

# Synthesis and Efficacy of Divalent Metal Complexes with Ligand Derived from 2,2-Dihydroxyindane-1,3-dione and ethylenediamine against Gram Positive and Gram Negative Bacteria

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**Abstract-**With the rise of pollution and the resistance developed by the microbes towards the pre-existing antimicrobial drugs, there is a demand of new, economical and effective antimicrobial drugs. As an extension of the work, a new series of divalent copper, nickel, iron and cobalt complexes were prepared templately using 2,2-dihydroxyindane-1,3-dione and ethylenediamines. Our own experience and through the literature survey, it is observed that the complexes of these metals have an antimicrobial tendencies. These complexes were found to have a distorted octahedral geometry by elemental analyses, molar conductance, electronic spectra and IR spectral data. The production of  $-C=N$  in the IR spectral data confirms cyclization through dehydration. Oxidation followed by estimation through volumetric analysis confirms the presence of a divalent metal, the molar conductance value indicates them to be non-electrolytes. Two Gram-positive bacteria and two Gram-negative bacteria were used for anti-bacterial study of the synthesized complexes and results were compared with the standard chloramphenicol and streptomycin. The complexes of copper were found to be anti-bacterial.

**Keywords-** 2,2-dihydroxyindane-1,3-dione, magnetic moments, Electronic spectra, efficacy, Octahedral, molar conductance, electronic excitation

## 1. INTRODUCTION

As researchers, we have been synthesizing and checking macrocyclic compounds for their antimicrobial activities<sup>(1-4)</sup>. Macrocyclic compounds and their derivatives are interesting ligand system. A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial and medical applications. Macrocyclic complexes are naturally occurring i.e. metalloproteins, porphyrins and cobalamine can also be synthetically prepared. Synthetic macrocyclic complexes mimic some naturally occurring macrocycles in their properties. Transition metal macrocyclic complexes have received great attention due to their biological activities, including antiviral, anticarcinogenic, antifertile, antibacterial and antifungal. Macrocyclic metal complexes of lanthanides e.g., Gd(III), are used as MRI (magnetic resonance imaging) contrast agents<sup>(6-8)</sup>. In a previous papers, the synthesis and characterization of macrocyclic complexes of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) derived from various diammines and dicarbonyl were reported. It was found that the compounds of Copper were showing anti bacterial activities. In search of better results, this work is being presented with the template synthesis and characterization

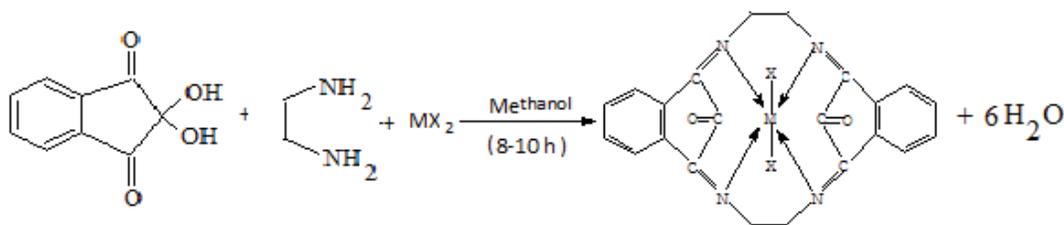
of Co(II), Ni(II), Fe(II), Cu(II), Zn(II) macrocyclic complexes derived from the condensation of 2,2-dihydroxyindane-1,3-dione and ethylenediamines. Complexes were characterized using various physicochemical techniques, such as IR and UV/Vis spectroscopy, elemental analyses and conductivity measurements.

All the synthesized macrocyclic complexes were tested for *In-vitro* antibacterial activity for Two Gram-positive bacteria *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508), two Gram-negative bacteria *Escherichia coli* (MTCC 51) and *Pseudomonas putida* (MTCC 121).

