Antibacterial and spectroscopic studies of   biologically active macrocyclic complexes of divalent metal ions

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ABSTRACT

The template methodology is frequently used for the synthesis of macrocyclic complexes as the metal ions are supposed to direct the stearic course of reaction towards the condensation reaction rather than polymerization. In continuation of our efforts ,we wish to report here the synthesis and characterization of macrocyclic complexes by template condensation reaction of Oxalyldihydrazide and Isatin resulting into the formation of the macrocyclic complexes of the type: $[M(C_{20}H_{14}N_{10}O_4)X_2]$; where M=Co(II), Ni(II), Cu(II), Zn(II) and X=Cl, NO_3, CH_3COO. The synthesized complexes have been characterized with the aid of elemental analysis, conductance measurements, magnetic susceptibility measurements, electronic, infrared, EPR and NMR spectral studies. The molar conductance measurements of the complexes in DMSO correspond to non electrolytes. On the basis of IR, Electronic and EPR spectral studies a distorted octahedral geometry has be assigned to these complexes. The complexes were also evaluated against the growth of bacteria in vitro.

Keywords: Macrocyclic complexes; infrared; NMR; mass spectra; Antibacterial activities.
Abbreviations*: B.M., Bohr Magneton; CFU, colony forming unit; DMF, N,N-dimethylformamide; DMSO, dimethylsulphoxide; IR infrared; ESR, Electronic Spin Resonance; NMR; Nuclear Magnetic Resonance MIC, Minimum Inhibitory Concentration; MHB Mueller Hinton Broth*

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INTRODUCTION

Macrocyclic complexes are thermodynamically more stable and more selective metal ion chelates than open chain analogue so the study of macrocyclic complexes is a growing class of research[1]-[3] Synthetic macrocyclic ligands are best designed with help of metal ions as template to direct the condensation reaction toward the ring closure [4]. Transition metal complexes of nitrogen donor ligands have been studied in detail on account of their wide practical utility [5] and a number of nitrogen donor macrocyclic derivatives have long been used in analytical industrial and have medical applications [6]. Macrocyclic Schiff base metal complexes are of great importance due to their resemblance to many natural systems such as porphyrins, cobalamines [7]. Macrocyclic nickel complexes find use in DNA recognition and oxidation while the macrocyclic copper complexes find use in DNA binding and cleavage[8]- [9]. Macrocyclic metal complexes are also well known for their use as dyes and pigments as well as NMR shift reagents [10]. Schiff base Cu (II) complexes with Isatin Ligand are potential anti-tumour agents [11]. Prompted by these, in the present paper, synthesis and characterization of cobalt (II), nickel (II), copper (II), zinc (II), macrocyclic complexes derived from Oxalyldihydrazide and isatin have been discussed. The synthesized complexes have been characterized with the aid of elemental analysis, conductance measurements, magnetic susceptibility measurements, electronic, IR and NMR studies.

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MATERIALS AND METHODS

2.1 Reagents

All the chemicals and solvents used in this study were of Analytical grade. Oxalyldihydrazide, isatin and metal salts were purchased from S.D fine, Merck, Ranbaxy and were used as received.

2.2 Analytical and physical Measurements

Microanalysis (C, H, and N) were estimated by an elemental analyzer (Perkin Elmer 2400) at CDRI lucknow. Magnetic susceptibility measurements were carried out at SAIF, IIT Roorkee, on a vibrating sample magnetometer (MODEL PAR 155). IR spectra were recorded on FT-IR spectrophotometer (PerkinElmer) from 4000 to 200cm^{-1} using Nujol mull /KBr pellets. ^{1}H-NMR spectra (at room temp in DMSO-d6) were recorded on a Bruker AVANCE II 400 NMR spectrometer (400 MHz), Me$_4$Si reference (0.0ppm) at SAIF Punjab University Chandigarh. Electronic Spectra (in DMSO) were recorded on a Hitachi 330 spectrophotometer (850 -200 mm) at room temperature. The EI mass spectra (at room temperature) were recorded on TOF ME ES + mass spectrometer. The metal content in the complexes was determined by literature methods [12]. Molar conductivities were measured on a digital conductivity meter (HPG system, G-3001). Melting points were determined using capillaries in an electrical melting point apparatus.

