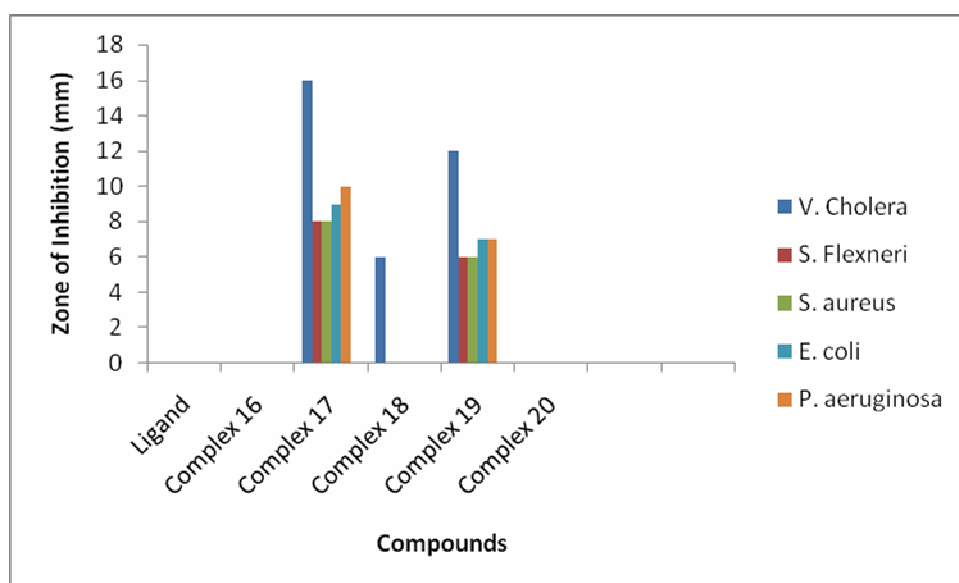
The complex 19 displayed antimicrobial activity against *Vibrio cholerae* (12 mm), *P.aeruginosa* and *E. coli* (7 mm each) and *S. flexneri* and *S. aureus* (6 mm each). Similarly these complexes also possessed higher to moderate active against all microbes.

The complex 18 exhibited minimal bactericidal activity of 7 mm against *Vibrio cholerae*, and no antibacterial property against any of other microorganisms.

New macrocyclic multidentate Schiff-base ligand metal complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) reported for biological activity against selected Gram positive and Gram negative bacterial strain revealed that the metal complexes become potentially important for antimicrobial activity compared to free ligand(11).

Complex **16** and **20** does not showed bactericidal activity against any of the microbes, similar to that of ligand's antibacterial activity.

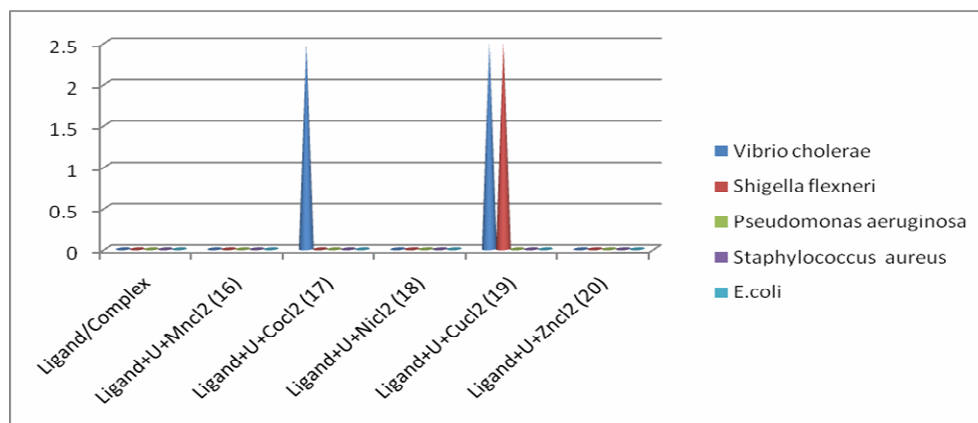
**Fig. 1.9. Graph showing Zone of inhibition (mm) for the ligand and the complexes (16-20) against bacterial strain culture**



