

## Biological Activity Studies on Metal Complexes of Macrocyclic Schiff Base Ligand: Synthesis and Spectroscopic Characterization

Parveez Gull\* and Athar Adil Hashmi

Department of Chemistry, Jamia Millia Islamia, 110025 New Delhi, India

In this study, we prepared the macrocyclic Schiff base ligand (L) derived from 1,4-dicarbonyl-phenyl-dihydrazide and pentane-2,4-dione (2:2) and its Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes. The compounds were characterized by the analytical and spectroscopic methods like elemental analysis, molar conductance measurements, mass spectrometry, <sup>1</sup>H nuclear magnetic resonance (NMR), and Fourier transform infrared (FTIR) spectroscopy. The ligand behaves as a tetradentate ligand and coordinates to the metal ions via the nitrogen atoms and the complexes have the mononuclear structures. The analytical and spectroscopic results indicated that the complexes are non-electrolytes in nature and may be formulated as [M(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)X<sub>2</sub>], where M = Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> and X = Cl<sup>-</sup>. The antimicrobial activities of the ligand and its complexes, as growth inhibiting agents, have been screened *in vitro* against different species of bacteria and fungi and the results concluded that the metal complexes are effective drugs against the tested strains as compared to the macrocyclic ligand.

**Keywords:** tetradentate, non-electrolyte, antimicrobial activity, macrocycle

### Introduction

Macrocyclic compounds have attracted increasing interest owing to their mixed soft-hard donor character, versatile coordination behavior and in the understanding of molecular processes.<sup>1,2</sup> Macrocyclic metal complexes are of significant attention in terms of structural and coordination chemistry. The study of metal complexes of macrocyclic ligands appears to be fascinating in view of the possibility of obtaining coordination compounds of unusual structure and stability. Transition metal macrocyclic complexes have received exceptional consideration because of their active part in metalloenzymes and as biomimetic model compounds due to their closeness to natural proteins and enzymes. Synthetic tetraaza macrocycle (N<sub>4</sub>) molecules are considered typically good models for oxygen carriers due to the presence of four nitrogen donor sites confined to a single four-fold or a slightly four-fold plane in a ring structure, appropriate for metal ligand binding. The research field dealing with macrocyclic metal complexes is very broad due in part to their potential interest for a number of interdisciplinary areas that include bioinorganic chemistry, catalysis, and magneto chemistry.<sup>3-5</sup> A number of important macrocyclic molecules which show biological activities including antibacterial, antifungal,<sup>6-15</sup> antidiabetic,<sup>16</sup>

antitumor,<sup>17-19</sup> antiproliferative,<sup>20,21</sup> anticancer,<sup>20,21</sup> herbicidal,<sup>22</sup> and anti-inflammatory activities<sup>13</sup> have been reported. Due to their capability to form complexes with different transition metals, macrocyclic metal complexes can act as catalysts for different reactions.<sup>23-27</sup> Synthetic macrocycles are emerging class of compounds with varying chemistry, different molecular topologies and sets of donor atoms. It is known fact that N atom plays a key role in the coordination of metals at the active sites of numerous metallobiomolecules.<sup>9</sup> Due to the demand of new metal based antibacterial and antifungal compounds, metallorganic chemistry is becoming an emerging area of research.<sup>28,29</sup> Important characteristics that can be correlated with good antimicrobial activities are the lipophilicity and penetration of complexes through the lipid membrane. A survey of the literature reveals that no work has been carried out on the synthesis of metal complexes with macrocyclic hydrazone Schiff base ligand derived from 1,4-dicarbonyl-phenyl-dihydrazide with pentane-2,4-dione. The coordination abilities of Schiff base have attracted our attention and aroused our interest in elucidating the structure of Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup>.

The present study describes the coordination behavior of macrocyclic system derived from the condensation of 1,4-dicarbonyl-phenyl-dihydrazide with pentane-2,4-dione towards some transition elements. For this purpose the complexes of Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> ions with ligand were

\*e-mail: parveezgull@gmail.com

studied and the structure of the complexes were elucidated using elemental analyses, infrared (IR),  $^1\text{H}$  nuclear magnetic resonance (NMR), magnetic moment, molar conductance, and thermal analysis measurements. Besides the characterization of complexes by physicochemical technique, biological activities of the synthesized complexes were examined against some microbial strains for evaluation of antibacterial and antifungal activities.

