

Biologically Active Tetraazamacrocyclic Complexes Of Co (II), Ni (II), Cu (II), and Zn (II)

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Abstract: A new series of macrocyclic complexes of type $[M(C_{14}H_{24}N_4)X_2]$ Where $M=Co(II), Ni(II), Cu(II), Zn(II)$, and $X=Cl^{-1}, NO_3^{-1}, CH_3COO^{-1}$ has been synthesized by [2+2] condensation reaction of ethylenediamine with and acetyl acetone in the presence of divalent metal ions. The complexes have been characterized with the help of conductance measurements, electronic, NMR and infrared spectral studies. On the basis of these studies, six coordinate octahedral geometry has been proposed for all the complexes. The complexes were tested for their in vitro antibacterial activity. Some of the complexes showed remarkable antibacterial activity against some selected bacterial strains.

1. Introduction

The multifarious role played by the naturally occurring macrocycles in biological systems is well known. The chemistry of synthetic macrocyclic complexes is also of great importance due to their use as dyes and pigments, MRI contrast agents and models for naturally occurring macrocycles [1-4]. Macrocyclic nickel complexes find use in DNA recognition and oxidation [5] while macrocyclic copper complexes find use in DNA binding and cleavage [6]. Some macrocyclic complexes have been reported showing antibacterial, antifungal and anti-inflammatory activities [7-8]. Macrocyclic metal chelating agents (DOTA) are useful to detect tumor lesions [9]. Prompted by these, in the present paper a new series of macrocyclic complexes of Co(II), Ni(II), Cu(II), and Zn(II) (chloride, nitrate acetate salt of cobalt, nickel copper and acetate salt of zinc) obtained by template

condensation reaction of ethylenediamine have been characterized with the help of various physicochemical techniques like elemental analyses, IR, NMR, magnetic susceptibilities, electronic spectra and molar conductance. These macrocyclic complexes were also screened for their in vitro antibacterial activity against some pathogenic bacteria.

2. Experimental

2.1. Chemistry

All the reported macrocyclic complexes were prepared by template method. To a stirring methanolic solutions (-50cm^3) of ethylenediamine (10mmol) was added divalent cobalt, nickel copper and zinc salt (5mmol) (chloride, nitrate, acetate for cobalt, nickel, copper and acetate for zinc) dissolved in methanol (20cm^3). After that acetylacetone (10mmol) dissolved in 20cm^3 methanol was added in the mixture and again stir for 1h. Colored complexes formed which were filtered,

washed with methanol, acetone and ether and dried in vacuo (Yield 45-50%). Scheme-I presents the non template synthesis of the complexes. The complexes were soluble in DMF and DMSO, but were insoluble in common organic solvents and water. They were found thermally stable up to -250°C and then decomposed.

2.2. In Vitro Antibacterial Activity (Biological assay)

The synthesized macrocyclic complexes were tested for in vitro antibacterial activity against some bacterial strains using spot-on-lawn on Muler Hinton Agar.

2.2.1 Test Pathogens

Four test pathogenic bacterial strains viz Bacillus cereus (MTCC739) and staphylococcus aureus (MTCC1144) were considered for determination of MIC (Minimum Inhibitory Concentration) of selected complexes.

2.2.2 Culture Conditions

The test pathogens were subcultured aerobically using Brain Heart Infusion Agar (HiMedia, Mumbai, India), at 37°C/24hrs. Working cultures were stored at 4°C in Brain Heart Infusion(BHI) broth (HiMedia, Mumbai, India), while stock cultures were maintained at -70°C in BHI broth contacting 15%(v/v)glycerol (Qualigenes, Mumbai, India). Organism was grown overnight in 10 ml BHI broth centrifuged at 5000g for 10min and the pellet was suspended in 10ml of phosphate buffer saline (PBS, pH 7.2). Optical density at 545 nm (OD-545) was adjusted to obtained 10⁸ cfu/ml followed by plating serial dilution onto plate count agar (HiMedia, Mumbai, India).