2.3 Isolation of Complexes

All the complexes were synthesized by the template method i.e., by condensation of Oxalyldihydrazide and isatin in the presence of the respective divalent metal salts. To a hot stirring methanol solution (~50 ml) of Oxalyldihydrazide (5m mole) was added divalent nickel, cobalt, copper and zinc salt (2.5mmol) dissolved in the minimum quantity of methanol (~20 ml). The resulting solution was refluxed for 0.5 h. and after that isatin (5mmol) was added in the refluxing mixture and refluxing was continued for 8-10hr. The mixture was concentrated to half of its volume and kept in desiccators overnight. On overnight cooling, orange colour precipitates formed, which were filtered, washed with methanol, acetone and diethyl ether and dried in a vacuum, yield ~ 41-65% was obtained. The complexes were soluble in DMF and DMSO. They were found to be thermally stable in the temperature range 160-285°C above which they got decomposed.

The template condensation of Oxalyldihydrazide and isatin in the presence of divalent metal salts in the molar rates 2:2:1 is represented by the following **Figure 1**
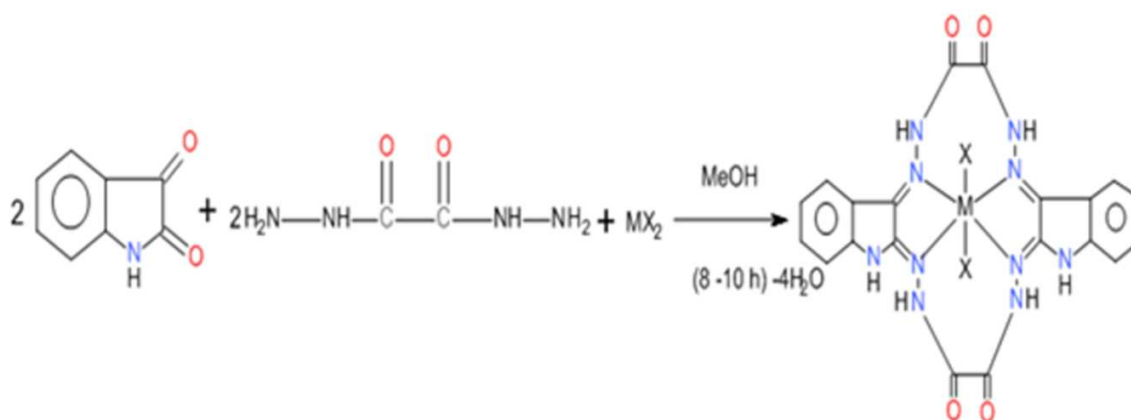
Where M = Co (II), Ni (II), Cu (II), Zn (II) ; X = Cl⁻, NO₃⁻, CH₃COO⁻

Figure 1 Scheme for synthesis of Complexes derived from Oxalyldihydrazide and Isatin with divalent cobalt nickel copper and zinc metal salts

3.0 Biological assay

3.1 Test microorganisms

Four bacterial strains were selected on the basis of their clinical importance in causing diseases in humans. *Agrobacterium tumifaciens* (MTCC 431), *Bacillus subtilis* (MTCC 8509), *Escherichia coli* (MTCC 51) and - *Pseudomonas putida* (MTCC 121)   were screened for antibacterial activity of the compounds.

3.2 *In vitro* Antibacterial Activity

3.2.1 Primary screening.

The bacterial activities were evaluated by agar well diffusion method [13]. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately 1.5×10^8 CFUmL^{-1} [14]. Twenty milliliters of Mueller Hinton agar

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media were poured into each Petri plate and plates were swabbed with 100 μL inoculate of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8-mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μL volume of 4 mg mL^{-1} of each compound reconstituted in DMSO. All the plates were incubated at 37°C for 24h. Antimicrobial activity was evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (Hi Antibiotic zone scale). The medium with DMSO as solvent was used as a negative control, whereas Streptomycin and Chloramphenicol was used as positive control. The experiments were performed in triplicate.

3.2.2 Determination of Minimum Inhibitory Concentration of the complexes

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of microorganisms after overnight incubation. The MICs of the macrocyclic complexes were tested against bacterial strains through a macro dilution tube method [**14**]. In this method, the test concentration of complexes was made from 128 to 0.25 μg mL^{-1} in the sterile tubes No. 1-10. Mueller Hinton Broth (MHB) medium was prepared and 100 μL sterile MHB medium was poured in each sterile tube, followed by addition of 200 μL of the complex in tube 1. Two fold serial dilutions were carried out from tubes 1-10 and excess broth (100 μL) was discarded from the last tube, no.10. To each tube, 100 μL of standard inoculums (1.5×10^{8} CFU/mL) was added. Streptomycin and Chloramphenicol (antibacterial drug) was used as control. All the tubes were incubated for 24 h at 37°C.