## Experimental

### Materials and methods

All chemicals used were of AnalaR grade. Pentane-2,4-dione was purchased from Sigma Aldrich and diethyl terephthalate was purchased from Loba Chemie India. Hydrazinhydrate and metal(II) chlorides were obtained from Merck and were used as received without any further purification. All necessary precautions were taken to exclude moisture during the synthesis and handling of the compounds. Elemental analysis of ligand and its metal complexes were carried out using Perkin-Elmer elemental analyzer. Molar conductance of the complexes was measured using a coronation digital conductivity meter. Fourier transform infrared (FTIR) spectra of the ligand and its metal complexes were recorded in the spectral range 4000-400  $\text{cm}^{-1}$  (using KBr) with a Perkin-Elmer Series 2400 apparatus and FTIR/far infrared (FIR) Perkin Elmer (Frontier) spectrometer in the range 700-30  $\text{cm}^{-1}$  (using CsI), respectively. Electronic spectra were recorded on a Perkin-Elmer spectrophotometer. Magnetic susceptibility measurements were done using Gouy balance.  $^1\text{H}$  NMR spectra were run at Bruker 300 MHz spectrometer in dimethylsulfoxide (DMSO) against tetramethylsilane (TMS) as internal reference. Thermal analysis data was studied under nitrogen atmosphere using an A63000 SII technology instrument.

### Synthesis of 1,4-dicarbonyl-phenyl-dihydrazide

To 2.22 g of diethyl ester of terephthalic acid (**1**) in 20 mL of ethanol was added hydrazine hydrate (**2**) (98%, 2 mL) in ethanol. The solution was refluxed for 3-4 h. The reaction mixture was allowed to cool to the room temperature and then poured onto ice cold water. The terephthalohydrazide (**3**) thus obtained, was filtered and recrystallized from ethanol.

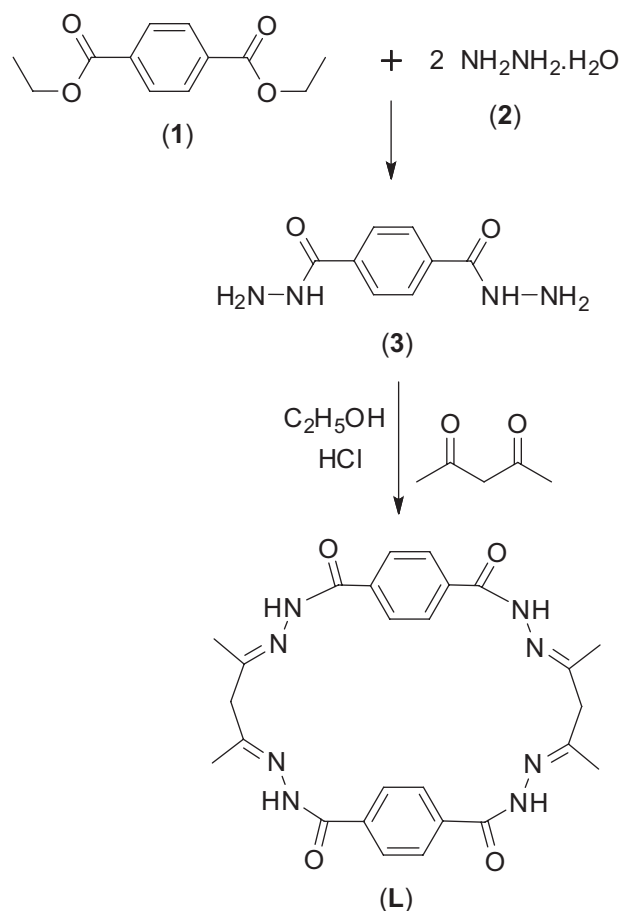
### 1,4-dicarbonyl-phenyl-dihydrazide (**3**)

Yield: 68%; buff color; IR (KBr)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  3432, 1716;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  7.25-8.01 (m, 4H,  $J$  4.24,

1.72 Hz, Ar-H), 7.87 (t, 1H,  $J$  8.0, 4.0 Hz, N-H), 1.98 (d, 2H,  $\text{NH}_2$ ); MS (ESI) calcd. for  $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$   $[\text{M}]^+$ : 194.19; found: 194.08.

