

# Synthesis, Spectroscopic and Anti-microbial studies of Binuclear Schiff Base Complexes derived from the Ligand prepared from Isoniazid and Benzilmonohydrazone

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## ABSTRACT

The binuclear Schiff base complexes have been prepared newly using different transition metals at their stable oxidation states as Cu(II), Ni(II), and Co(II) with the ligand (L) obtained by the condensation of isoniazid and benzilmonohydrazone. The composition and structure of the complexes have been established by analytical and spectral data, thermal analysis, magnetic and conductivity measurements. The metallo- ligands as well as the macrocyclic complexes are found to have good antimicrobial activities against the pathogenic fungus *Aspergillus niger*, *Helminthosporium oryzae* and *Fusarium oxysporium*. The binuclear metal complexes were found to possess potent antimicrobial, antifungal activity better than the ligand.

**Keywords:** Schiff base complex, Thermal analysis, Antimicrobial activity, Conductivity measurements.

## 1. Introduction

The chemistry of transition metal complexes of Schiff bases has played a significant role in the development of coordination chemistry. Multidentate Schiff bases have been widely used as ligands, as they can easily attach to metal ions due to the formation of highly stable coordination compounds. Metal complexes of S-, N-, and O-chelating ligands have attracted considerable attention owing to their interesting physicochemical properties, pronounced biological activities and for their use as models of metalloenzyme active sites<sup>1,2</sup>. This has led to a rapid surge in the quantum of research in this area. These complexes have numerous applications, such as in the treatment of cancer<sup>3</sup>, as antibactericide agents<sup>4</sup>, as antiviral agents<sup>5</sup>, as fungicide agents<sup>6</sup> and for other biological properties<sup>7</sup>. In the past couple of decades, the properties of Schiff base metal complexes have stimulated huge interest for their noteworthy contributions to material science<sup>8</sup>, catalysis of many reactions<sup>9</sup> and their industrial applications<sup>10</sup>.

In view of above application of Schiff base complexes we report the synthesis of a series of binuclear complexes of Cu(II), Ni(II) and Co(II) with the titled ligand.

## 2. Experimental

All the chemicals and solvents used are either of Glaxo or Merck grade. The solvents were purified before its use in the reaction. The metal contents of all the complexes were estimated by standard methods<sup>11</sup>. The percentage of nitrogen was determined by semi micro Kjeldahl's method. Carbon and hydrogen were estimated with CHN micro analyzer. The IR spectra of the metal complexes were recorded on a Varian spectrophotometer,

Australia, in KBr pellets in the region 4000 - 400  $\text{cm}^{-1}$ . The electronic spectra of the complexes in DMSO were recorded on a Perkin Elmer-398 Spectrophotometer. The conductivity of the complexes in DMF was measured with a Philips conductivity bridge (model CLO-06, cell constant 0.5  $\text{cm}^{-1}$ ) using  $1 \times 10^{-3}$  M solution of the complex in DMF. The room temperature magnetic moment values were determined by Gouy method using mercury tetrathiocyanatocobaltate (II),  $\text{Hg}[\text{Co}(\text{SCN})_4]$  as calibrant. The TG-DTG thermograms of the complexes were recorded on NETZSCH- Geratebau GmbH system. The EPR spectra of Cu(II) complexes were recorded on Bruker EMX Plus at room temperature.

## 2.1 Synthesis of metal complexes with benzil monohydrazone

The complexes were prepared by two different methods as described below. First method (Method- I) involves a two step process i.e. preparation of Schiff base ligand (L), followed by complexation with corresponding  $\text{Co}^{\text{II}}$ ,  $\text{Ni}^{\text{II}}$  and  $\text{Cu}^{\text{II}}$  ions whereas the second method (Method- II) involves *in situ* synthesis of metal complexes<sup>12,13</sup>. However, in both the cases, complexes of same stoichiometry were isolated.

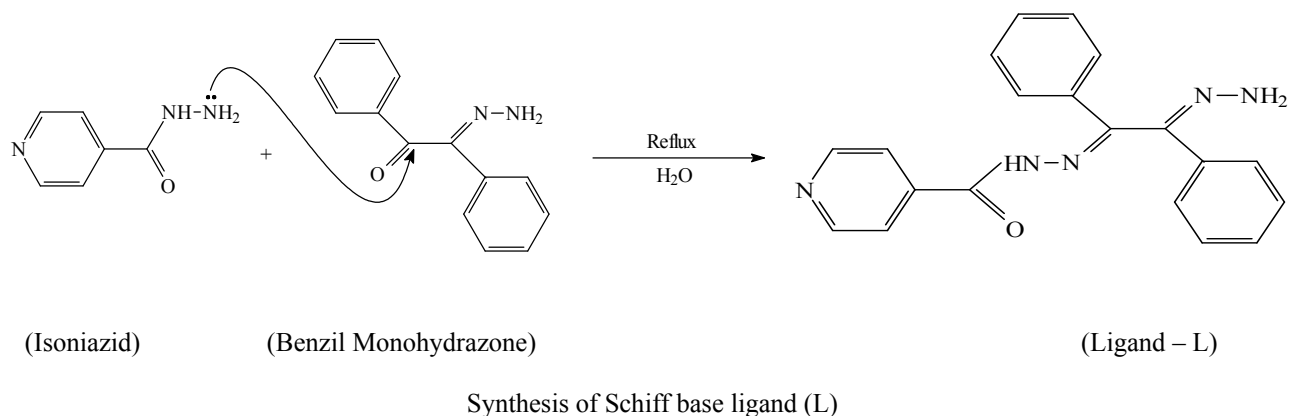
### 2.1.1 Method-I

#### Synthesis of Schiff base ligand

A solution of isoniazid (6.8g or 0.05mol) in ethanol (50 ml) was prepared by heating the mixture for about 10 min in a 100 ml two necked round bottom flask fitted with a reflux condenser. To this solution benzil monohydrazone (11.21g or 0.05mol) was added slowly and the resulting mixture was refluxed at 60-70<sup>o</sup> C for 4 hours (Scheme- 1). The reaction mixture was then allowed to cool to room temperature, when a pale yellow solid compound separated. It was filtered and washed with small quantities of ethanol several times. It was then recrystallized from ethanol and dried in a desiccator over fused  $\text{CaCl}_2$ .