2. Analyses

Melting point were determined using capillaries in electrical melting point apparatus. The molecular weight was determined by rast camphor method. The metal contents were estimated using standard methods.

3. Physical Measurements

Electronic spectra of metal complexes were recorded in the region 1100-200 nm on a Hitachi U-2000 spectrophotometer. IR spectra

were recorded on Beckman IR-20 spectrophotometer in KBr/Nujol mull in the range 4000-200cm⁻¹. Proton NMR spectra was recorded in DMSO (d6) on Bruker ACF 300 spectrometer at 300 MHz reference to Me₄Si(0.0 ppm). Magnetic moments studies were carried out at SAIF, IIT, Roorkee, on Guoy balance. The conductivity was measured on digital conductivity meter (HPG System, G-3001).

4. Result and Discussion

4.1 Chemistry

The analytical data suggest the formula of macrocyclic complexes as: [M(C₁₄H₂₄N₄)X₂] Where M=Co(II), Ni(II), Cu(II), Zn(II), and X=Cl⁻¹, NO₃⁻¹, CH₃COO⁻¹. The test for anions is positive after decomposing the complex with conc. HNO₃, indicating their presence inside the coordination sphere. Conductivity measurements in DMSO indicate them to be non electrolytic in nature (10-20 ohm⁻¹cm²mol⁻¹)[10-12]. (Table 1)

Table-1: Magnetic movements and molar conductivity of complexes

Complexes of acetyl acetone	$\lambda_M \Omega^{-1}cm$	Magnetic moment μ_B
[CuLCl ₂]	22	1.78
[CuL(NO ₃) ₂]	20	1.77
[CuL(CH ₃ COO) ₂]	19	1.78
[CoLCl ₂]	23	4.83
[CoL(NO ₃) ₂]	22	4.83
[CoL(CH ₃ COO) ₂]	19	4.82
[NiLCl ₂]	22	2.80
[NiL(NO ₃) ₂]	21	2.82
[NiL(CH ₃ COO) ₂]	21	2.80
[ZnLCl ₂]	29	-
[ZnL(NO ₃) ₂]	28	-
[ZnL(CH ₃ COO) ₂]	27	-

4.1.1. Infrared Spectra

A pair of the band at -3230 cm^{-1} and -3260 cm^{-1} of medium intensity was observed in the spectrum of ethylenediamine corresponding to $\nu(\text{NH}_2)$, but was found absent in the spectra of all the complexes. A strong peak was observed at -1700 cm^{-1} of strong intensity in the spectrum of acetyl acetone which may be attributed due to $>\text{CO}$ group. This peak was found absent in the spectra of all the complexes. This indicates the absence of $>\text{CO}$ groups of acetyl acetone moiety in all complexes. This confirms the condensation of carbonyl groups of acetyl acetone and amino groups of ethylenediamine [13-14] his fact is also supported by appearance of new strong absorption band in the region $-1590-1615\text{ cm}^{-1}$ which may be attributed due to $\nu(\text{C}=\text{N})$ [15-16]. These results provide strong evidence for the formation of macrocyclic frame [17]. The lower value of $\nu(\text{C}=\text{N})$ indicates coordination of azomethine nitrogens to metal [18]. The bands present at $-1350-1000\text{ cm}^{-1}$ of medium intensity may be assigned due to $\nu(\text{C}-\text{N})$ vibrations. The bands present at $-2900-3130\text{ cm}^{-1}$ may be assigned due to various $\nu(\text{C}-\text{N})$ vibrations.

The infrared spectra show bands in the region $-420-460\text{ cm}^{-1}$ originate from $\nu(\text{M}-\text{N})$ vibrations in all the complexes [19-20]. The presence of bands in all the complexes in the region $-420-460\text{ cm}^{-1}$ originates from $\nu(\text{M}-\text{N})$ azomethine vibrations modes and gives an idea about coordination of azomethine nitrogen [21]. The bands present at $-300-310\text{ cm}^{-1}$ may be assigned as being due to $\nu(\text{M}-\text{Cl})$ vibrations [22,23] the bands present at $-210-250\text{ cm}^{-1}$ in all nitrate complexes are assignable to $\nu(\text{M}-\text{O})$ vibrations of nitrate group. (Table 2)