## II. EXPERIMENTAL

All the complexes are synthesized by template method i.e. by condensation of 2,2-dihydroxyindane-1,3-dione and ethylenediamines in the presence of the respective divalent metal salts. To a hot stirring methanolic solution ( $\sim 50 \text{ cm}^3$ ) of 2,2-dihydroxyindane-1,3-dione (10 mmol) was added divalent cobalt, nickel, copper, iron and zinc salts (5 mmol) dissolved in the minimum quantity of methanol. The resulting solution was refluxed for 0.5hs. Ethylenediamine (10 mmol) was added in the refluxing mixture and refluxing was continued for 8-10 h. The mixture was concentrated to half of its original volume, cooled at room temperature and kept in a desiccator for overnight. Dark colored precipitates were formed. These precipitates were filtered, washed with methanol, acetone, diethylether and dried *in vacuo*. 60-70 % yield was obtained in. The complexes are soluble in dimethylformamide and dimethylsulphoxide. They were found thermally stable up to  $\sim 270\text{-}290^\circ\text{C}$  and then decomposed. They do not show any sharp melting point.

The template condensation of 2,2-dihydroxyindane-1,3-dione and ethylenediamine in the presence of divalent metal salts, in the molar ratio 2:2:1 has been shown as follows:



Where M = Co(II), Ni(II), Fe(II), Cu(II), Zn(II)

X =  $\text{Cl}^-$ ,  $\text{Br}^-$ .

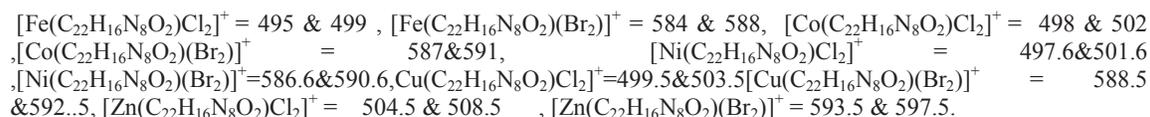
## III. RESULTS AND DISCUSSION

The formula for dark coloured solid macrocyclic complexes was derived on the basis of the analytical data presented in table 1.1 as:  $[\text{M}(\text{C}_{22}\text{H}_{16}\text{N}_8)\text{X}_2]$ ; Where M = Co(II), Ni(II), Fe(II), Cu(II) and Zn(II); X =  $\text{Cl}^-$ ,  $\text{Br}^-$ . The molar conductance value was low ( $10\text{-}17 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ). It was determined in dimethylsulphoxide as solvent indicates their non-electrolyte nature<sup>(4)</sup>. The test for anions was positive only after decomposing the complexes. This indicates that the anions are present inside the coordination sphere and are contributing to the secondary valency of the transition metal.

Data from mass spectroscopy was helpful in determining the molecular mass of the complexes as various attempts for crystallization using mixtures of solvents, low temperature crystallization were unsuccessful to obtain a single crystal suitable for X-ray crystallography. However, the analytical data, spectroscopic and magnetic data also supported us to predict the possible structure of the synthesized complexes.

### Mass Spectra:

The fast atom bombardment mass spectra were recorded using nitrobenzyl alcohol matrix for the synthesized macrocyclic complexes. All the spectra exhibit parent peaks due to molecular ions  $[\text{M}]^+$ . The molecular ion  $[\text{M}]^+$  &  $[\text{M}+4]^+$  peaks obtained for various complexes are as follows:



The proposed molecular formula of these complexes was confirmed by comparing their molecular formula weights with  $m/z$  values. The data is in good agreement with the proposed molecular formula for these complexes i.e.  $[\text{M}(\text{C}_{22}\text{H}_{16}\text{N}_8\text{O}_2)\text{X}_2]$ . This confirms the formation of the macrocyclic frame. In addition to the peaks due to the molecular ions, the spectra exhibit peaks assignable to various fragments arising from the thermal cleavage of the complexes.  $[\text{M}+4]^+$  peaks confirm the presence of two halogen atoms (Cl/Br) in the compound. The peak intensity gives an idea of the stability of the fragments.

