

Synthesis, Characterization and Biological Evaluation of Unsymmetrical 12-Membered Pentaaza Macrocyclic Complexes

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ABSTRACT: A new series of unsymmetrical 12-membered pentaaza macrocyclic complexes $[M(L)X_2]$ (where, $M = Co^{II}$, Ni^{II} and Cu^{II} , $L =$ ligand and $X = Cl^-$) have been prepared via the template condensation reaction using bis(benzil)ethylenediamine, semicarbazide hydrochloride and metal chlorides in a 1:1:1 molar ratio. These complexes have been characterized by elemental analyses, molecular weight determinations, molar conductance, IR and electronic spectral studies. Co^{II} and Ni^{II} complexes may be considered to possess an octahedral geometry whereas Cu^{II} complexes possess tetragonal geometry. On the basis of the IR and NMR spectral data the involvement of amido nitrogen in coordination to the central metal ion has been confirmed. These transition metal complexes synthesized have also been tested for their antimicrobial potentials against five bacterial pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Shigella Flexneri*, *Pseudomonas aeruginosa* and *Vibrio cholerae*. Compared to the control antibiotics used, Ni^{II} and Cu^{II} metal complexes exhibited best antimicrobial activity against *Shigella flexneri* (30mm each). They also showed an observable activity even at the least concentration value of 0.078mg/ml for *Shigella flexneri*. The role of transition metal complexes in bactericidal effect has been discussed.

KEYWORDS: Bis(benzil)ethylenediamine; Co^{II} ; Ni^{II} ; Cu^{II} ; Semicarbazide hydrochloride.

I. INTRODUCTION

The chemistry of macrocycles has attracted the attention of many inorganic chemists during the last few decades. The great significance of macrocyclic metal complexes are due to their resemblances with many natural systems such as porphyrins and cobalamines and used in DNA recognition oxidation and cleavage [1-3]. The novel approach to macrocyclic synthesis, therefore, overcomes the usual limitations of the more traditional template routes where the metal tends to be "locked" into the macrocycle with demetallation often resulting in decomposition [4]. The influence of variation of the structural feature on metal complexation, namely the length of the alkyl bridge linking the benzil nitrogen atoms, was investigated [5]. The use of metal ions as templates in such reactions has led to the synthesis of many metal complexes of macrocyclic ligands [6]. These ligands display a number of features of chemical interest. They are relatively rigid in nature and thus impose specific coordination geometry on the metal ion [7]. In this paper, we report the synthesis and characterization of new bivalent transition metal complexes from the reaction of bis(benzil)ethylenediamine with semicarbazide hydrochloride which show the marked effect of any build-up of ligand strain on the coordination geometry of the central metal.

II. MATERIALS AND METHODS

Melting points were determined in capillary tubes and are uncorrected. All the reagents used in the preparation of macrocyclic ligand and their metal complex were of reagent grade (Merck). The solvents used for the synthesis of macrocyclic ligands and metal complex were distilled before use. All other chemicals were of AR grade and used without further purification.

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The microelemental analysis was carried out using CHN-Rapid Analyser. 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 moments were measured out by using gouy balance. IR spectra (KBr) of the sample were recorded on a shimadze FTIR-8400s spectro-photometer. The electronic spectra (chloroform) were recorded on the Lamda 35 spectrometer.

2.1. Synthesis of the Complexes:

A hot methanolic solution (20 ml) of Co^{2+} , Ni^{2+} and Cu^{2+} metal chlorides (4.3mmol) is mixed with a hot methanolic solution of mixture of 1,2,7,8-Tetraphenyl-3,6-diazahepta-2,6-diene-1,8-dione (L) (2g, 4.3mmol) and Semicarbazide hydrochloride (0.4796g, 4.3mmol) with constant stirring. This mixture is refluxed on water bath at $\sim 80^\circ\text{C}$ for 5 hours. On cooling, brightly coloured complexes $[\text{C}_{31}\text{H}_{26}\text{CoN}_5\text{OCl}_3]$, $[\text{C}_{31}\text{H}_{26}\text{NiN}_5\text{OCl}_3]$, $[\text{C}_{31}\text{H}_{26}\text{CuN}_5\text{OCl}_3]$ are precipitated out. These are filtered, washed several times with hot water, then with cold methanol, dried in vacuum over anhydrous calcium chloride. The complexes are recrystallised using methanol + benzene mixture (1/1) to get the complex in pure form.