#### Mechanism of formation of Schiff base ligand

##### Scheme-1



#### Synthesis of Metal Complexes Cu (II) complexes:

### i) Synthesis of $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$

A solution of the ligand(L) (3.43g or 0.01mol) in ethanol (50ml) was taken in a two necked round bottom flask. An ethanolic solution of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (1.70g or 0.01mol) was added slowly to it (Scheme- 2). The resulting mixture was then refluxed for 4 hours until an intense green coloured compound separated out. The compound was then filtered, washed several times with cold ethanol and finally was dried over fused  $\text{CaCl}_2$  in a desiccator.

### ii) Synthesis of $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$

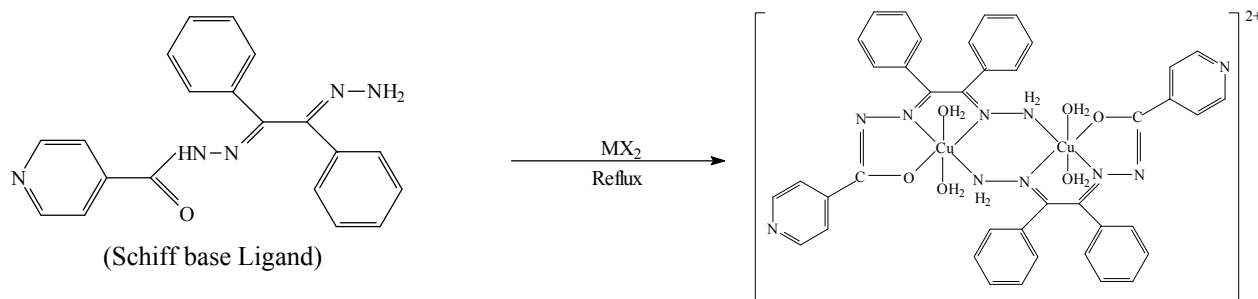
An ethanolic solution of  $\text{Cu}(\text{CH}_3\text{COO})_2 \cdot \text{H}_2\text{O}$  (2.0g or 0.01mol) was added slowly to a solution of isolated Schiff base ligand (L) (3.43g or 0.01mol) in ethanol (50ml) taken in a two necked round bottom flask (Scheme- 2). The resulting mixture was refluxed for 4 hours until a dark coffee coloured compound separated out. The compound was then filtered, washed several times with cold ethanol and finally it was dried over fused  $\text{CaCl}_2$  in a desiccator.

### iii) Synthesis of $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$

A solution of isolated Schiff base ligand(L) (3.43g or 0.01mol) in ethanol (50ml) was taken in a two necked round bottom flask. An ethanolic solution of  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  (2.41g or 0.01mol) was added slowly to it (Scheme- 2). The resulting mixture was then refluxed for 4 hours until a parrot green coloured compound separated out. The compound was then filtered, washed several times with cold ethanol and finally was dried over fused  $\text{CaCl}_2$  in a desiccator.

The other binuclear Schiff base complexes of Ni(II) and Co(II) were prepared in a similar manner as described above.

#### Scheme-2



Synthesis of Cu(II) complex( X – Cl,  $\text{CH}_3\text{COO}$ ,  $\text{NO}_3$ )

#### 2.1.2 Method- II (In situ process)

In this method, calculated amount of isoniazid was taken in a round bottom flask containing ethanol. To this ethanolic solution, benzil monohydrazone was added and refluxed for 1 hour. The metal salt was then added to it in stoichiometric proportion. The isoniazid, benzil monohydrazone and metal salt were in (1:1:1) ratio. The mixture was then refluxed for 5 hours, when the desired compound separated out. It was filtered, washed several times with ethanol to achieve the maximum purity and dried over fused calcium chloride (Scheme-3).

The analytical data of the ligand and its metal complexes are given in Table-1.

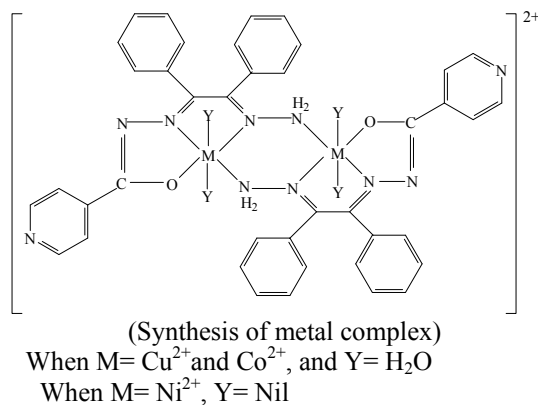
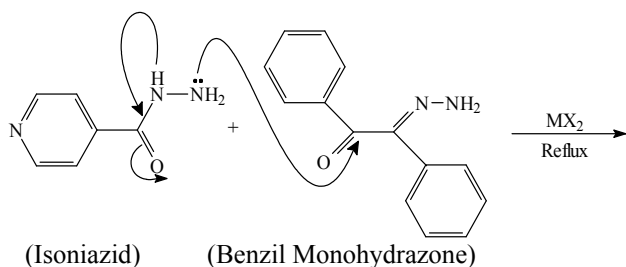
**Table 1: Analytical and physicochemical data of complexes**