#### *Infrared Spectra:*

In the infrared spectra of all the metal complexes, appearance of a strong band at  $\sim 1720 \text{ cm}^{-1}$  may be assigned due to carbonyl  $>\text{C}=\text{O}$  group which was formed by dehydration of geminal hydroxyl groups due to heating. It was noted that a pair of bands at  $\sim 3500$  and  $3400 \text{ cm}^{-1}$  corresponding to free asymmetric and symmetric N-H stretching modes of  $-\text{NH}_2$  group present in infrared spectra of ethylenediamine were absent in the infrared spectra of all the metal complexes. There were bands present in the range  $\sim 1350\text{-}1000 \text{ cm}^{-1}$  in all the complexes were assigned to  $\nu(\text{C-N})$  stretching vibrations. A strong absorption band was seen in the region  $\sim 1595\text{-}1640 \text{ cm}^{-1}$  which may be attributed to the  $\nu(\text{C=N})$  group<sup>(9,10)</sup>. The lower values of  $\nu(\text{C=N})$  stretching frequency is due to the drift of lone pair density of azomethine nitrogen towards the metal atom. These results provided strong evidence for the formation of macrocyclic frame, probably due to the condensation of  $-\text{NH}_2$  groups of ethylenediamine with  $>\text{C}=\text{O}$  group of ninhydrin. The presence of stretching bands at  $\sim 1462$  and  $\sim 1500 \text{ cm}^{-1}$  may be assigned to  $>\text{C}=\text{C}<$  skeleton of benzenoid rings. The spectra exhibit a ring breathing mode at  $\sim 990 \text{ cm}^{-1}$ , an out-of-plane  $\nu(\text{C-H})$  deformation at  $\sim 810 \text{ cm}^{-1}$ , an in-plane  $\nu(\text{C-C})$  deformation at  $\sim 620 \text{ cm}^{-1}$  and out-of-plane deformation at  $\sim 410 \text{ cm}^{-1}$ , respectively<sup>(21)</sup>. The far infrared spectra showed bands in the region  $\sim 420\text{-}470 \text{ cm}^{-1}$  corresponding to  $\nu(\text{M-N})$  vibrations<sup>(11)</sup>. The presences of these bands in all complexes give an idea about the coordination of azomethine nitrogen to the central metal atom<sup>(11)</sup>. The bands present at  $\sim 290\text{-}310 \text{ cm}^{-1}$  may be assigned as being due to  $\nu(\text{M-Cl})$  vibrations<sup>(11)</sup>.

*<sup>1</sup>H NMR Spectra:* These complexes did not form a clear solution hence NMR could not be obtained.

#### *Magnetic measurements and electronic spectra:*

**Iron (II) Complexes:** The effective magnetic moments of iron complexes were found to be in the range of 5.1-5.5 B.M., owing to the spins of the four unpaired electrons and a small, second order orbital contribution in a high spin  $d^6$  system. The values were consistent with the spin free octahedral nature of these complexes<sup>(12)</sup>. The electronic spectra of the iron complexes showed a double peak at  $\sim 10,800 \text{ cm}^{-1}$  and a shoulder at  $\sim 8,350 \text{ cm}^{-1}$  with a number of very small peaks at  $\sim 20,000 \text{ cm}^{-1}$ . The double peak may be due to  ${}^5\text{T}_{2g} \rightarrow {}^5\text{E}_g$  transition split by  $2,000 \text{ cm}^{-1}$  owing to Jahn-Teller distortion which derives from a  ${}^3\text{T}_{2g} \rightarrow {}^3\text{E}_g$  configuration. The small peaks were assigned to spin-forbidden bands. The assignments were based on approximate  $\text{D}_{4h}$  symmetry of the complexes, since the spectra were consistent with the lower symmetry of these complexes<sup>(13)</sup>. The energy level sequence for iron(II) complexes may be drawn as:  ${}^5\text{B}_{2g} < {}^5\text{E}_g < {}^5\text{A}_{1g} < {}^5\text{B}_{1g}$ <sup>(14)</sup>. The spectra resemble to those reported for six coordinate distorted octahedral iron(II) complexes<sup>(13,15,16)</sup>.