2.2. Biological Activity

2.2.1. Bacterial cultures used

The microorganisms used as test organisms are bacteria isolated from clinical samples. One gram positive *Staphylococcus aureus* and four gram negative *Vibrio Cholerae*, *Shigella Flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli*. The test organisms were secluded from the clinical samples from patients attending Government Hospital, Kanchipuram, Tamilnadu, India.

2.2.2. Antimicrobial testing by disc diffusion method

The organisms were isolated in nutrient agar medium and selectively cultured at 37°C for 24hrs. The bacterial strains were identified by biochemical and standard antibiogram tests as per the directions from Berge's manual for determinative bacteriology. Circular disc of 6mm diameter were prepared from the Whatman No. 1 filter paper. Discs were soaked with equal volume (50 μl) of each metal complexes dissolved in DMSO at a concentration of 100 $\mu\text{g}/\text{ml}$. The discs were aseptically placed over plates of Muller Hinton agar (MHA, Difco) seeded with each of test pathogens, and the inoculums were adjusted to 0.5 McFarland turbidometry. The plates were incubated in a vertical position at 37°C for 24 hours and the zone of inhibition was calculated (in mm diameter). Inhibition zones with diameter less than 12mm were considered to have low antibacterial activity. Diameters between 12 and 16mm were considered to be moderately active, and those with diameters $> 16\text{mm}$ were considered to be highly active. The clinical strains were also tested for their sensitivity against the standard antibiotics, Ampicillin (10mcg), nalidixic acid (10mcg), Methicillin (10mcg) by the disc diffusion method. The solvent control (DMSO) was also maintained throughout the experiment.

2.2.3. Determination of minimum inhibitory concentration (MIC)

The prepared complex is subjected to microbiological testing by using standard strains of *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli*. Their activities are compared with the parent compounds. The efficacy is determined by the MIC values.

The complex sample was separately dissolved in the DMSO and Vortexed vigorously under aseptic condition. The MIC assay was performed aseptically in a sterile U bottom 96 well microtitre plate in the laminar hood. In order to determine the minimum inhibitory concentration (MIC), a serial dilution of complexes (2.56mg/ml, 1.25mg/ml, 0.625mg/ml, 0.312mg/ml, 0.15mg/ml, 0.078mg/ml, 0.039mg/ml, 0.019mg/ml and 0.009mg/ml) was prepared and 100 μL nutrient broth bacterial suspension was also added to get a final concentration of 0.5 McFarland OD (10^8 CFU/ml). The above procedure of serial dilutions is repeated to obtain preparation for the sample. At the same time, solvent control, positive and negative controls are also run along with the test. The positive and negative controls are free from drug and solvent control were free from macrocyclic complex. Only the positive control wells were inoculated with 1 full loop of the broth culture. After inoculation, the dilutions are incubated at 37°C for 24 hours. After incubation of 24hrs, the turbidity (growth) was checked in the microtitre plates for MIC value. The highest serial dilution which showed no turbidity was taken as the MIC value of the compound.

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III. RESULTS AND DISCUSSION

3.1. General properties of the macrocyclic complexes

The physical and the analytical data of the complexes are listed in Table 1. All the complexes are coloured. The percentage yield and the melting points of the complexes are also given in this Table. The elemental analysis (Table 2) shows the stoichiometry of the metal complexes. The complexes were highly soluble in chloroform, acetonitrile, DMF and DMSO. In other common organic solvents they are sparingly soluble.