| Complexes  | Colour       | Mol. Wt. | % of Metal Calcd. (Found) | % of C Calcd. (Found) | % of H Calcd. (Found) | % of N* Calcd. (Found) | % of Cl Calcd. (Found) | $\Lambda$ in $\text{ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$ | Melting point in $^{\circ}\text{C}$ |
|--|--------------|----------|---------------------------|-----------------------|-----------------------|------------------------|------------------------|--|-------------------------------------|
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | Dark Green   | 954      | 13.31 (14.14)             | 50.31 (51.45)         | 4.19 (4.45)           | 14.67 (14.85)          | 7.44 (7.90)            | 131  | 294                                 |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | Parrot Green | 1007     | 12.61 (12.87)             | 47.66 (48.63)         | 3.97 (4.05)           | 16.68 (16.91)          | -                      | 134  | 297                                 |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | Dark Coffee  | 1001     | 12.68 (12.75)             | 52.74 (51.47)         | 4.59 (4.71)           | 13.98 (14.18)          | -                      | 132  | 295                                 |
| $[\text{Ni}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | Light Green  | 944      | 12.39 (12.85)             | 50.84 (51.05)         | 4.23 (4.30)           | 14.83 (15.25)          | 7.52 (7.97)            | 124  | 285                                 |
| $[\text{Ni}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | Yellow       | 997      | 11.73 (12.02)             | 48.14 (48.73)         | 4.01 (4.09)           | 16.85 (17.60)          | -                      | 127  | 283                                 |
| $[\text{Ni}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | Brick Red    | 991      | 11.80 (12.48)             | 53.27 (54.01)         | 4.64 (5.25)           | 14.12 (14.90)          | -                      | 125  | 286                                 |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | Light Violet | 945      | 12.48 (13.05)             | 50.79 (51.29)         | 4.23 (4.50)           | 14.81 (15.25)          | 7.51 (7.99)            | 137  | 272                                 |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | Pink         | 998      | 11.82 (12.07)             | 48.09 (49.08)         | 4.00 (4.09)           | 16.83 (17.19)          | -                      | 135  | 274                                 |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | Brick Red    | 992      | 11.89 (12.31)             | 53.22 (53.96)         | 4.63 (4.95)           | 14.11 (15.09)          | -                      | 138  | 271                                 |

### 3. Results and Discussion

The hydrazone and carbonyl functions in benzilmonohydrazone exist in *trans* configuration with respect to C–C bond. The stereochemical requirement demands both the groups to come closer in presence of the metal ion to the extent that they attain *cis* configuration so that the condensation of the carbonyl group with isoniazid occurs accompanied by formation of the corresponding metal complex. The complexes are intensely coloured and have melting point greater than  $250^{\circ}\text{C}$ . They are insoluble in most of the organic solvent but dissolve in N, N- dimethyl formamide (DMF). The high values of molar conductance of binuclear Schiff base complexes at room temperature ( $135\text{--}145 \text{ ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$ ) indicate that the complexes are electrolytic in nature with the presence of anions outside the coordination sphere. The analytical data of the complexes are recorded in Table -1. The scheme of the reaction is presumed to be as follows:

#### Scheme- 3



The ligand and its metal complexes were characterized by elemental analysis, UV, IR, EPR and molar conductance data. The Schiff base ligand and its complexes were investigated for anti-fungal properties.

### 3.1 IR Spectra

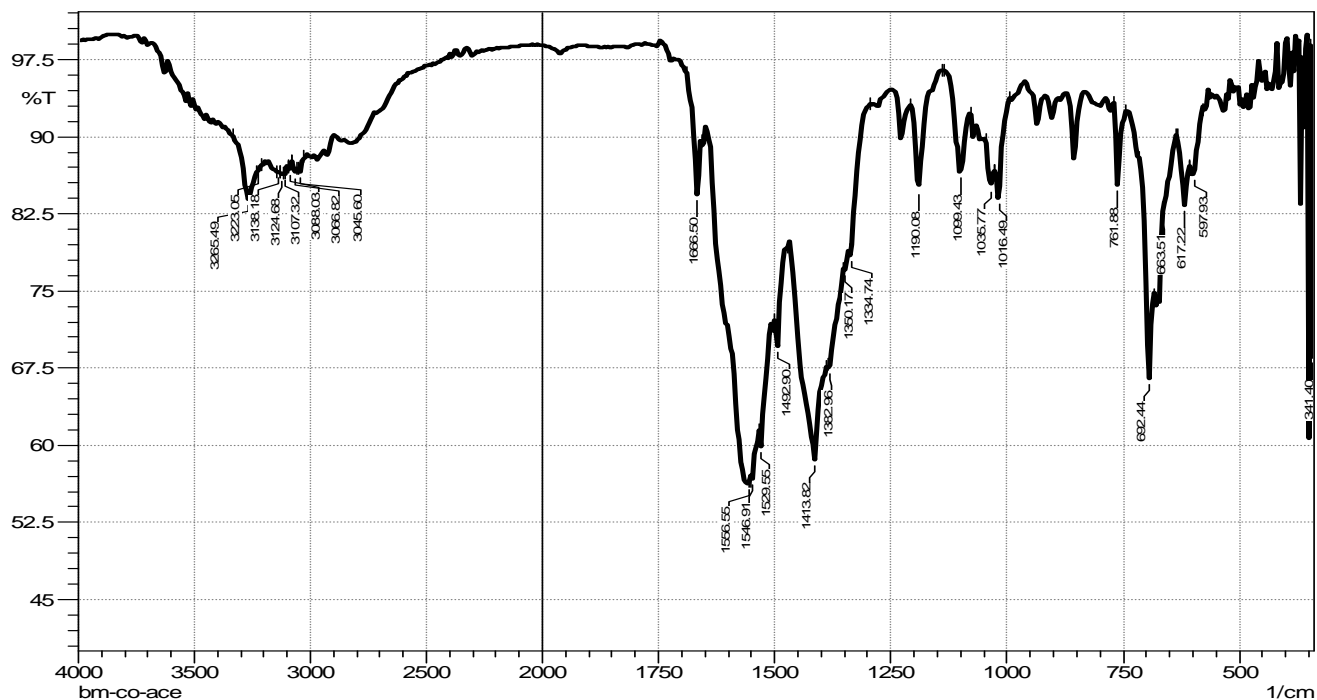
IR spectra provide valuable information regarding the nature of functional groups attached to the metal ion in the complexes. The IR spectra of metal complexes are very complicated. However attempts have been made to identify bands which provide vital information on the mode of bonding of the ligand with the metals<sup>14</sup>, by comparing their spectra with the ligand and other related reported complexes.