**Cobalt(II) complexes:** The magnetic moments of the cobalt complexes were measured at room temperature and were found in the range of 4.90-4.95 B.M., which corresponds to three unpaired electrons<sup>(89,256)</sup>. The electronic spectra of the cobalt(II) complexes recorded in DMSO, resemble to those complexes reported to be distorted octahedral<sup>(13)</sup>. These spectra exhibits three absorption peaks in the region  $8,150\text{-}9,280 \text{ cm}^{-1}$  ( $\nu_1$ ),  $13,500\text{-}15,550 \text{ cm}^{-1}$  ( $\nu_2$ ) and  $18,500\text{-}20,500 \text{ cm}^{-1}$  ( $\nu_3$ ), respectively. Thus, assuming the effective symmetry to be  $\text{D}_{4h}$ , the various bands may be assigned to the transitions:  ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}$  (F), ( $\nu_1$ );  ${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}$  (F), ( $\nu_2$ );  ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{1g}$  (P), ( $\nu_3$ ), respectively. The assignment of the first spin-allowed band seems plausible since the first band appears approximately at half the energy of the visible<sup>(13)</sup>. Various bands do not follow any regular pattern and seem to be anion independent. Various ligand field parameters,  $\text{Dq}$ ,  $\text{B}$ ,  $\beta$ , and  $\beta\%$  were calculated for the complexes and has been listed in the Table. The value of  $\text{B}$  is  $971 \text{ cm}^{-1}$  for free cobalt (II) ion. The Nephelauxetic parameter  $\beta$  was calculated for the

complexes and the values of  $\beta$  lie in the range of 0.52-0.56. These values indicate the presence of covalent character of the metal ligand 'σ' bond. The values of the  $v_2/v_1$  ratio lie between 1.66-1.73 and suggest the octahedral structure for these complexes<sup>(15)</sup>.

**Nickel(II) Complexes:** The magnetic moments of the nickel complexes at room temperature were found in the range of 2.94-3.10 B.M. These values indicate a high spin configuration and showed the presence of two unpaired electrons in the Ni(II) ion in all complexes<sup>(15,18)</sup>. The spectra of Ni(II) complexes recorded in DMSO solution exhibit a well discerned band with a shoulder on the low energy side. The other two bands generally observed in the region 16,500-17,200  $\text{cm}^{-1}$  ( $v_2$ ), and 27,800-28,300  $\text{cm}^{-1}$  ( $v_3$ ), were assigned to  ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$  ( $v_2$ ), and  ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$  ( $v_3$ ), respectively. The first two bands result from the splitting of one band,  $v_1$  and were in the range of  $\sim 9,650$ - $10,200$  and  $11,850$ - $12,350$   $\text{cm}^{-1}$ , which may be assigned to  ${}^3B_{1g} \rightarrow {}^3E_g$  and  ${}^3B_{1g} \rightarrow {}^3B_{2g}$ , assuming the effective symmetry to be  $D_{4h}$  (component of  ${}^3T_{2g}$  in  $O_h$  symmetry)<sup>(13)</sup>. The intense higher energy bands at  $34,540$   $\text{cm}^{-1}$  may be due to a  $\pi\text{-}\pi^*$  transition of the (C=N) group. Various ligand field parameters, Dq, B,  $\beta$  and  $\beta\%$  were calculated for the complexes, and have been listed in the Table. The value of B for free nickel(II) ion is  $1040$   $\text{cm}^{-1}$ . The Nephelauxetic parameter  $\beta$  was calculated for the complexes and the values of  $\beta$  lie in the range of 0.58-0.62. These values indicate that the presence of covalent character of the metal ligand 'σ' bond. The values of the  $v_2/v_1$  ratio lie between 1.37-1.42 and suggest the complexes as octahedral structure geometry<sup>(17)</sup>.