Table-2: IR spectra of complexes

Compound	$\nu(\text{C}=\text{N})$	$\nu(\text{C}-\text{N})$	$\nu(\text{M}-\text{N})$
$[\text{CuLCl}_2]$	1610	1288	420

4.1.2. NMR Spectra

The ^1H NMR spectrum provides a signal at 3.10 ppm due to $-\text{NCH}_2\text{CH}_2\text{N}-$ protons, 2.60 ppm due to $-\text{CCH}_2\text{C}-$ protons while $-\text{CH}_3$ protons appeared at 2.50 ppm. (Table 3)

Table-3: ^1H NMR Spectroscopic data of the complexes Table-3

Complex	$-\text{CH}_3$	$-\text{NCH}_2\text{CH}_2\text{N}-$	$-\text{CCH}_2\text{C}-$	$-\text{CCH}_2\text{CH}_2\text{C}-$
$\text{ZnL}(\text{NO}_3)_2$	2.40	3.15	2.65	-

4.1.3. Magnetic Measurements and Electronic Spectra

The magnetic moments of cobalt complexes at room temperature was found to be in the range of 4.82-4.85 μB . These data corresponds to three unpaired electrons. The electronic spectra of cobalt complexes show band at $-8100-9100(\nu_1)$, $12000-15550(\nu_2)$, and $18050-20220\text{ cm}^{-1}(\nu_3)$ or $(12.3-11 \times 10^2)(\nu_1)$, $(8.3-6.4) \times 10^2(\nu_2)$, $(5.5-4.9) \times 10^2(\nu_3)$ nm, respectively. The spectral data resembles to those reported to be octahedral. Thus assuming the effective symmetry to be D_{4h} , the various bands can be assigned to $^4\text{T}_{1g} \rightarrow ^4\text{T}_{2g}(\text{F})$, (ν_1) , $^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g}(\text{F})$, (ν_2) , $^4\text{T}_{1g} \rightarrow ^4\text{T}_{1g}(\text{P})$, (ν_3) respectively. It appears that the symmetry of these complexes is not idealized octahedral but is D_{4h} . The assignment of the first spin-allowed band seems plausible since the first band appears approximately at half the energy of the visible band.

The nickel complexes show magnetic moments in the 2.80-2.84 μB . range at room temperature showing octahedral environment around the divalent nickel ion in all complexes exhibit a well discernable band with a shoulder on the low energy side. The other two bands observed in the region at $\sim 16700-17020\text{ cm}^{-1}$ or $(6.5-5.9) \times 10^2$ nm (ν_2) , and $26800-28000\text{ cm}^{-1}$ or $(3.7-3.6) \times 10^2$ nm (ν_3) , are assigned to $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}(\text{F})$ (ν_2) , and $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g}(\text{P})$ (ν_3) respectively. The first two bands result from the splitting of one band ν_1 , and are in the range at $\sim 9700-10400$

cm^{-1} or $(10.3-9.6) \times 10^2 \text{ nm}$ and $11850-12500 \text{ cm}^{-1}$ or $(8.4-8) \times 10^2 \text{ nm}$, which can be assigned to ${}^3\text{B}_{1g} \rightarrow {}^3\text{E}_g$ and ${}^3\text{B}_{1g} \rightarrow {}^3\text{B}_{2g}$, assuming the effective symmetry to be D_{4h} (component of ${}^3\text{T}_{2g}$ in O_h symmetry). The intense higher energy band at $\sim 35550 \text{ cm}^{-1}$ or $(2.8) \times 10^2 \text{ nm}$ may be due to a $\pi-\pi$ transition of the (C=N) group. Various bands do not follow any regular pattern and seem to be anion independent. The spectra are consistent with distorted octahedral nature of these complexes.