### Synthesis of macrocyclic ligand (L)

Pentane-2,4-dione (0.324 g, 2 mmol) in ethanol (20 mL) was added to a solution of terephthalohydrazide (**3**) (0.388 g, 2 mmol) in ethanol (20 mL) containing a few drops of concentrated HCl as depicted in Scheme 1. The reaction mixture was refluxed for 4 h. The mixture was cooled to room temperature and the solvent removed *in vacuo* until a solid product was formed that was washed with cold ethanol and dried under vacuum.



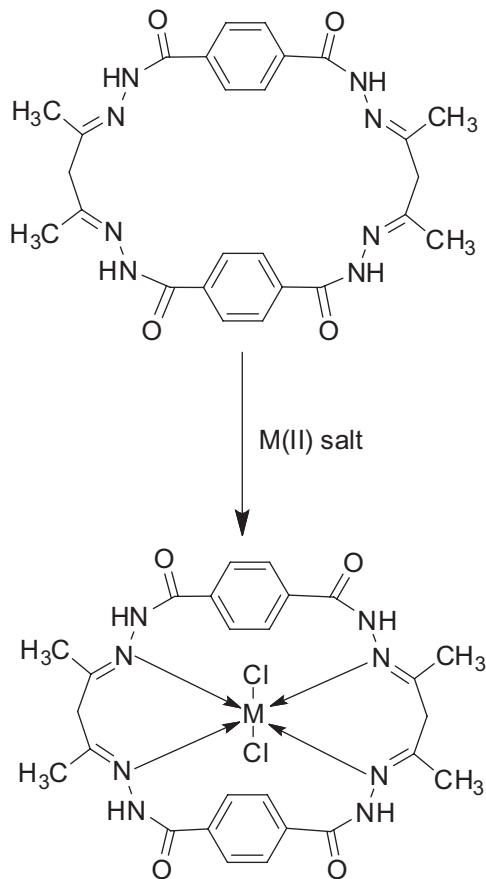
**Scheme 1.** Synthesis of macrocyclic Schiff base ligand (L).

### Macrocyclic ligand L ( $\text{C}_{26}\text{H}_{28}\text{N}_8\text{O}_4$ )

Yield: 72%; brown solid; IR (KBr)  $\nu_{\text{max}}$  /  $\text{cm}^{-1}$  3336, 1625;  $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  7.18-8.76 (m, 4H,  $J$  4.25, 1.73 Hz, Ar-H), 7.84 (t, 1H,  $J$  8.0, 4.0 Hz, N-H), 0.8-1.0 (s, 12H,  $J$  8.0, 4.0 Hz,  $-\text{CH}_3$ ), 1.0-1.4 (s, 4H,  $J$  8.0, 4.0 Hz  $-\text{CH}_2$ ); MS (ESI) calcd. for  $\text{C}_{26}\text{H}_{28}\text{N}_8\text{O}_4$   $[\text{M}]^+$ : 516; found: 517  $[\text{M} + \text{H}]^+$ .

### Synthesis of the Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes

Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes were prepared by the general method. To a solution of 1 mmol of the appropriate M(Cl)<sub>2</sub> metal salts in 20 mL of ethanol was slowly added with stirring a solution of 1 mmol of L in 20 mL of ethanol and the reaction mixture was refluxed for 3 h. The precipitate was filtered off, washed with ethanol and dried under vacuum over anhydrous CaCl<sub>2</sub>; yield 60-65% (Scheme 2).