The IR spectra of the ligand (Table 2) produced a broad band in the region 3470-3465  $\text{cm}^{-1}$  characteristic of the presence of  $\text{NH}_2$  group. On complexation, this band is shifted to lower frequency in the range (3420-3432  $\text{cm}^{-1}$ ) indicating the involvement of primary amine nitrogen in coordination to metal ion for all the complexes. The  $\nu_{\text{C}=\text{N}}$  stretching frequency of the azomethine group in the free ligand appears at 1597  $\text{cm}^{-1}$ , which is shifted to lower frequency in the spectra of all the complexes (1556-1597  $\text{cm}^{-1}$ ). This may be attributed to the involvement of azomethine nitrogen in coordination to the metal ion<sup>15</sup>.

In all the complexes a broad band relatively in the region of higher frequency between 3400- 3200  $\text{cm}^{-1}$  is observed indicating the presence of co-ordinated water molecules. The broadness of the band has been assigned to the combined  $\nu_{(\text{O-H})}$  and  $\nu_{(\text{N-H})}$  stretching mode of vibrations and intermolecular hydrogen bonding. The presence of coordinated water<sup>16</sup> is further supported by the appearance of another band at about 1025  $\text{cm}^{-1}$  due to  $\delta\text{H}_2\text{O}$ .

In order to ascertain the nature of the  $\text{NO}_3^-$  group (coordinated or free), the spectra of the nitrate complexes were recorded. However, no bands in the vicinity of 1285  $\text{cm}^{-1}$  and 1040  $\text{cm}^{-1}$  were observed, thereby ruling out the possibility of coordination of the  $\text{NO}_3^-$  group<sup>17</sup> to the metal ions and supporting the ionic nature of the complexes consistent with their high molar conductance values. Further the absorption at 1350-1370  $\text{cm}^{-1}$  in the acetate complexes was assigned to uncoordinated acetate ion.

The fundamental frequencies due to C–C and N–N have been noticed in the region of 1050–1030  $\text{cm}^{-1}$ . Another band in the region 3075–3050  $\text{cm}^{-1}$  appears for  $\nu_{\text{C-H}}$  of the phenyl ring. The disappearance of  $\nu_{\text{C}=\text{O}}$  (amidic) of the ligand (1680  $\text{cm}^{-1}$ ) in the spectra of the complexes and appearance of a medium intensity band in the region 900–880  $\text{cm}^{-1}$  due to  $\nu_{\text{C}=\text{O}}$  amply suggests amido-imidol tautomerism<sup>18</sup> existing in the system. Thus, the ligand acts as a tetradentate chelating agent, offering one primary amino group, two azomethine nitrogen atoms and one deprotonated imidol oxygen for coordination with metal ions (Scheme- 2). Assignment of the proposed coordination sites is further supported by the appearance of medium bands at 475- 487  $\text{cm}^{-1}$  and 560- 540  $\text{cm}^{-1}$  which could be attributed to  $\nu_{\text{M-N}}$  and  $\nu_{\text{M-O}}$  respectively<sup>19,21</sup>. The IR spectra of  $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$  and the spectral data of the ligand and its binuclear metal complexes are given below in Fig-1 and Table-2 respectively.


**Fig 1:** IR spectrum of  $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ 
**Table 2:** IR data of ligand and metal complexes

| COMPOUNDS  | Band positions in $\text{cm}^{-1}$ |                          |                                   |                   |                   |                   |
|--|------------------------------------|--------------------------|-----------------------------------|-------------------|-------------------|-------------------|
|  | $\nu(\text{O-H})+\nu(\text{N-H})$  | $\nu(\text{C}=\text{N})$ | $\nu(\text{C-C})+\nu(\text{N-N})$ | $\nu(\text{C-O})$ | $\nu(\text{M-O})$ | $\nu(\text{M-N})$ |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | 3200                               | 1597                     | 1024                              | 900               | 545               | 487               |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | 3226                               | 1593                     | 1026                              | 850               | 550               | 480               |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | 3369                               | 1579                     | 1060                              | 898               | 548               | 477               |
| $\text{Ni}_2(\text{L})_2\text{Cl}_2$                                     | 3236                               | 1591                     | 1024                              | 906               | 542               | 478               |
| $\text{Ni}_2(\text{L})_2(\text{NO}_3)_2$                                 | 3201                               | 1597                     | 1024                              | 904               | 548               | 475               |
| $\text{Ni}_2(\text{L})_2(\text{CH}_3\text{COO})_2$                       | 3261                               | 1597                     | 1022                              | 905               | 550               | 480               |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | 3300                               | 1560                     | 1050                              | 900               | 545               | 478               |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | 3250                               | 1595                     | 1025                              | 890               | 542               | 475               |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | 3265                               | 1556                     | 1035                              | 860               | 548               | 480               |

### 3.2 Thermal Analysis

Since the IR spectra of the complexes indicated presence of coordinated water, thermogravimetric analysis was carried out to ascertain their nature. The thermogram of the chloride complex of Copper(II) is recorded in Fig-2.