The magnetic moments of copper complexes lie in the range $1.77-1.79 \mu\text{B}$. The electronic spectra of the copper complexes exhibit bands in the region $\sim 17400-19050 \text{ cm}^{-1}$ or $(5.7-5.2) \times 10^2 \text{ nm}$ with a shoulder on the low energy side at $\sim 14450-16300 \text{ cm}^{-1}$ or $(6.9-6.1) \times 10^2 \text{ nm}$, and show that these complexes are distorted octahedral. Assuming tetragonal distortion in the molecule, the d-orbital energy level sequence for these complexes may be: $x^2-y^2 > z^2 > xy > xz > yz$ and the shoulder can be assigned to $z^2 \rightarrow x^2-y^2 ({}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g})$ and the broad band contains both $xy \rightarrow x^2-y^2 ({}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{2g})$ transitions. The band separation of the spectra of the complexes is the order 2500 cm^{-1} or $(40) \times 10^2 \text{ nm}$, which is consistent with proposed geometry of the complexes. Therefore it may be concluded that all the copper complexes are distorted octahedral.

4.1.4. EPR Spectra

EPR spectra of complexes were recorded in powder form at room temperature differ significantly g_{\parallel} , g_{\perp} , A_{\parallel} and A_{\perp} and G values of complexes are summarized in (table-4).

Table-4: EPR parameters for metal complexes

Complex	Gyrometric ratio g	A gauss	G	e/gm 10^{21}
[NiL(NO ₃) ₂]	$g_{\parallel} = 1.9967$	$A_{\parallel} = 11.11$	0.6235	1.5031
	$g_{\perp} = 1.9935$	$A_{\perp} = 16.66$		
	$g_{av} = 1.9945$			

Difference in values of line width

suggests that they are independent of temperature.

Nickel complexes shows isotropic sharp line width at 3344 gauss giving $g_{\parallel} = 1.9967$, $g_{\perp} = 1.9935$ and exchange interaction coupling constant $G = 0.6235$. Value of electron per gram is found to be 1.5031×10^{21} . g_{av} is less than 2.3 indicates sufficient covalent nature of metal ligand bond.

For complexes were $g_{\parallel} > g_{\perp}$ and G value observed to be less than 4 which indicates that electron lies on $dx^2 - y^2$ orbital. According to Hathway, if exchange interaction coupling constant If G is larger than 4 the exchange interaction are negligible because local tetragonal axes are aligned parallel and slightly misaligned and if it is less than 4 the exchange interaction are considerable and local tetragonal axes are misaligned. For present complexes G value is very small suggests exchange interactions are considerable and local tetragonal axes are misaligned.

4.1.5. Powder X-ray analysis

Powder XRD diffractogram of some selected complexes were recorded in 2θ range 20 to 80° cu-radiation source at wavelength 1.5447 \AA at room temperature are shown in figure. Major reflexes were used to determine corresponding interplaner distances. Diffractogram were then indexed independently. Miller indices were calculated and lattice parameters a, b, c and interfacial angles α , β , γ were determine by computer based powder programme. Unit cell volumes of complexes were determined. (Table 5)

XRD Diffractogram:

CuCl_2

Crystal System: Monoclinic Lattice Type: P2/M
Lattice Parameter: a = 7.8042 b = 8.6593 c = 7.3259

Lattice Parameter: $\alpha = 90.00$, $\beta = 124.68$, $\gamma = 90.00$

Unit cell volume = 476.29 cm^3

Table-5: Millar indices and interprener distances

Complex	h k l	^{2θ} observed	^{2θ} calculated	d
CuLCl ₂	1 0 1	23.590	23.597	3.7684
	-1 0 2	24.776	24.788	3.5906
	-2 0 2	25.250	25.252	3.5242
	-2 1 2	27.300	27.301	3.2641
	2 2 0	31.497	31.517	2.8381
	3 0 0	35.846	35.852	2.5031

4.1.6. Thermal analysis

From TG analysis, presence of lattice water, coordinated water and breakdown pattern of complexes are evaluated.(table-6)