**Scheme 2.** Formation of macrocyclic metal complexes (M = Cu, Co and Ni; X = Cl).

#### [Cu(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>]

Yield: 72%; dark brown; IR (KBr)  $\nu_{\max}$  / cm<sup>-1</sup> 3342, 1610, 446, 235; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.25-8.01 (m, 4H, *J* 4.25, 1.73 Hz, Ar-H), 7.87 (t, 1H, *J* 8.0, 4.0 Hz, N-H), 0.8-0.98 (s, 12H, *J* 8.0, 4.0 Hz, -CH<sub>3</sub>), 1.0-1.4 (s, 4H, *J* 8.0, 4.0 Hz, -CH<sub>2</sub>); MS (ESI) calcd. for [Cu(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>] [M]<sup>+</sup>: 651; found: 650.

#### [Co(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>]

Yield: 65%; reddish brown; IR (KBr)  $\nu_{\max}$  / cm<sup>-1</sup> 3343, 1620, 433, 232; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.25-8.15 (m, 4H, *J* 4.22, 1.71 Hz, Ar-H), 7.87 (t, 1H, *J* 8.1, 4.3 Hz,

N-H), 0.8-0.9 (s, 12H, *J* 8.1, 4.2 Hz, -CH<sub>3</sub>), 1.0-1.4 (s, 4H, *J* 8.1, 4.2 Hz, -CH<sub>2</sub>); MS (ESI) calcd. for [Co(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>] [M]<sup>+</sup>: 647; found: 648 [M + H]<sup>+</sup>.

#### [Ni(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>]

Yield: 68%; brown; IR (KBr)  $\nu_{\max}$  / cm<sup>-1</sup> 3346, 1613, 457, 237; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.25-8.10 (m, 4H, *J* 4.25, 1.73 Hz, Ar-H), 7.89 (t, 1H, *J* 8.0, 4.0 Hz, N-H), 0.8-0.98 (s, 12H, *J* 8.0, 4.0 Hz, -CH<sub>3</sub>), 1.0-1.4 (s, 4H, *J* 8.0, 4.0 Hz, -CH<sub>2</sub>); MS (ESI) calcd. for [Ni(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>] [M]<sup>+</sup>: 646; found: 647[M + H]<sup>+</sup>.

### Antimicrobial activity

The antimicrobial activities of the synthesized compounds (macrocyclic ligand and its metal complexes) have been screened *in vitro*, as growth inhibiting agents. The antibacterial and antifungal screening were carried out using disc diffusion method against some strains of bacteria like *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* and fungal species, including *Candida albicans*, *Fusarium sp.*, *Trichosporon sp.* and *Aspergillus flavus*. The compounds were dissolved in 1% DMSO to get the required test solutions. The nutrient agar and potato dextrose agar (PDA) were used as a required medium for these activities. After incubation for 24 h at 27 °C in the case of bacteria and for 48 h at 27 °C in the case of fungi, inhibition of the organisms was evidenced by clear zone surrounding each disk, which was measured.

### Results and Discussion

The two dione groups of pentane-2,4-dione were used for the condensation of two amino groups of 1,4-dicarbonyl-phenyl-dihydrazide to synthesize the macrocyclic Schiff base ligand. The condensation between 1,4-dicarbonyl-phenyl-dihydrazide and pentane-2,4-dione lead to the formation of a tetradentate macrocyclic ligand. It is stable in air and is partially soluble in ethanol, methanol and completely soluble in chloroform, tetrahydrofuran (THF), dimethylformamide (DMF) and DMSO. The metal(II) complexes are non-hygroscopic, soluble in DMSO, DMF and sparingly soluble in methanol and ethanol. The elemental analysis data of ligand and its metal complexes along with molar conductance values are given in Table 1.

#### Molar conductance

The metal(II) complexes of macrocyclic Schiff base ligand (10<sup>-3</sup> mol dm<sup>-3</sup>) were dissolved in DMF and molar conductivities of the solutions at room temperature were

**Table 1.** Analytical data of the Cu<sup>II</sup>, Co<sup>II</sup>, and Ni<sup>II</sup> complexes

Complex	Elemental analysis calculated (found) / %					$\mu_{\text{eff}}$ (BM)	Molar conductivity / ( $\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ )
	C	H	N	O	M		
C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> (L)	60.45 (60.40)	5.46 (5.38)	21.69 (21.60)	12.39 (12.29)	–	–	–
[Cu(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	47.97 (47.84)	4.34 (4.29)	17.21 (17.18)	9.83 (9.78)	9.76 (9.70)	1.94	2.4
[Co(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	48.31 (48.25)	4.37 (4.28)	17.34 (17.26)	9.90 (9.81)	9.12 (9.08)	4.95	3.8
[Ni(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	48.33 (48.29)	4.37 (4.28)	17.34 (17.25)	9.90 (9.83)	9.08 (9.01)	3.1	6.3