3.2. Molar conductance measurements

Table 1. Physical and analytical data of the complexes

Complex	Colour	Mol. Wt.	M.Pt. (°C)	Yield (%)
[C ₃₁ H ₂₆ CoN ₅ OCl ₃]	green	650	132	62
[C ₃₁ H ₂₆ NiN ₅ OCl ₃]	blue	650	121	51
[C ₃₁ H ₂₆ CuN ₅ OCl ₃]	green	655	154	56

The molar conductivity values of 10⁻³M solutions of the complexes in acetonitrile are exhibited in Table 2. These values for the complexes, [C₃₁H₂₆CoN₅OCl₃], [C₃₁H₂₆NiN₅OCl₃], [C₃₁H₂₆CuN₅OCl₃] are measured as 20.73, 25.29 and 30.94 mho cm² mol⁻¹ respectively indicating non-electrolyte nature of these complexes. From the conductivity measurements, it was recognized that the chloride ions are coordinated to metal ions, indicating that they are ligands and not simple ions.

Table 2. Elemental analysis, magnetic moment and molar conductance of the complexes

Complex	Elemental Analysis % Found (Calculated)				μ_{eff} BM	Molar Conductance ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)
	C	H	N	M		
C ₃₁ H ₂₆ CoN ₅ OCl ₃	57.19	3.96	10.71	9.01	4.94	20.73
	(57.23)	(4.00)	(10.77)	(9.06)		
C ₃₁ H ₂₆ NiN ₅ OCl ₃	57.20	3.97	10.72	8.96	3.31	25.29
	(57.23)	(4.00)	(10.77)	(9.03)		
C ₃₁ H ₂₆ CuN ₅ OCl ₃	56.74	3.92	10.62	9.64	1.94	30.94
	(56.79)	(3.97)	(10.68)	(9.70)		

3.3. Infrared spectral studies

The IR spectra of metal complexes are interpreted by comparing with the ligand in order to characterize their structures and mode of coordination of ligand. The results are listed in Table 3.

At 1772cm⁻¹ a strong band is observed in the IR spectra of ligand, L, which is assigned to C=O stretching mode of vibration. The appearance a new strong absorption band in complexes lie in the range 1632-1640cm⁻¹ which is attributable to the characteristic stretching frequencies of the imino linkage [8] $\nu(\text{C}=\text{N})$ and the absence of stretching and deformation frequencies of the amino and the carbonyl groups in the IR spectra of these macrocyclic complexes, provide strong evidence for the presence of cyclic product.

The presence of bands in the range of 1598-1600cm⁻¹ are attributed to the >C=O group of amide. The strong as well as sharp bands in the range of 1167-1169cm⁻¹ are due to the $\nu(\text{C}-\text{N})$ vibration. The medium intensity absorption bands in the range of 3309-3315cm⁻¹ are attributed to $\nu(\text{C}-\text{H})$ vibration. The band characteristic of the benzil moiety appeared in the range of 1443-1444cm⁻¹ ($\nu_{\text{asym}} \text{C}_6\text{H}_5$) and 1313-1316cm⁻¹ ($\nu_{\text{sym}} \text{C}_6\text{H}_5$). An important feature is the appearance of a new medium intensity band in the range of 430-462cm⁻¹, attributed to $\nu(\text{M}-\text{N})$ which provide strong evidence for the involvement of nitrogen in coordination [9].