RESULT AND DISCUSSION

The analytical data show the formula for macrocyclic complexes as: [M (C$_{20}$H$_{14}$N$_{10}$O$_{4}$) X$_{2}$] where M= Co (II), Ni (II), Cu (II), Zn (II) and X= Cl^{-}, NO$_{3}$$^{-}$ and CH$_{3}$COO^{-}. The low value of conductance of all complexes in DMSO indicated them to a non-electrolytic in nature [**15**] and the test for anion are positive only after decomposing the metal complexes with conc. HNO$_{3}$ indicating their presence inside the coordination sphere. The analytical, spectroscopic and magnetic data enable us to predict the possible structure of the synthesized complexes. All the macrocyclic complexes are dark colored solids and are soluble in DMF or DMSO. All complexes give satisfactory elemental analyses as shown in **Table 1.**

Table 1 Analytical data of the synthesized Co (II), Ni (II), Cu (II) and Zn (II) complexes derived from Oxalyldihydrazide and Isatin

Sr. No	Complexes	Colour	μ$_{eff.}$ B.M.	%M Found (Calcd)	%C Found (Calcd)	%H Found (Calcd)	%N Found (Calcd)	^M
1	[Co(C$_{20}$H$_{14}$N$_{10}$O$_{4}$) Cl$_{2}$]	Orange	4.75	10.00(10.02)	40.81(40.82)	2.32(2.38)	23.81(23.81)	45
2	[Co(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(NO$_{3}$)$_{2}$]	Orange	4.77	9.11(9.19)	37.44(37.44)	2.17(2.18)	21.84(21.84)	44
3	[Co(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(OAc)$_{2}$]	Dark brown	5.01	9.23(9.28)	37.70(37.79)	2.16(2.20)	22.01(22.04)	46
4	[Ni(C$_{20}$H$_{14}$N$_{10}$O$_{4}$) Cl$_{2}$]	Orange	2.99	9.90(9.98)	40.80(40.83)	2.32(2.38)	22.81(23.82)	15
5	[Ni(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(NO$_{3}$)$_{2}$]	Brown	2.92	9.14(9.16)	37.71(37.71)	2.16(2.18)	21.82(21.84)	26
6	[Ni(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(OAc)$_{2}$]	Dark Brown	2.94	9.14(9.24)	37.76(37.79)	2.21(2.20)	22.05(22.05)	36
7	[Cu(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)Cl$_{2}$]	Orange	2.02	10.66(10.72)	51.14(51.18)	2.32(2.36)	23.61(23.62)	15
8	[Cu(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(NO$_{3}$)$_{2}$]	Brown	1.78	9.78(9.8)	37.12(37.15)	2.12(2.16)	21.61(21.68)	28
9	[Cu(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(OAc)$_{2}$]	Brown	2.01	9.69(9.93)	37.49(37.50)	2.16(2.18)	21.79(21.80)	40
10.	[Zn(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(OAc)$_{2}$]	Dark Brown	-	9.70(9.91)	37.50(37.53)	2.16(2.18)	21.86(21.89)	35

4.1 Infrared Spectra

The presence of a single medium band in the region 3250-3350 cm^{-1} in the infrared spectra of all the complexes may be assigned due to N –H stretch [**16**]-[**17**]. It was noted that a pair of bands corresponding to ν(NH$_{2}$) at 3250 cm^{-1} and 3300 cm^{-1} were present in the spectrum of Oxalyldihydrazide but were absent in the infrared spectra of all the complexes. Further no strong absorption band was observed near 1700 cm^{-1} indicating the absence of > C=0 ground of isatin moiety. The disappearance of these bands and appearance of absorption band near 1590-1610 cm^{-1} confirms the condensation of carbonyl group of isatin and amino group of Oxalyldihydrazide and formation of macrocyclic Schiff base [**18**]as these band may be assigned due to ν (C= N) [**19**] azomethine nitrogen. The lower value of ν(C= N) may be explained on the basis of drift of lone pair density of azomethine nitrogen towards the metal atoms [**20**] indicating that coordination take place through nitrogen of C=N groups. The band present in the range 1680-1699 cm^{-1} may be assigned due to > C=O group of CONH moiety [**21**] in all the complexes. This indicates that coordination to metal atom through oxygen of carbonyl group is ruled out. The various bands present in the range ~1300-1000cm^{-1} in all the complexes are assigned due to ν(C-N) stretch. The **Far infrared** Spectra show bands in the region ~420-450 cm^{-1} corresponding to ν(M-N) vibration.[**22**] The presence of bands in the region 420-450 cm^{-1} in all the complexes originate from (M-N) azomethine vibration modes and indicate the coordination of azomethine nitrogen [**23**]The bands present in the region 220-240cm^{-1} in all the nitrato complexes are assignable to ν(M-O) [24] The bands present at 300-250cm^{-1} may be assigned due to ν(M-Cl) vibration.[**25**]