BM: Bohr Magneton.

measured. The conductance data (Table 1) indicate that all the metal complexes are having conductivity values in accordance with non-electrolytes.<sup>30,31</sup>

### IR spectra

The IR spectra of the macrocyclic ligand and its metal complexes were obtained on a Perkin-Elmer Series (2400) apparatus in the range 4000-400 cm<sup>-1</sup> and FTIR/FIR Perkin Elmer (Frontier) spectrometer in the range 700-30 cm<sup>-1</sup>. The peak observed at 1690 cm<sup>-1</sup> in pentane-2,4-dione is assigned for >C=O group. 1,4-Dicarbonyl-phenyl-dihydrazide has strong bands at 3432 cm<sup>-1</sup> corresponding to the –NH<sub>2</sub> stretching frequency. In macrocyclic Schiff base ligand these peaks are absent, the imine >C=N band is superimposed with the >C=O group and appears as a strong band at 1625 cm<sup>-1</sup>,<sup>32</sup> confirming the formation of macrocyclic ligand (Supplementary Information Figure S1). The strong band observed at 1625 cm<sup>-1</sup> is shifted to lower wavenumber by 16-8 cm<sup>-1</sup> in the spectra of metal(II) complexes. This indicates the coordination of imino (>C=N) groups to the metal atom in complexes. In the spectra of macrocyclic ligand and its complexes the band due to ring  $\nu(\text{NH})$  is observed at 3340-3330 cm<sup>-1</sup>. In the spectra of the complexes, appearance of new bands in the region 460-420 and 220-240 cm<sup>-1</sup> has been attributed to M–N and M–Cl bonds, respectively.<sup>33</sup> From the IR spectral data it is concluded that macrocyclic ligand acts as a tetradentate ligand in Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes, coordinating through imino nitrogen atoms. Important IR spectral bands of macrocyclic ligand and its metal complexes are summarized in Table 2.

**Table 2.** IR spectral data of Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes

Compound	$\nu(\text{N-H})$	$\nu(\text{C=N})$	$\nu(\text{C=O})$	$\nu(\text{M-N})$
C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> (L)	3336	1625	1630	–
[Cu(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	3342	1610	1635	446
[Co(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	3343	1620	1640	433
[Ni(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	3346	1613	1635	457

### Mass spectra

The mass spectra of ligand and its Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes were recorded and their stoichiometric compositions were compared. The mass spectrum of macrocyclic ligand (C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>) shows a well-defined molecular ion peak at  $m/z$  517 which coincides with the formula weight of the Schiff base. The mass spectra of all the synthesized macrocyclic complexes displayed molecular ion peaks [M + H]<sup>+</sup> at  $m/z$  650, 648 and 647 a.m.u. corresponding to their molecular formulae [Cu(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>], [Co(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>] and [Ni(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub>], respectively. The mass spectrum of macrocyclic Co<sup>II</sup> complex shows a molecular ion peak at  $m/z$  648, which corresponds to [Co(C<sub>26</sub>H<sub>28</sub>N<sub>8</sub>O<sub>4</sub>)Cl<sub>2</sub> + H]<sup>+</sup> as the calculated mass is 647. The series of peaks have been observed at  $m/z$  516, 420, 270, 162, and 147 a.m.u., corresponding to various fragments. The mass spectra of Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> macrocyclic complexes have been recorded (Table 3, Figures S2-S4). This data is in good agreement with the proposed molecular formula for these complexes. In addition to the peaks due to the molecular ion, the spectra exhibit peaks assignable to various fragments arising from the thermal cleavage of the complexes.