**Copper(II) Complexes:** The magnetic moments of copper(II) complexes was found in the range of 1.76-1.80 B.M. The electronic spectra of the Cu(II) complexes exhibit bands in the region  $17,750$ - $19,500$   $\text{cm}^{-1}$ , with a shoulder on the low energy side at  $\sim 14,500$ - $16,000$   $\text{cm}^{-1}$ , and showed that these complexes were distorted octahedral<sup>(15)</sup>. 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 may be assigned to:  $z^2 \rightarrow x^2-y^2$  ( ${}^2B_{1g} \rightarrow {}^2B_{2g}$ ) and the broad band contains both the  $xy \rightarrow x^2-y^2$  ( ${}^2B_{1g} \rightarrow {}^2E_g$ ) and  $xy, yz \rightarrow x^2-y^2$  ( ${}^2B_{1g} \rightarrow {}^2A_{2g}$ ) transition<sup>(19)</sup>. The band separation of the spectra of the complexes was of the order of  $2,500$   $\text{cm}^{-1}$ , which was consistent with proposed geometry of these complexes<sup>(19)</sup>. Therefore, it may be concluded that all the complexes formed by macrocycles with Cu(II) metals were distorted octahedral.

**Zinc(II) Complexes:** Zinc are considered to have no d-d transition as the electronic configuration of  $\text{Zn}^{+2}$  ion is  $3d^{10}, 4s^0$ . So no electronic spectra are available for zinc complexes.

#### EPR Spectra:

The EPR spectra of the copper(II) complexes were recorded at room temperature in solid state, on X-band, at frequency of 9.5 GHz under the magnetic field of 3000 G. The spectrum of the complex exhibits a single anisotropic broad signal. The analysis of the spectra gives  $g_{\parallel}$  values in the range 2.2125-2.2308- and  $g_{\perp}$  values in the range 2.1625-2.1850 (Table-2.14). The trend  $g_{\parallel} > g_{\perp} > 2.0023$  ( $g_e$ ), observed for the complex indicate that the unpaired electron is localized in  $d_{x^2-y^2}$  orbital of the copper(II) ion, this suggests distortion in the copper(II) complexes from  $O_h$  symmetry to  $D_{4h}$  symmetry. The observed  $g_{\parallel}$  value for the copper(II) complexes is less than 2.3 which is in agreement with the covalent character of the metal ligand bond.

#### IV. ANTIMICROBIAL ACTIVITIES OF DIVALENT COBALT, NICKEL, COPPER, ZINC AND CADMIUM COMPLEXES DERIVED FROM ETHYLENEDIAMINE AND NINHYDRIN.

Two Gram-positive bacteria *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508), two Gram-negative bacteria *Escherichia coli* (MTCC 51) and *Pseudomonas putida* (MTCC 121) were used for biological study of the synthesized complexes formed by the condensation of 2,2-dihydroxyindane-1,3-dione and ethylenediamine in the presence of the respective divalent metal salts. The complexes of copper show a comparable amount of antibacterial activities for *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508), one Gram-negative bacteria *Pseudomonas putida* (MTCC 121). The complexes of copper, cobalt and nickel as chlorides show a comparable amount of antibacterial activities for *Bacillus stearothermophilus* (MTCC 8508) only.