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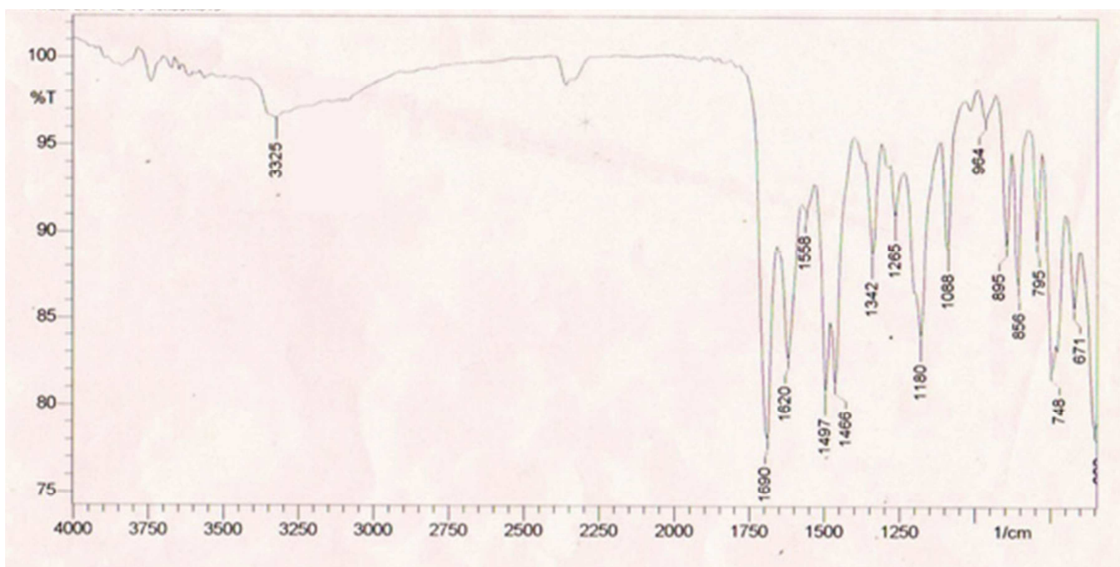
Figure 2 IR Spectra of [Ni (C$_{20}$H$_{14}$N$_{10}$O$_{4}$) Cl$_{2}$]
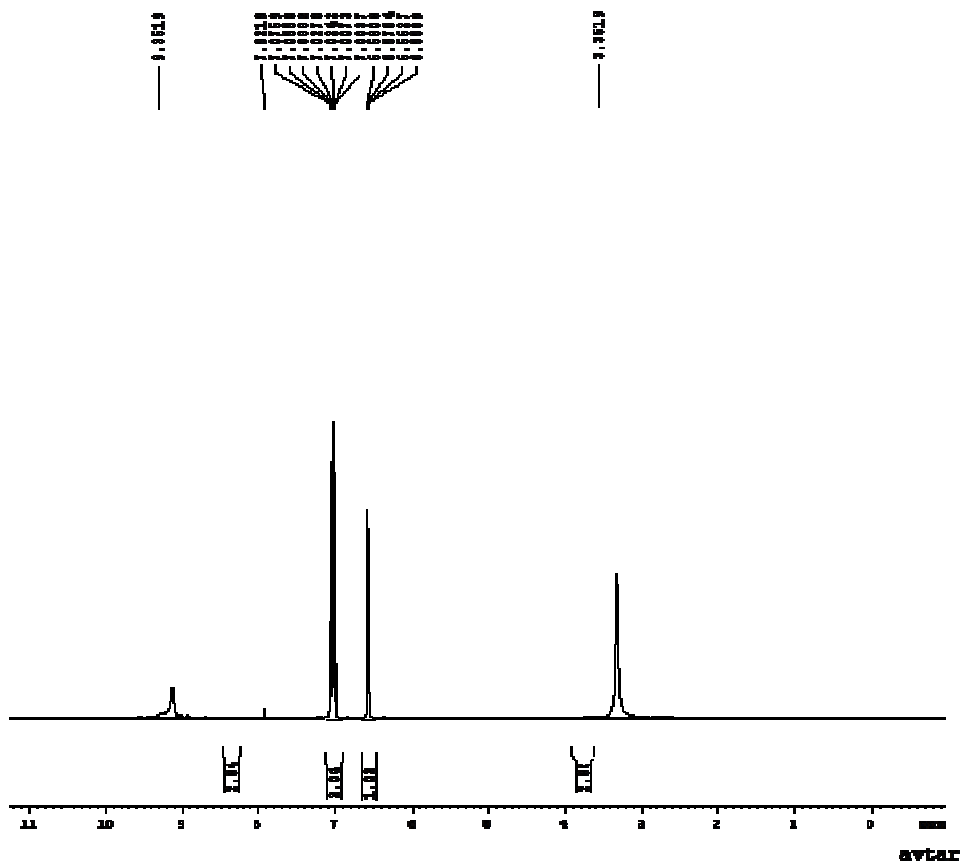
Figure 2 NMR Spectra of [Zn(C$_{20}$H$_{14}$N$_{10}$O$_{4}$)(OAc)$_{2}$]