**Table 3.** Mass spectral data of the macrocyclic metal complexes

Complex	Molecular weight	Molecular ion peak [M] <sup>+</sup>
C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> (L)	516.55	[M] <sup>+</sup> = 517
[Cu(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	651	[M] <sup>+</sup> = 650
[Co(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	647	[M + H] <sup>+</sup> = 648
[Ni(C <sub>26</sub> H <sub>28</sub> N <sub>8</sub> O <sub>4</sub> )Cl <sub>2</sub> ]	646	[M + H] <sup>+</sup> = 647

### <sup>1</sup>H NMR

A survey of literature reveals that the NMR spectroscopy has been proved useful in establishing the structure and nature of many Schiff base ligands and its metal complexes. The <sup>1</sup>H NMR spectra of Schiff base ligand was recorded in DMSO-*d*<sub>6</sub> solution using TMS as internal standard

(Figure S5). The  $^1\text{H}$  NMR data for all complexes show a multiplet in the 6.70-7.00 ppm region which may be assigned to the secondary amide protons (C–NH; 4H). A multiplet in the 1.0-1.4 ppm region may be assigned<sup>34</sup> to the methylene protons ( $-\text{CH}_2-$ ; 4H). However, a multiplet in the region 7.18-8.76 ppm may be assigned to aromatic ring protons,<sup>35,36</sup> while a singlet at 0.8-1.0 ppm may be ascribed to the methyl protons of the pentane moiety ( $-\text{CH}_3$ ; 12H).

#### Electronic spectral studies and magnetic measurements

The electronic spectrum of the macrocyclic Schiff base  $\text{Cu}^{\text{II}}$  complex recorded at room temperature, in DMF solution, shows broad band absorption in the range 14,220-14,492, 20,398-20,610 and 22,725-23,252  $\text{cm}^{-1}$ , which may be assigned to  $^2\text{B}_{1g} \rightarrow ^2\text{A}_{1g}$ , ( $d_{x^2-y^2} \rightarrow d_z^2$ ) ( $\nu_1$ ),  $^2\text{B}_{1g} \rightarrow ^2\text{B}_{2g}$ , ( $d_{x^2-y^2} \rightarrow d_{yz}$ ) ( $\nu_2$ ), and  $^2\text{B}_{1g} \rightarrow ^2\text{E}_g$ , ( $d_{x^2-y^2} \rightarrow d_{zy}, d_{yz}$ ) ( $\nu_3$ ) transitions, respectively, which confirms the octahedral geometry of the complex.<sup>37</sup> The most probable geometric configuration indication of the synthesized metal complexes is their magnetic moment value, which lies at 1.98 BM for  $\text{Cu}^{\text{II}}$  complex corresponding to the presence of one unpaired electron and it supports an octahedral geometry.<sup>38</sup>

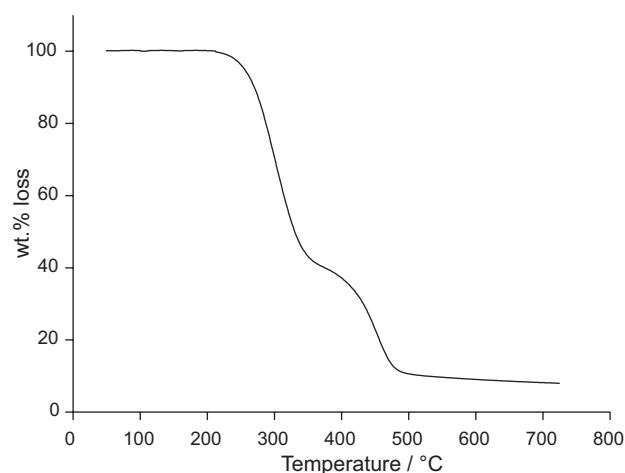
The electronic spectrum of the macrocyclic  $\text{Co}^{\text{II}}$  complex exhibits absorption bands in the range 12,656-12,901, 15,382-15,745 and 22,219-22,725  $\text{cm}^{-1}$ , which may be assigned to  $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{2g}(\text{F})$  ( $\nu_1$ ),  $^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g}(\nu_2)$  and  $^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{1g}(\text{P})$  ( $\nu_3$ ) transitions, respectively, confirm an octahedral geometry around a  $\text{Co}^{\text{II}}$  ion, in the complexes under study. The geometry of the complex is supported by the magnetic moment measurements which lie at 4.98 BM.<sup>39</sup>