The complex undergoes two step decomposition processes. The first step is observed at around  $\sim 180^{\circ}\text{C}$  which corresponds to the weight loss due to the loss of four molecule of coordinated water.

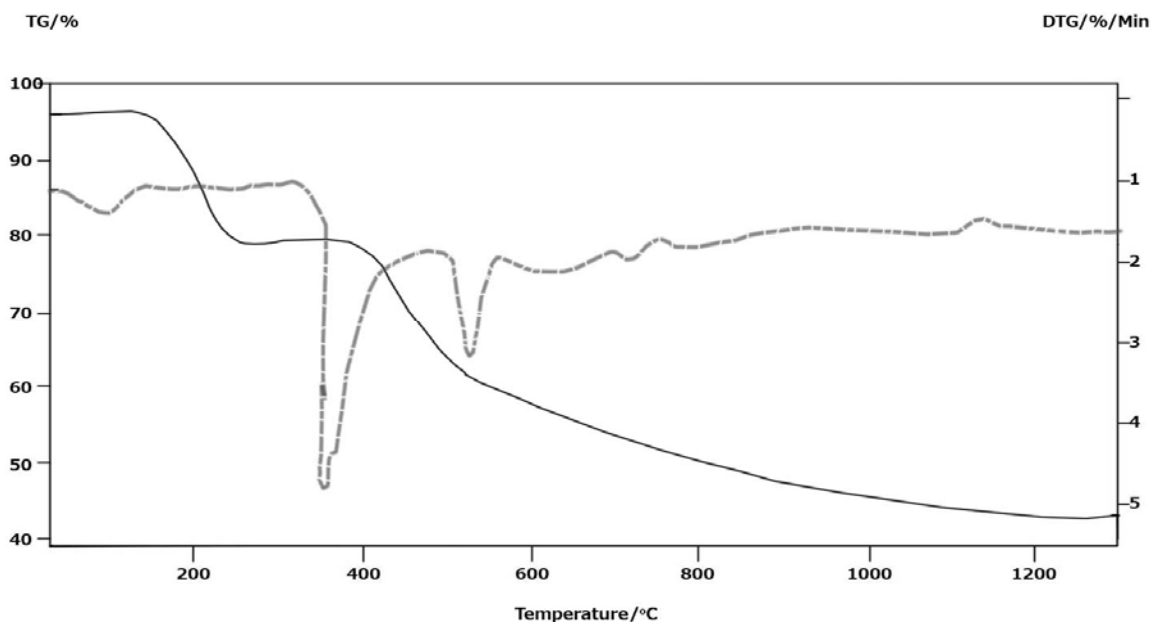
The second step of decomposition observed at around  $360^{\circ}\text{C}$  indicates the disassociation of the ligand molecule and their subsequent decomposition leaving behind copper oxide as the final product. The endothermic peaks occurring in the second and third step at  $380^{\circ}\text{C}$  and  $\sim 530^{\circ}\text{C}$ , respectively, correspond to the decomposition of organic constituents, leaving behind the respective metal oxides which are in conformity with system undergoing bond breaking in thermal process. The same thermal behaviour is also exhibited by Co – Co systems. The thermal stability of the complexes are in the order of  $\text{Cu} - \text{Cu} > \text{Co} - \text{Co}$ .

The thermoanalytical data of the binuclear metal complexes are given in Table-3.

**Table 3:Thermoanalytical data of binuclear metal complexes**

| Complex  | Temperature range of decomposition | % of Wt. loss found (Calc) | Remarks                      |
|--|------------------------------------|----------------------------|------------------------------|
| [Cu(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> Cl <sub>2</sub> | 180 <sup>0</sup> C                 | 7.34<br>(7.54)             | Loss of four water molecules |
|  | 360 <sup>0</sup> C                 | 16.17<br>(16.67)           | Formation of metal oxides    |
| [Co(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> Cl <sub>2</sub> | 170 <sup>0</sup> C                 | 7.38<br>(7.61)             | Loss of four water molecules |
|  | 350 <sup>0</sup> C                 | 15.73<br>(15.85)           | Formation of metal oxides    |

The TG– DTG curves of binuclear Schiff base Cu(II) complex is shown below in Fig-2.



**Fig 2: TG - DTG curves of [Cu (L)(H<sub>2</sub>O)<sub>2</sub>]<sub>2</sub>Cl<sub>2</sub> complex**

### 3.3 Electronic Spectra

The electronic spectra of the complexes  $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{X}_2$  display a broad band at  $19760 \text{ cm}^{-1}$  ( $506 \text{ nm}$ ) indicating Cu(II) centre to be in an approximately distorted octahedral environment. However, instead of getting three bands due to  ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$  ( $\nu_1$ ),  ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$  ( $\nu_2$ ),  ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$  ( $\nu_3$ ) transitions for  $d^9$  system<sup>22</sup>, only one broad band is observed suggesting that all the  $\nu_1$ ,  $\nu_2$ , and  $\nu_3$  bands are superimposed due to small energy difference into a single broad band which is analogous to  ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$  transition. The width and asymmetry of the band provide evidence for Jahn-Teller distortion. Further non occurrence of bands below  $10,000 \text{ cm}^{-1}$  ( $1000 \text{ nm}$ ) rules out the possibility of tetrahedral or pseudo tetrahedral geometry for the Cu(II) centre. The more intense band at higher frequency region near  $26,380 \text{ cm}^{-1}$  ( $379 \text{ nm}$ ) is believed to arise due to charge transfer (CT). The observed magnetic moment values of the complexes lie in the range 1.71 – 1.83 BM which is commensurate with spin free two hexa coordinated Cu(II) centre.