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4.2 ^{1}H NMR Spectra

^{1}H NMR spectra of the macrocyclic complex [Zn $(C_{20}H_{14}N_{10}O_4)$ (OAc) $_2$] shows multiplets in the range 6.55-7.97 ppm corresponding to aromatic ring protons of isatin ring moiety. A broad singlet appears at 3.326 ppm corresponding to NH protons (2H) of isatin. The broad signal appears at 9.323 ppm which may be assigned due to amide protons as shown in figure 3.

4.3 Mass Spectra

The EI mass spectra of Co (II), Cu (II), Ni (II), Zn (II) macrocyclic complexes have been recorded. The spectra exhibit parent peaks due to the molecular ion [M] $^+$ and [M+2] $^+$. The proposed molecular formulas of these complexes were confirmed by comparing their molecular weights with m/z values. The molecular ion [M]$^+$ peaks obtained for various complexes have been shown in Table 2. The data were in good agreement with the proposed molecular formula for the complexes i.e. [M $(C_{20}H_{14}N_{10}O_4)$ X_2]. This indicates the formation of the macrocyclic frame. In addition to molecular ion peaks, the spectra exhibit other peaks assign able to various fragments arising from the thermal cleavage of the complexes shown in **Table 2.**

Table 2 Mass Spectra Data of   Co (II), Ni (II), Cu (II) and Zn (II) complexes derived from Oxalyldihydrazide and Isatin

Compounds No.	Molecular ion peak [M]$^+$ and [M+2]$^+$ at m/z	Important peaks due to complex fragmentation
1	[M]$^+$=586.9(^{35}Cl) [M+2]$^+$=588.9(^{37}Cl)	[Co$(C_{20}H_{14}N_{10}O_4)$Cl]$^+$=551.43;[Co$(C_{20}H_{14}N_{10}O_4)$-H]$^+$=515.71; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
2	[M]$^+$=639.9	[Co$(C_{20}H_{14}N_{10}O_4)$NO$_3$]$^+$=577.91;[Co$(C_{20}H_{14}N_{10}O_4)$-H]$^+$=515.91;[$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
3	[M]$^+$=633.9	[Co$(C_{20}H_{14}N_{10}O_4)$ OAc]$^+$=575.9;[Co$(C_{20}H_{14}N_{10}O_4)$-H]$^+$=515.9; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
4	[M]$^+$=586.7(^{35}Cl) [M+2]$^+$=588.7(^{37}Cl)	[Ni$(C_{20}H_{14}N_{10}O_4)$Cl]$^+$=551.21;[Ni$(C_{20}H_{14}N_{10}O_4)$-H]$^+$=515.71; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
5	[M]$^+$=639.7	[Ni$(C_{20}H_{14}N_{10}O_4)$NO$_3$]$^+$=577.71;[Ni$(C_{20}H_{14}N_{10}O_4)$H]$^+$=515.71; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
6	[M]$^+$=633.7	[Ni$(C_{20}H_{14}N_{10}O_4)$ OAc]$^+$=575.7;[Ni$(C_{20}H_{14}N_{10}O_4)$-H]$^+$=515.7; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
7	[M]$^+$=591.5(^{35}Cl) [M+2]$^+$=593.5(^{37}Cl)	[Cu$(C_{20}H_{14}N_{10}O_4)$Cl]$^+$= 556.5;[Cu$(C_{20}H_{14}N_{10}O_4)$-H]$^+$= 521.5; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
8	[M]$^+$=644.5	[Cu$(C_{20}H_{14}N_{10}O_4)$NO$_3$]$^+$=582.5;[Cu$(C_{20}H_{14}N_{10}O_4)$H]$^+$=520.5; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
9	[M]$^+$=638.9	[Cu$(C_{20}H_{14}N_{10}O_4)$ OAc]$^+$=579.5;[Cu$(C_{20}H_{14}N_{10}O_4)$-H]$^+$=520.5; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456
10	[M]$^+$=638.3	[Zn$(C_{20}H_{14}N_{10}O_4)$OAc]$^+$=580.3;[Zn$(C_{20}H_{14}N_{10}O_4)$H]$^+$=520.3; [$C_{20}H_{14}N_{10}O_4$]$^+$=457; [$(C_{20}H_{14}N_{10}O_4)$-2H]$^+$=456