The magnetic moment of the macrocyclic  $\text{Ni}^{\text{II}}$  complex at room temperature lies at 2.97 BM which shows the presence of an octahedral environment around the  $\text{Ni}^{\text{II}}$  ion. The electronic spectra of the  $\text{Ni}^{\text{II}}$  complexes exhibit three absorption bands, in the range of 10,200-11,109, 15,149-15,382 and 26,313-27,395  $\text{cm}^{-1}$  which may be assigned to three spin allowed transitions:  $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{2g}(\text{F})$  ( $\nu_1$ ),  $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{F})$  ( $\nu_2$ ), and  $^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{P})$  ( $\nu_3$ ), respectively.<sup>39</sup>

#### Thermal analysis

The thermal stabilities of the metal complexes were investigated using thermogravimetric analysis (TGA) under nitrogen atmosphere with a heating rate of 20  $^\circ\text{C min}^{-1}$  from 35 to 800  $^\circ\text{C}$ . The thermograms of all the complexes do not show any weight loss up to 225  $^\circ\text{C}$  indicating the absence of water molecules in these complexes. The  $\text{Co}^{\text{II}}$  complex

started to decompose at 315  $^\circ\text{C}$  and gradual decrease in the weight loss occurs up to 580  $^\circ\text{C}$ . After that a straight line is obtained indicating the formation of cobalt as residue. The  $\text{Ni}^{\text{II}}$  complex gradually decreases its weight from 355  $^\circ\text{C}$  and forms the metal oxide at 625  $^\circ\text{C}$ . The  $\text{Cu}^{\text{II}}$  complex was stable below 335  $^\circ\text{C}$ , after that it gradually decomposes to form the corresponding metal oxide at 700  $^\circ\text{C}$ . The gradual decrease of the weight loss in the complexes may be due the removal of chlorine atoms as HCl gas, carbon and nitrogen in the organic moiety as their oxides. From the data it is confirmed that all the complexes are stable at ordinary temperature and  $\text{Ni}^{\text{II}}$  complex is more stable than the other complexes (Figure 1).



**Figure 1.** TGA curves of  $\text{Ni}^{\text{II}}$  metal complexes.

#### Antimicrobial activity

The macrocyclic ligand and its  $\text{Co}^{\text{II}}$ ,  $\text{Cu}^{\text{II}}$  and  $\text{Ni}^{\text{II}}$  complexes were tested *in vitro* against the bacterial species *Escherichia coli* (MTCC 1687), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424), and *Staphylococcus aureus* (MTCC 96); and fungal species *Candida albicans* (MTCC 183), *Fusarium* sp. (MTCC 3326), *Trichosporon* sp. (MTCC 6179) and *Aspergillus flavus* (MTCC 2206) by the disc diffusion method. The standards used were amikacin and nystatin. The minimum inhibitory concentration (MIC) was determined by the Clinical and Laboratory Standards Institute recommended broth microdilution method M27-A3.<sup>40</sup> The test tubes containing 5 mL of sterile nutrient/Sabouraud broth were inoculated with 0.02 mL of 24 h old culture of bacteria and fungi. Different amount of compounds in DMSO were aseptically added with the help of sterile pipettes from the stock solution 200  $\mu\text{g mL}^{-1}$  to 5 mL quantities of respective media so as to reach the concentration from 1  $\mu\text{g mL}^{-1}$  to 50  $\mu\text{g mL}^{-1}$ . All test