**Table 1.6. Minimum Inhibitory concentrations for the ligand and the complexes (16-20)**

Ligand/Complex	MIC at mg/ml				
	<i>Vibrio cholerae</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>E.coli</i>
Ligand	--	--	--	--	--
[Mn(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] ( <b>16</b> )	NA	NA	NA	NA	NA
[Co(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] ( <b>17</b> )	2.5	NA	NA	NA	NA
[Ni(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] ( <b>18</b> )	NA	NA	NA	NA	NA
[Cu(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] ( <b>19</b> )	NA	NA	NA	NA	NA
[Zn(C <sub>31</sub> H <sub>24</sub> N <sub>4</sub> O)Cl <sub>2</sub> ] ( <b>20</b> )	NA	NA	NA	NA	NA

The mechanism of improved toxicity of the complexes can be attributed due to increase in the lipophilic nature of the complexes arising from complexation. Complexation reduces the polarity of the metal atom largely because of partial sharing of its positive charge with the donor groups and probable  $\pi$ -electron delocalization within the whole complex(12). It also increases the lipophilic nature of the central metal atom, which later on favors the permeation through the lipid layer of cell membrane. The mode of action of complexes involves the formation of hydrogen bonds with the amide group by the active sites leading to interference with the cell wall synthesis. This hydrogen bond formation damages the cytoplasmic membrane and the cell permeability and concurrently leading to cell death(13).

**Fig. 1.10. Graph showing Minimum Inhibitory Concentration of ligand and complexes (16-20) against bacterial strains culture**

Among all the complexes, only complex **17** showed moderate zone of inhibition activity against the microbe, *Vibrio cholerae* with a zone of inhibition value of 16mm and a comparative antimicrobial activity against *Pseudomonas aeruginosa* with a zone of inhibition value of 10mm. This was the only complex found to be active against all the microbes compared to other complexes. And also, complex **17** exhibited MIC value of 2.5 mg/ml concentration after 24 hrs incubation at 35°C ± 2 against *Vibrio cholerae* species.

#### 4. REFERENCES:

1. Fujiwara M, Wakita H, Matsushita T, and Shono T., Preparation of 14-, 18-, and 22-Membered Tetraaza Macrocycles and Their Complexing Ability for Copper(II) and Nickel(II) Ions, Bull. Chem. Soc., 1990, 63, 3443-3449.
2. Bashall A, Partlin M.C, Murphy B.P, Fenton D.E, Kitchen S.J. and Tasker P.A., The Interaction of Lead (II) with Oxa-aza Macrocycles: The X-Ray Crystal Structures of Lead(II) Complexes of an N<sub>3</sub>O<sub>3</sub> Schiff Base Macrocycle and of the Corresponding Saturated Macrocycle, J. Chem. Soc. Dalton Trans., 1990, 505-509.
3. Atkinson I.M, Baillie P.J, Choi N, Fabbrizzi F, Lindoy L.F, Partlin M.C. and Tasker P.A., Comparative Study of the Interaction of 'Lop-Sided' 14- to 17-Membered Tetraaza Macrocycles Containing Fused Dibenzo Substituents with Nickel(II), J. Chem. Soc. Dalton Trans., 1996, 3045-3051.
4. Chandra S. and Singh R., Synthesis and Characterization of Nickel(II), Copper(II) and Palladium(II) Complexes of Dibenzo-tetraaza Tetradentate Macrocyclic ligand, Indian J. Chem., 1995, 34A, 1003-1004.
5. Prasannabalaji N, Muralitharan G, Sivanandhan R.N, Kumaran S. and Pugazhendhan S.R., Antibacterial activities of some Indian traditional plant Extracts, Asian Pacific Journal of Tropical Disease., 2012, S291-S295.
6. Nakamoto K., Infrared Spectra of Inorganic and Coordination Compounds, Wiley-Interscience, New York, 1970.
7. Rao C.N.R., Chemical Applications of Infrared Spectroscopy, Academic, New York, 1963.
8. Bersuker I.B., Coord. Chem. Rev., 1975, 14, 367.
9. Mondal N, Dey D.K, Mitra S. and Abdul Malik K.M, Polyhedron, 2000, 19, 2707.
10. Lever A.B.P., Crystal Field Spectra, Inorganic Electronic Spectroscopy, 1<sup>st</sup> ed., Elsevier, Amsterdam, 1968, 249.
11. Ahmad I. and Beg A.Z., Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens, Journal of Ethnopharmacology., 2001, 74, 113-123.
12. De S.I, Atac A. and Yurdakul S, Journal of Molecular Structure., 2002, 605, 103.
13. McCarthy P.J, Hover R.J, Uexo K. and Martelo A.E, Journal of Chemical Society., 1955, 77, 5820.

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