4.4 Magnetic Measurements and Electronic Spectra:

The magnetic moments of nickel complexes at room temperature were 2.94-2.99 B.M. These values are Consistent with two unpaired electrons [26] and show the presence of an octahedral environment around the Ni (II) ion in all complexes. The spectra of Ni (II) complexes in DMSO solution exhibit peaks with a shoulder on the low energy side. The other two bands observed at 16530-17210 cm^{-1}(v_2) and 27,800-28,230 cm^{-1} (v_3) are assigned due to $^3A_{2g}$ → $^3T_{1g}$ (F) and $^3A_{2g}$ → $^3T_{1g}$ (P) (v_3), respectively and the first two bands result from the splitting of one band v_1 are in the range of ~ 9,650-10,230 cm^{-1} and 11,850-12440 cm^{-1} which can be assigned to $^3B_{1g}$→^3E_g and $^3B_{1g}$→$^3B_{2g}$, assuming the effective symmetry to be D$_{4h}$ (components of $^3T_{2g}$ in O$_h$ symmetry) [27] The intense higher energy bands at 34,540 cm^{-1} may be due to a π-π* transition of the (C=N) group. Various bands do not follow any regular pattern. The spectra were consistent with distorted octahedral nature of these complexes.

The magnetic moments of cobalt complexes was measured at room temperature and found in the range 4.75-5.01 B.M. which corresponds to three unpaired electrons [28]. The spectra of the cobalt (II) complexes recorded in DMSO exhibits three absorption peaks in the region 8,100-9,185 cm^{-1} (v_1), 12,500-15740 cm^{-1} (v_2) and 18,560- 20, 500 cm^{-1} (v_3) respectively. The various bands may be assigned to the transition $^4T_{1g}$ → $^4T_{2g}$ (F) (v_1); $^4T_{1g}$ → $^4A_{2g}$ (F),(v_2) ; $^4T_{1g}$ → $^4T_{1g}$ (P) (v_3) respectively. The spectra is similar to those cobalt complexes reported to be distorted Octahedral [29]. Thus assuming the effective symmetry to be D$_{4h}$ It appears that the symmetry of these complexes was not idealized octahedral (O$_h$). The assignment of the first spin allowed band seems plausible since the first band appears approximately at half the energy of the visible band [27].

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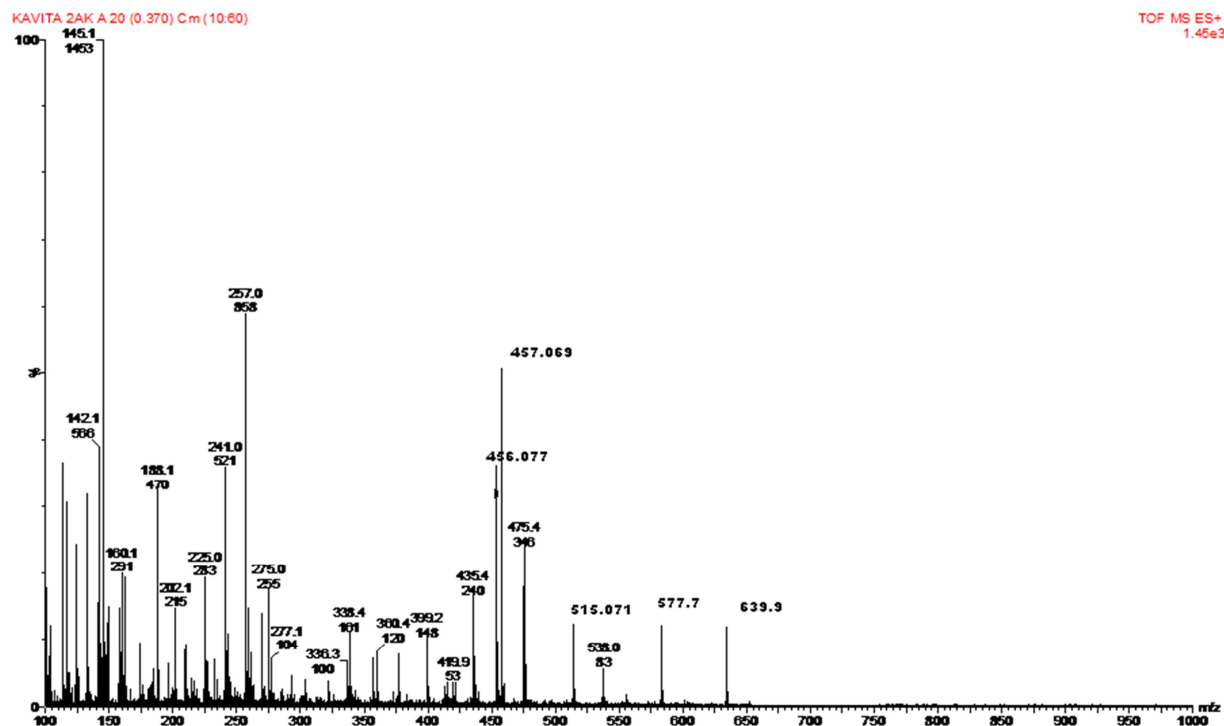
Figure 4 MASS Spectra of [Ni (C$_{20}$H$_{14}$N$_{10}$O$_4$)(NO$_3$)$_2$]