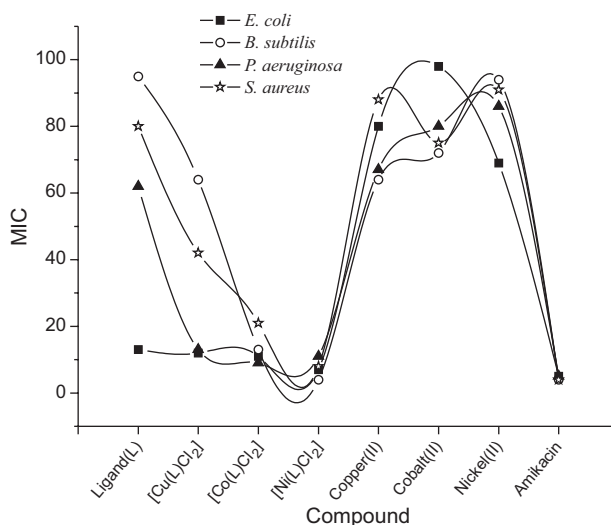


tubes were incubated at 37 °C and at room temperature for bacteria and fungi, respectively. Test tubes inoculated with organisms were observed for presence of turbidity after 24 and 48 h, respectively. The lowest concentrations of compounds inhibiting the growth of organisms were determined as MIC value (Tables S1 and S2).

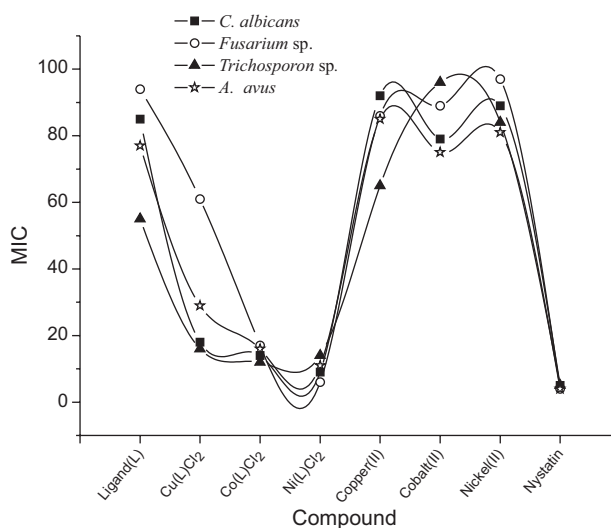
From the results of the antibacterial and antifungal activity of metal complexes, it is apparent that the metal complexes show greater antimicrobial activity than that of the free macrocyclic ligand; this advanced antibacterial activity of the metal complexes, compared with that of Schiff bases, is conceivably owing to modification in structure due to coordination, and chelating tends to make metal complexes act as more influential and powerful bacteriostatic agents, thus inhibiting the growth of the microorganisms. Overtone's concept and chelation theory<sup>41</sup> explains the increased antimicrobial effect as the chelation has a tendency to make the ligand a more powerful and potent bacterial agent. On chelation, the polarity of the metal ion will be reduced to a better range due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of  $\pi$ -electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.<sup>42</sup> In general, metal complexes are more active than the ligands because metal complexes may serve as a vehicle for activation of ligands as the principle cytotoxic species.<sup>43</sup> The synthesized compounds exhibit moderate to strong antimicrobial activity. The Cu<sup>II</sup> complex exhibits a higher activity than the other metal complexes towards fungal species. Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes have low activity compared to the standard. In the case of fungal species, Ni<sup>II</sup> complex shows remarkable activity against *Fusarium* sp. and *C. albicans* compared to the standard drug (Figures 2 and 3).

## Conclusions

The ligand and its Co<sup>II</sup>, Cu<sup>II</sup> and Ni<sup>II</sup> complexes were synthesized, characterized and tested for their antimicrobial inhibition potential. The outcome of antimicrobial studies showed that the macrocyclic ligand possessed mild activity and metal(II) complexes possessed moderate to significant activities against different bacterial and fungal strains which might be due to azomethine (–C=N–) linkage and/or heteroatoms present in these compounds. The



**Figure 2.** Antibacterial activity of macrocyclic ligand and its metal complexes.



**Figure 3.** Antifungal activity of macrocyclic ligand and its metal complexes.

biological activity findings exhibited that majority of the macrocyclic ligands possessed increased activity upon coordination with different metal ions. The enhancement in biological activity upon coordination may be elucidated on the basis of Overtone's concept and chelation theory. The cytotoxicities values designate that metal complexes present significant cytotoxic activities that might help in the development of potent antimicrobial drugs. Further structural optimization revisions might thus represent a rationale for further investigation.

## Supplementary Information

Supplementary data are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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