The electronic spectral studies of the complexes of  $\text{Ni}_2(\text{L})_2\text{X}_2$  exhibit only one band at  $22,230 \text{ cm}^{-1}$  ( $450 \text{ nm}$ ). The width of the band manifests that the band represents a group of two to three transitions arising out of  ${}^1\text{A}_{2g} \rightarrow {}^1\text{A}_{1g}$ ,  ${}^1\text{A}_{2g} \rightarrow {}^1\text{B}_{2g}$  and  ${}^1\text{A}_{2g} \rightarrow {}^1\text{E}_g$  transition under a square planar environment and superimposed into a single one<sup>23</sup>. This is confirmed from diamagnetic nature of the complexes.

In the complexes  $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2\text{X}_2$  three bands are observed in the region  $\sim 12,100 \text{ cm}^{-1}$  ( $826 \text{ nm}$ ),  $14,790 \text{ cm}^{-1}$  ( $676 \text{ nm}$ ) and  $16,400 \text{ cm}^{-1}$  ( $610 \text{ nm}$ ). The first band may be assigned to  ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$ , second band to  ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}$  and the third band to  ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{P})$  transitions respectively in an approximately octahedral field around cobalt(II) centres<sup>24</sup>. The high frequency band at  $27700 \text{ cm}^{-1}$  ( $361 \text{ nm}$ ) has been assigned to charge transfer (CT). The magnetic moment values of the complexes lie in the range 4.76- 4.87 BM. The magnetic moments in all the complexes are at higher values than the normal range. This is due to high spin octahedral structure for the complexes. The position of the electronic absorption bands indicates the presence of octahedral environment around Co(II) ion in all the complexes, which is supported by magnetic moment values. The electronic spectra and magnetic properties of complexes are given in Table – 4.

**Table 4: Electronic spectra and magnetic properties of complexes**

| Complexes  | Band Position in $\text{cm}^{-1}$ | Assignment   | $\mu_{\text{eff}}$ in BM |
|--|-----------------------------------|--|--------------------------|
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | 19760<br>26380                    | ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$<br>CT   | 1.81                     |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | 19538<br>26400                    | ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$<br>CT   | 1.71                     |
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | 19870<br>26900                    | ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$<br>CT   | 1.83                     |
| $\text{Ni}_2(\text{L})_2\text{Cl}_2$                                     | 22230                             | ${}^1\text{A}_{2g} \rightarrow {}^1\text{A}_{1g}$<br>${}^1\text{A}_{2g} \rightarrow {}^1\text{B}_{2g}$<br>${}^1\text{A}_{2g} \rightarrow {}^1\text{E}_g$ | Diamagnetic              |
| $\text{Ni}_2(\text{L})_2(\text{CH}_3\text{COO})_2$                       | 22470                             | ${}^1\text{A}_{2g} \rightarrow {}^1\text{A}_{1g}$<br>${}^1\text{A}_{2g} \rightarrow {}^1\text{B}_{2g}$<br>${}^1\text{A}_{2g} \rightarrow {}^1\text{E}_g$ | Diamagnetic              |



|  |                                   |   |             |
|--|-----------------------------------|---|-------------|
| $\text{Ni}_2(\text{L})_2(\text{NO}_3)_2$                                 | 22530                             | ${}^1\text{A}_{2g} \rightarrow {}^1\text{A}_{1g}$<br>${}^1\text{A}_{2g} \rightarrow {}^1\text{B}_{2g}$<br>${}^1\text{A}_{2g} \rightarrow {}^1\text{E}_g$  | Diamagnetic |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$               | 12,100<br>14790<br>16400<br>27700 | ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$<br>${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}$<br>${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$<br>CT | 4.76        |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2(\text{CH}_3\text{COO})_2$ | 12,400<br>14490<br>16670<br>27300 | ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$<br>${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}$<br>${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$<br>CT | 4.83        |
| $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2(\text{NO}_3)_2$           | 12600<br>14800<br>16940<br>27500  | ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$<br>${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}$<br>${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$<br>CT | 4.87        |

The electronic spectra of the complexes are given below in Fig-3(a), 3(b) and 3(c).

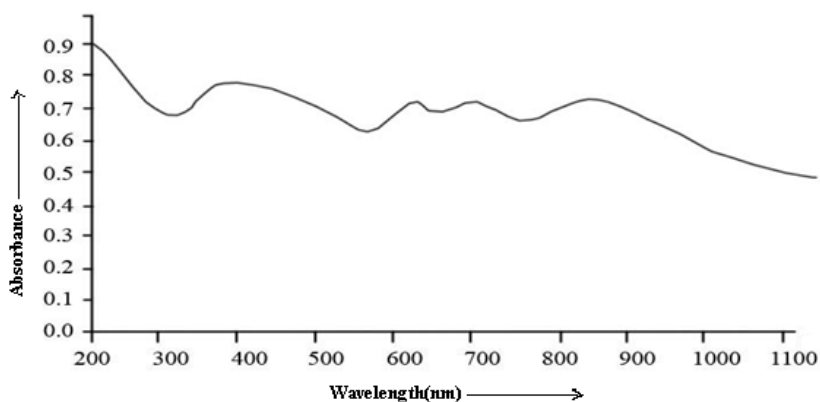


Fig 3(a): Electronic spectra of  $[\text{Co}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$