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Table 3. Characteristic IR bands (cm⁻¹) of the ligand and its complexes

Ligand / Complex	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{N})$	$\nu(\text{C}=\text{O})$ of amide	$\nu(\text{C}-\text{H})$	$\nu(\text{C}-\text{N})$	$\nu_s\text{C}_6\text{H}_5$	$\nu_a\text{C}_6\text{H}_5$	$\gamma \text{ M-N}$
$\text{L}_1(\text{C}_{30}\text{H}_{24}\text{N}_2\text{O}_2)$	1772	1667	-	3317	1169	1312	1439	-
$\text{C}_{31}\text{H}_{26}\text{CoN}_5\text{OCl}_3$	-	1638	1600	3309	1167	1313	1444	448
$\text{C}_{31}\text{H}_{26}\text{NiN}_5\text{OCl}_3$	-	1632	1598	3312	1168	1316	1443	430
$\text{C}_{31}\text{H}_{26}\text{CuN}_5\text{OCl}_3$	-	1640	1599	3315	1169	1316	1443	462

3.4. Magnetic properties

The magnetic moments, μ_{eff} of the macrocyclic complexes are given in Table 2. The Co^{II} complex show magnetic moment of 4.94 B.M. which lies in the range of 4.62 - 5.04 B.M., while Ni^{II} complex show magnetic moment of 3.31 B.M. which lies in the range of 3.13 - 3.36 B.M. at room temperature. The values are in tune with a high spin configuration and show the presence of an octahedral environment around Co^{II} and Ni^{II} ion.

The magnetic moment of Cu^{II} complex at room temperature is found to be 1.94 B.M. which lies in the range of 1.92 - 2.02 B.M., corresponding to one unpaired electron. The copper complex is considered to have tetragonally elongated octahedral geometry.

3.5. Electronic spectral analysis

The cobalt(II) complex showed a magnetic moment value of 4.94 B.M. at room temperature 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 exhibited three bands of medium intensity observed at 507nm (19723cm⁻¹), 868nm (11520cm⁻¹) and 992nm (10080cm⁻¹) are assigned to ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$ (ν_1), ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$ (ν_2) and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ (ν_3) transitions respectively. This is indicative of an octahedral geometry around Co^{II} centre.

The electronic spectrum of nickel(II) chloride complex exhibits main d-d band at 397nm (25188cm⁻¹) which is assigned to ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$ transition. This transition confirms octahedral geometry around the Ni (II) ion. The value of the magnetic moment (3.31 B.M) also supports the octahedral environment around the Ni(II) ion.

The observed electronic spectrum of copper(II) chloride complex shows a broad band in the range 13297cm⁻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 [10-12].

3.6. Structure of the complexes

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, μ_{eff} of the macrocyclic complexes reveal that they are all of high spin type with paramagnetic nature and hence Co, Ni and Cu 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 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 macrocyclic complexes (Fig. 1).

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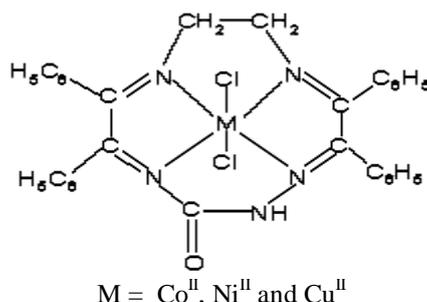


Fig. 1. Proposed structure of the complexes

3.7. Antibacterial activity

The ligand and the complexes were evaluated against different species of bacteria like *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli* at $30 \pm 2^\circ\text{C}$ as per procedure reported earlier [13-15]. DMSO was served as a control. Generally, the macrocyclic compounds are more active than the open chain derivatives. The antimicrobial assay values by disc diffusion and minimum inhibitory concentrations of the ligand and the complexes were expressed in Tables 4, 5 and Figures 2 & 3.

Co(II) complex showed moderate bactericidal activity against all the five microbes namely, *Vibrio cholerae* (11mm), *Shigella flexneri* (10mm), *Staphylococcus aureus* (9mm), *Pseudomonas aeruginosa* (8mm) and *Escherichia coli* (7mm).

Cu(II) complex exhibited antibactericidal activity against three microbes namely, *Shigella flexneri* (30mm), *Vibrio cholerae* and *Escherichia coli* (8mm each). Also the MIC value shows 0.078 mg/ml as the lowest concentration with optimal MIC value to inhibit pathogenic *Shigella flexneri* organism.

