

## SYNTHESIS AND SPECTROSCOPIC CHARACTERIZATION OF AMINO ACID BASED SCHIFF BASE LIGANDS AND ITS COMPLEXES AS MICROBIAL GROWTH INHIBITORS

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### ABSTRACT

Metal chelates of novel Schiff bases obtained by the condensation of Glutaraldehyde with L-Glutamine and L-Leucine have been synthesized. The structure, complexation behavior and morphology of the synthesized compounds were obtained from their elemental analyses, TG-DTA, magnetic susceptibility and molar conductivity measurements, powder XRD, IR, UV-Vis,  $^1\text{H}$  NMR spectral studies and SEM analysis. The stoichiometric reaction between the metal (II) ions and ligands in the molar ratio M:L (1:1) [where M = Zn and Cd] resulted in the formation of six-coordinate octahedral complexes. The bioefficacy of the ligands and their complexes have been examined against the growth of microbes *in vitro* to evaluate their antimicrobial potential. The results indicate that the metal complexes possess notable antimicrobial properties than the corresponding ligands

**Keywords:** Schiff base; Metal chelate; Spectral studies; Powder X-ray diffraction.

### 1. INTRODUCTION

Complexes of transition metal ions with multidentate organic ligands have been the subject of intensive research because they not only have interesting spectral and magnetic properties but also possess a diverse spectrum of biological activities. The unique ability of transition metal ions and their complexes to control the chemistry of environmental, industrial, and biological processes has increased the importance of clarifying their mechanistic behavior in simple and complex chemical processes. Schiff bases are compounds containing azomethine group (-CH=N-) and have often been used as chelating ligands in the field of coordination chemistry and their metal complexes are of great interest for many years. They are the condensation products of ketones or aldehydes with primary amines and was first investigated by Hugo Schiff<sup>1</sup>. Metal Complexes of N, O-donor ligands in particular, have played a prominent role in this area because many of these complexes may serve as model for biologically important species such as metalloenzymes and also have a variety of applications in biological, clinical, analytical and industrial fields in addition to their important roles in catalysis and organic synthesis. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities. Formation of Schiff base generally takes place under acid or base catalysis or with heat. Bases obtained from amines and aldehydes have applications in various disciplines of chemistry<sup>2,3</sup>. Several of these biomimetic Schiff bases are gifted with antimicrobial and antitumor properties and could be used against HIV<sup>4,5</sup>. Schiff bases derived from amino acid are highly unstable and are generated on site<sup>6</sup>. Metal Schiff base complexes with biomolecules have been widely studied because they have a wide range of activities including industrial, anticancer, antiviral and herbicidal applications<sup>7,8</sup>. They could perform as models for many biological species and find applications in many biomimetic catalytic reactions<sup>9</sup>. Zinc can function as active site of hydrolytic enzymes, where it is ligated by hard donors (N or O). It has long been recognized as an important co-factor in biological molecules, either as a structural template in protein folding or as a Lewis acid catalyst that can readily adopt the coordination numbers 4, 5, or 6. The catalytic role of Zinc comprises Lewis acid activation of substrate, generation of a reactive nucleophile (Zn-OH) and stabilization of the leaving group. There is substantial interest in the coordination chemistry of Cadmium complexes because of the toxic environmental impact of Cadmium. The

mobilization and immobilization of Cadmium in the environment, in organisms, and in some technical processes (such as in ligand exchange chromatography) have been shown to depend significantly on the complexation of the metal center by chelating nitrogen donor ligands. A report has shown that cadmium Schiff-base complexes have an acceptable potential for inhibition of corrosion. Recently in analytical chemistry point of view, the utility of zinc, cadmium, and mercury Schiff-base complexes for construction of some ion selective electrodes have been reported. The variety of possible Schiff base metal complexes with wide choice of ligands and coordination environments has prompted to undertake research in this area. Here the syntheses, characterization, antimicrobial studies and complexation behavior of two Schiff bases obtained from Glutaraldehyde with L-Glutamine and L-Leucine have been reported.

## 2. EXPERIMENTAL

### 2.1 Chemicals

All the chemicals and solvents used for the synthesis were of reagent grade and were obtained commercially from Merck with the exception of the Cadmium nitrate, which was obtained from Aldrich. The solvents were purified by standard methods. The ligands were synthesized and characterized according to a published method<sup>11</sup> from Glutaraldehyde with L-Glutamine and L-Leucine.

### 2.2 Physical measurements

Microanalytical data of the compounds were recorded using a Vario EL III elemental analyzer at Sophisticated Analytical Instruments facility, CUSAT, Kochi. Ultraviolet spectra were recorded using Shimadzu double beam visible spectrophotometer in the visible region. The molar conductance of the metal complexes were determined in DMSO on SYSTRONICS digital conductivity meter. Magnetic susceptibility of the complexes were measured by Guoy balance using Copper sulphate as calibrant. IR spectra of the Schiff bases and its complexes in the range of 4000 to 400  $\text{cm}^{-1}$  were recorded on a Perkin Elmer FT-IR spectrometer MODEL 1600 as KBR discs.  $^1\text{H}$  NMR spectra (300 MHz) of the samples in  $\text{DMSO-d}_6$  were recorded on by employing TMS as internal standard at NIIST Trivandrum. Powder XRD were recorded on a computer controlled X-ray diffractometer system JEOL JDX 8030 at NIIST Trivandrum. SEM images were recorded at Sophisticated Analytical Instruments facility, CUSAT, Kochi. Thermal behavior of the samples were recorded in a thermal analyzer at NIIST Trivandrum. Double distilled water was used throughout the experimental work. The In vitro and in vivo antimicrobial study of the compounds were tested by Kirby-bauer Disc diffusion method.