#### In-vitro antibacterial activity

**Primary screening:** The antibacterial activities of the newly synthesized complexes were evaluated by Agar Well Diffusion Assay technique<sup>[283]</sup> against two Gram-negative bacteria: *Escherichia coli* (MTCC 51) and *Pseudomonas*

*putida* (MTCC 121) and two Gram- positive bacteria: *Bacillus subtilis* (MTCC 8509) and *Bacillus stearothermophilus* (MTCC 8508). The bacterial cultures were maintained on the nutrient agar media by sub-culturing them on the fresh slants after every 4-6 weeks and incubating them at the appropriate temperature for 24 h. All stock cultures were stored at 4°C. For the evaluation of antimicrobial activities of the synthesized compounds, suspension of each test microorganism was prepared. Turbidity of each suspension was adjusted to 0.5 McFarland units by suspending the cultures in sterile distilled water. The size of final inoculum was adjusted to  $5 \times 10^7$  cfu/ml. A volume of 20 ml of agar media was poured into each Petri plate and plates were swabbed with broth cultures of the respective micro-organisms and kept for 15 minutes for adsorption to take place. Wells of  $\approx 8$  mm diameter were punched in the seeded agar plates and a 100  $\mu$ l volume of each test compound reconstituted in DMSO was added into the wells. DMSO was used as control for all the test compounds. To allow diffusion of the compounds in to the agar, the plates were held at room temperature for 2 h. After that the plates were incubated at 37°C for 24 h. Antibacterial activities were determined by measuring the inhibition zone diameter. The entire tests were made in triplicates and mean of the diameter of inhibition was calculated.

**Determination of minimum inhibitory concentration (MIC):** The minimum inhibitory concentration (MIC) is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after overnight incubation.<sup>[21]</sup> Nutrient broth was adjusted to pH 7.0 used for the determination of MIC. The inoculum of the test microorganisms were prepared by using 16 h old cultures adjusted by reference to the 0.5 McFarland standards ( $10^8$  cells/ml).<sup>[22]</sup> These cultures were further diluted up to 10 folds with nutrient broth to get the inoculum size of  $1.2 \times 10^7$  cfu/ml. A positive control (containing inoculum but no compound) and a negative control (containing compound but no inoculum) were also prepared. A stock solution of 4 mg/ml of each compound was prepared in DMSO and further appropriately diluted to get final concentration ranging from 250 to 0.03  $\mu$ g/ml. Separate flasks were taken for each test dilution. To each flask was added the 100  $\mu$ l of inoculum. Then appropriately diluted test sample was added to each flask having broth and microbial inoculum. The contents of the flask were mixed and incubated for 24 to 48 h at 37°C. The test bacterial culture were spotted in a predefined pattern by aseptically transferring 5  $\mu$ l of each bacterial culture on the surface of solidified agar plates and incubated at 37°C for 24 h for determining the MIC values.

Table-1: Analytical data of divalent cobalt, nickel, copper, zinc and cadmium complexes derived from Ethylenediamine and Ninhydrin.

Sr. No.	Complexes	Found (Calcd.) %				Colour	Mol. Wt.
		M	C	H	N		
(1)	[Fe(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	11.1(11.31)	53.2(53.3)	3.09(3.23)	11.2(11.31)	Blackish grey	495
(2)	[Fe(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	95.5(95.89)	45.0(45.2)	2.60(2.73)	9.5(9.58)	Black	584
(3)	[Co(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	11.5(11.8)	52.9(53.1)	3.1(3.21)	11.2(11.4)	Dark grey	498
(4)	[Co(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	09.4(09.65)	44.7(44.9)	2.6(2.72)	9.5(9.54)	Black	587
(5)	[Ni(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	11.6(11.77)	52.9(53.05)	1.1(1.20)	11.0(11.2)	Black	497.6
(6)	[Ni(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	09.7(09.89)	44.9(45.00)	2.6(2.72)	9.3(9.54)	Black	586.6
(7)	[Cu(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	12.3(12.50)	52.5(52.8)	3.1(3.20)	11.0(11.2)	Dark grey	499.5
(8)	[Cu(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	10.7(10.79)	44.6(44.85)	2.6(2.72)	9.4(9.51)	Dark grey	588.5
(10)	[Zn(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	12.94(12.98)	52.0(52.3)	3.1(3.17)	10.9(11.1)	Brown	504.5
(11)	[Zn(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	10.9(11.03)	44.2(44.4)	2.6(2.69)	9.3(9.43)	greyish	593.5

Table-2: Ligand field parameters of the divalent cobalt and nickel complexes derived from Ethylenediamine and Ninhydrin.