Ni(II) complex exhibited best antimicrobial activity against *Shigella flexneri* (30mm). It also showed some bactericidal activity against *Vibrio cholerae* (8mm). The MIC value for inhibiting *Shigella flexneri* was 0.078mg/ml concentration.

In this case, all the complexes showed some bactericidal activity against microbes. Metal complexes have a greater effect than ligands against almost all bacteria.

Table 4. 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>Escherichia coli</i>
Ligand	--	--	--	--	--
C ₃₁ H ₂₆ CoN ₅ OCl ₃	11	10	8	9	7
C ₃₁ H ₂₆ NiN ₅ OCl ₃	8	30	--	--	--
C ₃₁ H ₂₆ CuN ₅ OCl ₃	8	30	--	--	8

The antibacterial activity values point out that the complexes possess higher growth of inhibition potential compared to those of their parent ligand. Similar to other ligand complexes, the antibacterial activity of the metal complexes increases with higher concentration of the complexes. It is suggested that the complexes having antibacterial activities slow down multiplication process of the microbes by blocking their active sites and inhibiting their growth rate [16]

The obtained results specified that the complexes were more effective against some microbes under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane [17] and can be explained by Tweedy's Chelation theory [18]. Chelation will augment the lipophilic character of the central metal atom which afterward favors its permeation through the lipid layers of the cell membrane [19] and blocking the metal binding sites on enzymes of microorganisms.

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Table 8. Minimum inhibitory concentrations for the ligand and the complexes

Ligand / Complex	MIC at mg/ml				
	<i>Vibrio cholerae</i>	<i>Shigella flexneri</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Ligand	--	--	--	--	--
C ₃₁ H ₂₆ CoN ₅ OCl ₃	NA	NA	NA	NA	NA
C ₃₁ H ₂₆ NiN ₅ OCl ₃	NA	0.078	NA	NA	NA
C ₃₁ H ₂₆ CuN ₅ OCl ₃	NA	0.078	NA	NA	NA

From the zone of inhibition and MIC studies it was found that the ligands did not showed any antibacterial activity against any of the microorganisms. Among the complexes, Ni(II) and Cu(II) complexes exhibited best antimicrobial activity against *Shigella flexneri* (30mm each). The MIC assay of the complexes indicated that Ni(II) and Cu(II) complexes showed an observable activity even at the least concentration value of 0.078mg/ml for *Shigella flexneri*.

IV. CONCLUSION

The molar conductance value for the $\sim 10^{-3}$ M solutions of the complexes showed that all are non-ionic in nature. The presence of chloride is apparent only after decomposition of the complexes, almost certainly due to their presence in the coordination sphere. The elemental analysis and other spectral studies designate the chemical formulae of the complexes. The IR spectral studies indicate the coordination sites of the complexes. The mode of coordination suggested based on the shifts observed in the stretching and bending frequencies of various donor atoms of the ligand on complexation. The appearance of new moderately intense band in the regions 430-462cm⁻¹ assigned to ν (M-N) further supports the involvement of nitrogen in coordination. The magnetic moments of the complexes tells that they are all of high spin type. The electronic spectral data supports that all the complexes were found to have octahedral/distorted octahedral geometry. The antimicrobial assay of the ligand and the complexes were carried out against the bacterial strains such as *Staphylococcus aureus*, *Vibrio cholerae*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli* by disc diffusion method. From the zone of inhibition and MIC studies it was found that the ligands did not showed any antibacterial activity against any of the microorganisms. Among the complexes, complexes Ni(II) and Cu(II) complexes exhibited best antimicrobial activity against *Shigella flexneri* (30mm each). The MIC assay of all the complexes indicated that Ni(II) and Cu(II) complexes showed an observable activity even at the least concentration value of 0.078mg/ml for *Shigella flexneri*. Thus, Ni(II), and Cu(II) complexes showed better bactericidal activity against the microbe *Shigella Flexneri*.

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