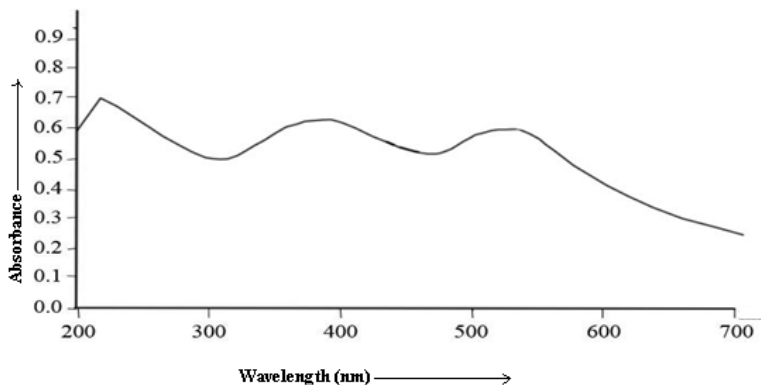


Fig 3(b): Electronic spectra of  $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$

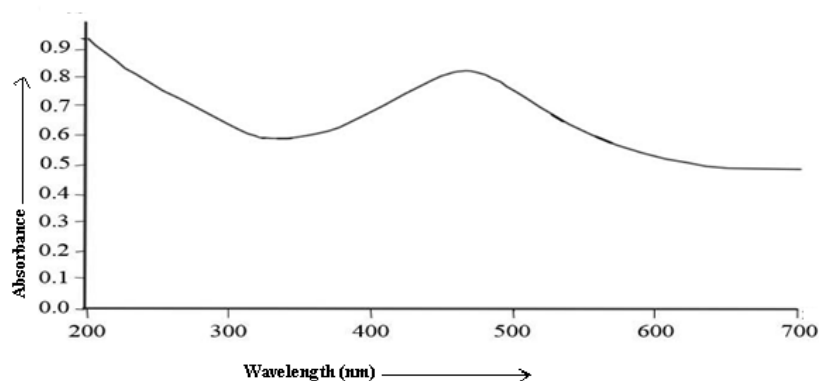


Fig 3(c): Electronic spectra of  $[\text{Ni}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$

### 3.4 ESR Spectra

The ESR spectra of metal complexes provide information about hyperfine and super hyperfine structures, which are of importance in studying the metal ion environment in complex, i.e., the geometry, nature of ligating sites of Schiff base and metal, and the degree of covalency of metal- ligand bonds .

The ESR spectra of Cu(II) complex were measured at X-band frequencies at 9.862117 GHz. ESR-spectrum of Cu(II) complex provides information about the coordination environment around Cu(II) ion. The spectrum of Cu(II) complex exhibits two signals at  $g_{\perp}$ ,  $g_{\parallel}$  and  $g_{\text{aver}} = (g_{\parallel} + 2g_{\perp}) / 3 = 2.214$ . These values indicate the presence of unpaired electron in  $d_{x^2-y^2}$  orbital giving octahedral geometry<sup>25</sup>.

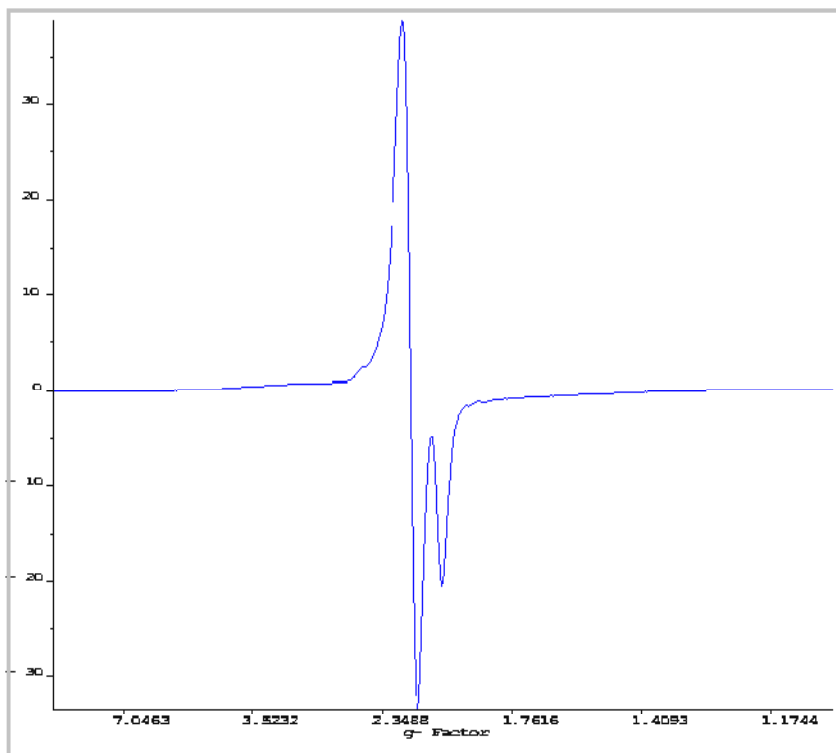
In the axial spectra, the  $g$ -values are related with exchange interaction coupling constant ( $G$ ) by the expression  $G = (g_{\parallel} - 2) / (g_{\perp} - 2)$ . The values of  $G$  reflect the spin interaction between Cu(II) centers of the binuclear Cu(II) complex. According to Hathaway and Billing<sup>26</sup> if  $G > 4$ , the spin exchange interaction is negligible and if  $G < 4$  considerable spin – exchange interaction prevails. In our present investigation  $G$  value comes out to be 4.91 which suggests negligible spin exchange interaction between the two copper ions that indicates the monomeric nature of the complex<sup>27</sup>. These data are in agreement with those obtained from the electronic spectra which confirm the octahedral geometry for Cu(II) Schiff base complexes.