Sr. No.	Complexes	Dq (cm <sup>-1</sup> )	B' (cm <sup>-1</sup> )	$\beta$	$\beta$ %	$\nu_2/\nu_1$	$\mu_{\text{eff}}$ (B.M.)
(1)	[Fe(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	245	916	0.872	77.2	1.293	5.1
(2)	[Fe(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	261	955	0.909	90.9	1.311	5.3
(3)	[Co(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	519	582	0.599	59.9	1.721	4.9
(4)	[Co(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	520	417	0.429	42.9	1.571	4.4
(5)	[Ni(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	698	1023	0.983	98.3	1.710	2.94
(6)	[Ni(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	709	1030	0.990	99.0	1.715	3.0
(7)	[Cu(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	290	2207	0.883	88.3	1.192	1.76
(8)	[Cu(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	275	2250	0.9	99.0	1.177	1.80

Table-3: Minimum inhibitory concentration (MIC) of divalent metal complexes derived from Ninhydrin and Ethylenediamine against test bacteria by using agar dilution assay.

S. N.	Complexes	MIC ( $\mu\text{g/ml}$ )			
		a	b	c	d
(1)	[Fe(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	>64	>64	30	>64
(2)	[Fe(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	>64	>64	32	64
(3)	[Co(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	32	32	>64	32
(4)	[Co(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	16	02	>64	>64
(5)	[Ni(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	16	08	32	16
(6)	[Ni(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	32	04	16	32
(7)	[Cu(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Cl <sub>2</sub> ]	04	08	04	08
(8)	[Cu(C <sub>22</sub> H <sub>16</sub> N <sub>8</sub> O <sub>2</sub> )Br <sub>2</sub> ]	08	16	32	16

(9)	$[\text{Zn}(\text{C}_{22}\text{H}_{16}\text{N}_8\text{O}_2)\text{Cl}_2]$	02	04	32	04
(10)	$[\text{Zn}(\text{C}_{22}\text{H}_{16}\text{N}_8\text{O}_2)\text{Br}_2]$	04	02	16	32
	<b>Chloramphenicol</b>	02	02	04	02
	<b>Streptomycin</b>	02	02	04	04

*a-Bacillus subtilis* (MTCC 8509)

*b-Bacillus stearothermophilus* (MTCC 8508)

*c- Pseudomonas putida* (MTCC 121)

*d- Escherichia coli* (MTCC 51)

*Chloramphenicol, Streptomycin* – Standard antibiotics

#### V.PROPOSED STRUCTURE

Therefore, based on various studies like elemental analyses, conductance measurements, magnetic susceptibilities, infrared, NMR, electronic and mass spectral studies a distorted octahedral geometry as shown in Fig.-1 may be proposed for all the divalent macrocyclic metal complexes.

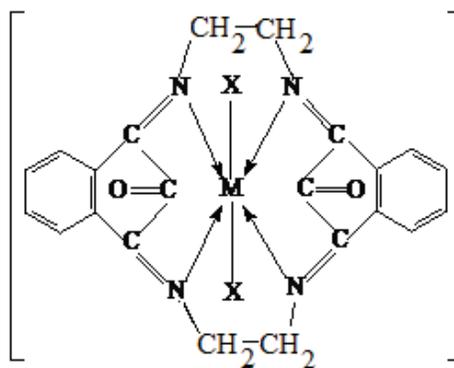


Fig-1

Where M = Fe(II), Co(II), Ni(II), Cu(II), Zn(II)

X = Cl<sup>-</sup>, Br

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