The magnetic moment of copper complexes laid in the range 1.78-2.02 B.M. [30]. The electronic spectra exhibits bands in the region ~ 17,630-18,840 cm^{-1} with a shoulder on the low energy side at ~ 13,520-15,020 cm^{-1}. The data show that these complexes are distorted octahedral [30]. Assuming tetragonal distorted the d-orbital energy level sequence for these complexes may be assigned as x^2-y^2>z^2>xy >xz>yz ; the shoulder may be assigned to z^2→x^2-y^2 (^{2}B$_{1g}$→^{2}B$_{2g}$); the broad band contains both xy→x^2-y^2 (^{2}B$_{1g}$→^{2}E$_g$) and xy→yz→x^2-y^2 (^{2}B$_{1g}$→^{2}A$_{2g}$) transition[31]. The spectra of these Cu (II) complexes show band separation of the order of 2500 cm^{-1} consistent with the proposed geometry of all these complexes [31] Therefore it is concluded that all the complexes formed in the presence of Copper (II) salt have distorted octahedral geometry.

4.5 ESR Spectra:

ESR spectra of the copper (II) complexes were recorded at room temperature in solid state, on X-band at a frequency of 9.5GHz under the magnetic field of 3000 G. The spectra of copper (II) complexes exhibit anisotropic signal. The analyses of spectra give g$_{II}$ value 2.250 and g$\perp$ value 2.132. The trend g$_{II>}$ g$\perp$> 2.0023(g$_e$) observed for these complexes indicate that the unpaired electron was localized in d$_x^{2}_{-y}$^{2} orbital of copper (II), with distortion in the copper (II) complexes from O$_h$ symmetry to D$_{4h}$ symmetry. The Observed g$_{II}$ value for the copper (II) complexes was less than 2.3, which was in agreement with covalent character of metal ligand bond [32]. The value of "G" less than 4 indicate considerable exchange interaction in the solid complexes [32].

4.6 Biological Result and Discussion

Minimum Inhibitory Concentration (MIC) of macrocyclic complexes against gram-positive and gram-negative bacteria namely *Agrobacteriums tumifaciens* (MTCC 431), *Bacillus subtilis* (MTCC 8509), *Escherichia coli* (MTCC 51) and *Pseudomonas putida* (MTCC 121) were determined by the method given by Andrews. Standard antibiotic namely streptomycin and Chloramphenicol, were used for comparison with the antibacterial activities shown by these complexes. All complexes possessed good antibacterial activity against gram positive and gram negative bacteria except the compound no 5 (**table 3,figure2**) which show the poor antibacterial activity. In the whole series, compound 6, 9 and 10 showed the highest MIC ranging from 1-2 µg/ml against *Bacillus subtilis* and *Escherichia coli* respectively.

The MIC of 4µg/ml was showed by Complexes 1 and 10 against *Bacillus subtilis* as well as Complexes 6 and 9 have MIC of 4µg/ml against gram negative bacteria *Escherichia coli*. Complexes 2,6 and 8 possessed MIC value of 8µg/ml against *Pseudomonas putida* and *Agrobacterium tumifaciens*. The complexes 1, 4 and 7 have MIC of 8µg/ml against *Bacillus subtilis* and *Escherichia coli*. It has been found that some of the compounds were more potent as compared to standard

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antibiotic against some species of bacteria. The gram positive organisms were much more susceptible to this series as compared as compared to gram negative organisms. Keeping in view the problem of antimicrobial resistance these compounds could be the good candidate for further studies.