Table-6 : Thermal decomposition and kinetic parameters of complexes

Complex	DTA peak °C	Temp. range °C	Mass loss (%) Obs. (Cal.)	Nature of decomp.	n	E _a , kJ/mole	ΔS J/k/mole	G KJ/mole	Z
CuLCl ₂	90 300	0-200	19.2 (19.5)	Water & Cl	1.9	16.804	-250.02	30.889	8.54x10 ⁻¹
		200-450	63.2(63.92)	Ligand & Cl	0.5	153.80	-9.902	154.53	3.92x10 ⁻¹²

In complex of Cu (II) two peaks are reflected, one is endothermic and other is exothermic. First endothermic mass loss corresponds to removal of water and chloride around 200°C and remaining part of ligand to be removed above 260°C and decomposition completed at 650°C, leading final product of copper oxide. Amount of copper oxide found in residue is close agreement with calculated.

The kinetic and thermodynamic parameters viz, order of reaction (n), energy of activation (E_a), pre exponential factor (Z) etc. for non- isothermal decomposition of metal complexes were determined by the Metzger-Horowitz approximation method. The data obtained are given in (table-6). The calculated values of the activation energy of the complexes are relatively low, indicating the autocatalytic effect of the metal ion on the thermal decomposition of the complex. The negative activation entropy values suggest that the activated complex were more ordered than the reactants and that the reaction were slow. The more ordered nature may be due to polarization

of bonds in activated state.

4.2. Biological Assay

For in vitro antimicrobial activity, the investigated compounds were tested against the bacteria salmonella typhi, staph aureus, Escherichia coli and Bacillus subtilis. The minimum inhibitory concentration values of compounds against the growth of microorganisms are summarized in table. From this it is observed that nickel and zinc complexes are more active in staphylococcus aureus and Escherichia coli respectively compared to other bacterial organisms. Copper and cobalt complexes are moderately active in all bacterial organisms. Moreover, all complexes are moderately active in above four bacterial organisms compared with standard cefadox and Linazoid.(Table 7)

Table-7: Antibacterial activity of complexes from acetyl acetone

Compound	S-typhi	S-aureo	E-coli	B-subt	Cefodox	Linazoid
CuLCl ₂	8	18	17	8	8	10
CuL(NO ₃) ₂	8	21	24	8	8	8
CuL(CH ₃ COO) ₂	9	19	19	8	8	11
COLCl ₂	11	26	25	8	10	11
COL(NO ₃) ₂	11	25	29	11	12	8
COL(CH ₃ COO) ₂	11	29	28	10	9	9
NiLCl ₂	10	24	32	17	9	12
NiL(NO ₃) ₂	13	26	30	19	10	13
NiL(CH ₃ COO) ₂	12	26	29	18	11	14
ZnLCl ₂	28	30	31	22	10	15
ZnL(NO ₃) ₂	26	27	29	24	12	12
ZnL(CH ₃ COO) ₂	27	26	25	26	11	14

5. Conclusion

The macrocyclic complexes synthesized by [2 + 2] condensation reaction of ethylenediamine with and acetyl acetone in presence of metal ion have been characterized with the help of conductance measurements, electronic, NMR and infrared, EPR, XRD, Thermal Analysis. Based these studies, a six coordinate distorted octahedral geometry has been proposed for all the complexes.

The complexes were tested for their in

vitro antibacterial activity. Some of the complexes showed remarkable antibacterial activity against some selected bacterial strains. It has been suggested that chelation/coordination reduces the polarity of the metal ion mainly because of partial sharing of its positive charge with donor group within the whole chelate ring system. This process of chelation thus increases the lipophilic nature of the central metal atom, which in turn, favours its permeation through the lipid layer of the membrane thus causing the metal complex to cross the bacterial membrane more effectively thus increasing the activity of the complexes. Besides from this many other factors such as solubility, dipole moment, conductivity influenced by metal ion may be possible reasons for remarkable antibacterial activities of these complexes[24-26]. It also has been observed that some moieties such as azomethine linkage or heteroaromatic nucleus introduced into such compounds exhibit extensive biological activities that may be responsible for the increase in hydrophobic character and liposolubility of the molecules in crossing the cell membrane of the microorganism and enhance biological utilization ratio and activity of complexes[27].

6. References

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