The ESR spectral data of binuclear Cu(II) complex is given below in Table-5.

Table 5: ESR spectral data of binuclear Cu(II) complex

| Complex  | $g_{\parallel}$ | $g_{\perp}$ | $g_{\text{aver}}$ | $\Delta g$ | $G$  |
|--|-----------------|-------------|-------------------|------------|------|
| $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$ | 2.457           | 2.093       | 2.211             | 0.364      | 4.91 |

The ESR spectrum of binuclear Cu(II) complex is given below in Fig- 4.


 Fig 4: ESR spectrum of  $[\text{Cu}(\text{L})(\text{H}_2\text{O})_2]_2\text{Cl}_2$ 

### 3.5 Fungicidal Activities

Schiff base ligand and their Cu(II), Ni(II) and Co(II) complexes were screened in vitro in order to evaluate their antifungal activity against *A. flavus* and *Fusarium* species (Table- 6) at two different concentrations. The results showed that the complexes are more toxic as compared to their parent ligands against the same microorganism under identical experimental conditions. The increased activity of the metal chelates can be explained on the basis of chelation theory<sup>28</sup>. The increase in the antifungal activity of metal chelates may be due to the effect of the metal ion on the normal cell process. A possible mode of toxicity increase may be considered in the light of Tweedy's chelation theory<sup>29</sup>. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layers of cell membrane. All the metal complexes possess higher antifungal activity than the free ligand.

The anti-fungal activities of the complexes were tested by the method of Horsfall<sup>30</sup>. The evaluation was carried out at concentration level 100 ppm in dioxane. The amount of germination or growth inhibition was determined after inoculation of the fungal spores into Czapekdox agar- agar media. Spores were also inoculated onto the agar- agar media containing the test sample. The whole system was kept in an incubator for five days. The percentage of inhibition was calculated as follows:

$$\% \text{ of inhibition} = 100 (P-Q)/p$$

Where P= area of colony growth without test sample and

Q= area of the colony growth with the test sample.

The binuclear copper (II) complexes are found to be more active than the other binuclear complexes and the ligand. The activity order is found to be Cu(II) > Co(II) > Ni(II) > L and with respect to anion the homo binuclear complexes follow the order  $\text{Cl}^- > \text{NO}_3^- > \text{CH}_3\text{COO}^-$ . The statistical calculations adopting two ways ANOVA test<sup>31</sup> have also indicated significant difference with respect to different treatment of complexes (Table-6).

**Table 6: Fungitoxicity of the Complexes at Concentration Level 100 ppm**  
Values are expressed as mean  $\pm$  S.E.M, (n=3)

| Complexes   | % inhibition over control |                                |                            |
|---|---------------------------|--------------------------------|----------------------------|
|   | <i>Aspergillus niger</i>  | <i>Helminthosporium oryzae</i> | <i>Fusarium oxysporium</i> |
| Ligand  | 19.547 $\pm$ 0.186        | 23.646 $\pm$ 0.131             | 26.610 $\pm$ 0.106         |
| [Cu(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> Cl <sub>2</sub>                    | 29.480 $\pm$ 0.091        | 34.346 $\pm$ 0.074             | 36.360 $\pm$ 0.084         |
| [Cu(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> (CH <sub>3</sub> COO) <sub>2</sub> | 23.517 $\pm$ 0.107        | 26.667 $\pm$ 0.213             | 30.560 $\pm$ 0.156         |
| [Cu(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>    | 20.277 $\pm$ 0.093        | 22.670 $\pm$ 0.107             | 29.630 $\pm$ 0.065         |
| Ni <sub>2</sub> (L) <sub>2</sub> Cl <sub>2</sub>  | 25.54 $\pm$ 0.173         | 22.617 $\pm$ 0.120             | 33.310 $\pm$ 0.058         |
| Ni <sub>2</sub> (L) <sub>2</sub> (CH <sub>3</sub> COO) <sub>2</sub>                     | 20.793 $\pm$ 0.111        | 20.483 $\pm$ 0.088             | 28.473 $\pm$ 0.088         |
| Ni <sub>2</sub> (L) <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>                        | 17.393 $\pm$ 0.098        | 19.467 $\pm$ 0.175             | 26.633 $\pm$ 0.136         |
| [Co(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> Cl <sub>2</sub>                    | 23.380 $\pm$ 0.083        | 21.603 $\pm$ 0.068             | 32.637 $\pm$ 0.234         |
| [Co(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> (CH <sub>3</sub> COO) <sub>2</sub> | 19.607 $\pm$ 0.176        | 19.480 $\pm$ 0.058             | 25.550 $\pm$ 0.115         |
| [Co(L)(H <sub>2</sub> O) <sub>2</sub> ] <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>    | 16.530 $\pm$ 0.153        | 18.493 $\pm$ 0.170             | 24.713 $\pm$ 0.069         |

#### 4. Conclusion

A series of macrocyclic Schiff base binuclear complexes were prepared via the template condensation of benzilmonohydrazone with isoniazid in the presence of Cu(II), Ni(II) and Co(II) metal ions. Based on the results of the investigation, octahedral binuclear structures are proposed for the metal complexes of Cu(II) and Co(II) and square planar structure for Ni(II). The copper(II) and cobalt(II) complexes are found to be more effective antifungal agents as compared to the Ni(II) complex. Keeping in view the rising problems of antimicrobial resistance these chemical compounds may be used for formulating novel chemotherapeutic agents and further investigation will be necessary to identify the active principle.

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