Table 3: Minimum inhibitory concentration (MIC) (in µg/ml)* of chemical compounds by using modified agar well diffusion method

| Sr. No	Compounds	*B. subtilis*	*A. tumifaciens*	*P. putida*	*E. coli*
1	$[Co(C_{20}H_{14}N_{10}O_4)\ Cl_2]$	04	>64	32	08
2	$[Co(C_{20}H_{14}N_{10}O_4)(NO_3)_2]$	32	08	08	16
3	$[Co(C_{20}H_{14}N_{10}O_4)(OAc)_2]$	32	16	08	16
4	$[Ni(C_{20}H_{14}N_{10}O_4)\ Cl_2]$	08	>64	>64	16
5	$[Ni(C_{20}H_{14}N_{10}O_4)(NO_3)_2]$	>64	>64	64	32
6	$[Ni(C_{20}H_{14}N_{10}O_4)(OAc)_2]$	**02**	**08**	**08**	**04**
7	$[Cu(C_{20}H_{14}N_{10}O_4)Cl_2]$	08	32	>64	>64
8	$[Cu(C_{20}H_{14}N_{10}O_4)(NO_3)_2]$	>64	08	16	>64
9	$[Cu(C_{20}H_{14}N_{10}O_4)(OAc)_2]$	02	08	08	04
10.	$[Zn(C_{20}H_{14}N_{10}O_4)(OAc)_2]$	04	32	>64	02
	Chloramphenicol	02	02	04	04
	Streptomycin	02	02	04	02
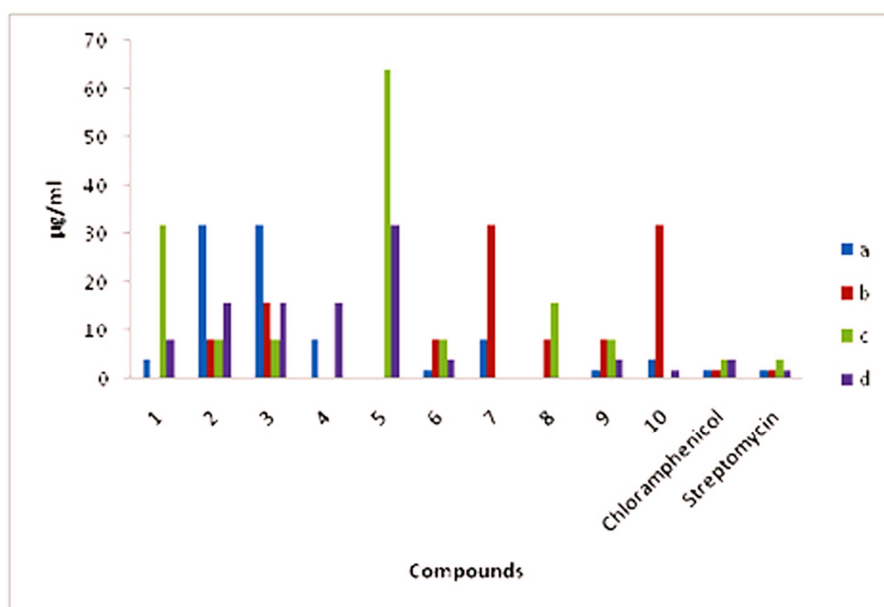
Figure5. Bar Graph representation of Minimum Inhibitory Concentration (MIC) of Complexes

CONCLUSION

On the basis of elemental analyses, conductance, magnetic susceptilities measurements, infrared, NMR, electronic and mass spectral studies, a distorted octahedral geometry, is proposed for the complexes as shown in figure 3. Among the series under test for the determination of minimum inhibitory concentration, all the complexes possessed good antibacterial activity however complex 4 were found to be best antibacterial agent and can further be used in pharmaceutical industries after testing their toxicity to human beings. It has also 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 hydrophobic character and liposolubility of the molecule in crossing the cell membrane of the microorganism and enhance biological utilization ratio and activity of complexes [33]-[34].

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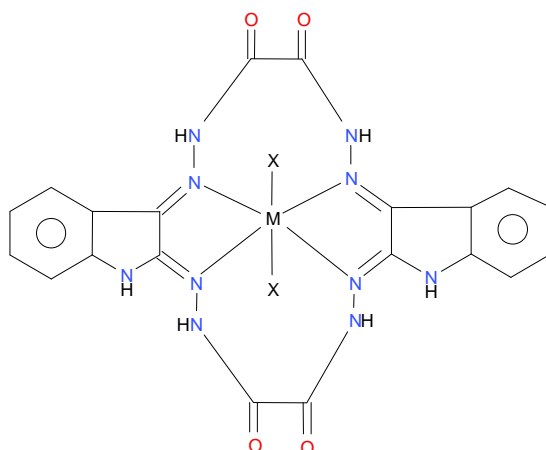
Where M = Co (II), Ni (II), Cu (II), Zn (II);   X = Cl⁻, NO₃⁻ CH₃COO⁻

FIGURE 3 Proposed Structure of Complex

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