### 2.3 Synthesis of Schiff base ligands

A solution of L-Glutamine (1 mmol in 20 mL methanol) was added drop-wise, under stirring to a solution of Glutaraldehyde (1 mmol in 10 mL methanol). A solution of 0.1 M NaOH was added to adjust the pH at 7-8 and the reaction mixture was refluxed for 3 h. The resulting yellowish mass product was filtered off, washed with distilled water, dried under vacuum recrystallised and finally preserved in desiccators. The other Schiff base from Glutaraldehyde and L-Leucine was also prepared by following the same method.

### 2.4 Synthesis of Schiff base transition metal complex

Metal (II) nitrates (1 mmol) were dissolved in 20  $\text{cm}^3$  of methanol. The filtered solutions were added dropwise into 20  $\text{cm}^3$  methanol solutions of the Schiff base ligands, the resulting mixtures were refluxed and stirred for 8 hours. After refluxing, the volume of the solutions were reduced to one third and the concentrate were cooled at 0°C. The precipitated complexes were filtered off, washed several times with cold ethanol and dried *in vacuo* over anhydrous  $\text{CaCl}_2$ .

### 2.5. Antimicrobial Activity

In vitro antimicrobial tests were carried out by an adapted agar-disc diffusion technique using 0.5 McFarland suspension of bacteria obtained from 24 h cultures. The antimicrobial activities of the synthesized compounds were determined against *Klebsiella sps*, *Escherichia coli*, *Staphylococcus aureus* (bacterias) and *Candida sps*, *Aspergillus niger* and *Aspergillus fumigates* (fungi) microbial strains. The compounds were solubilised in dimethylsulfoxide to a final concentration of 1 mg/mL. A volume of 10  $\mu\text{L}$  of each tested compounds solution was distributed directly on the solid medium previously seeded with the microbial inoculums. The inoculated petri plates were incubated for 24 h at 37°C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters.

### 3. RESULTS AND DISCUSSION

All the complexes were obtained as air-stable amorphous solids which decompose without melting. They were insoluble in water, partially soluble in methanol and ethanol, totally soluble in DMF and DMSO. They were identified by their physical and analytical data (Table 1).

**Table 1: Physical characterization, analytical, molar conductance and magnetic susceptibility data of the synthesized compounds**

Ligand/Metal Chelate	Empirical Formula	Colour	M:L ratio	Molar Cond. ( $\text{Ohm}^{-1} \text{cm}^2 \text{mol}^{-1}$ )	Elemental analysis % Found (calculated)			
					C	H	N	M
HGlu(glu) <sub>2</sub>	C <sub>15</sub> H <sub>24</sub> N <sub>6</sub> O <sub>4</sub>	Yellow	-	-	53.43 (53.0)	7.15 (7.18)	13.20 (13.17)	-
ZnGlu(glu) <sub>2</sub> .2 H <sub>2</sub> O	C <sub>15</sub> H <sub>28</sub> N <sub>4</sub> O <sub>8</sub> Zn	White	1:1	0.074	39.15 (39.0)	6.10 (6.19)	11.81 (12.28)	13.81 (13.93)
CdGlu(glu) <sub>2</sub> .2 H <sub>2</sub> O	C <sub>15</sub> H <sub>28</sub> N <sub>4</sub> O <sub>8</sub> Cd	Pale Yellow	1:1	0.069	39.45 (39.8)	6.30 (6.17)	11.89 (12.38)	13.91 (13.98)
HGlu(leu) <sub>2</sub>	C <sub>17</sub> H <sub>30</sub> N <sub>4</sub> O <sub>2</sub>	Yellow	-	-	59.98 (59.8)	9.34 (9.29)	9.67 (9.59)	-
ZnGlu(leu) <sub>2</sub> .2 H <sub>2</sub> O	C <sub>17</sub> H <sub>34</sub> N <sub>2</sub> O <sub>6</sub> Zn	White	1:1	0.075	47.81 (47.9)	8.01 (8.03)	6.48 (6.57)	14.55 (14.91)
CdGlu(leu) <sub>2</sub> .2 H <sub>2</sub> O	C <sub>17</sub> H <sub>34</sub> N <sub>2</sub> O <sub>6</sub> Cd	Pale Yellow	1:1	0.068	49.9 (48.9)	9.2 (9.89)	7.65 (7.34)	13.65 (13.87)

#### 3.1 UV-Visible Spectra

The electronic absorption spectra of the Schiff base ligands and its complexes were recorded in DMSO solution in the range of 200 to 800 nm regions. It is usually recorded as a plot of absorbance (A) versus wavelength ( $\text{cm}^{-1}$ ). The electronic spectra were determined at room temperature and the data obtained were correlated with magnetic moment values and ligand field parameters: splitting energy (10Dq), interelectronic repulsion parameter (B), and nephelauxetic ratio ( $\beta$ ). Upon the electronic spectrum of the Schiff base ligands, intense bands centered at 285 nm and 290 nm were observed which was assigned to  $\pi - \pi^*$  transition of the C=N chromophore. On complexation, the bands were shifted to lower wavelength region at 245 nm and 255 nm that suggests the coordination of azomethine nitrogen with the metal ions. Other transitions in the range of 340 and 350 nm, can be assigned to  $n - \pi^*$  transition. The weak bands at 400-500 nm were due to charge transfer band in the complex that does not exist in the ligands. The spectrum for the Cd(II) complex exhibited bands at 772 and 829 nm, indicative of charge transfer transition (M→L) which is expected for Cd(II) with filled 4d orbital in the ground state. An octahedral geometry is however proposed for the Cd(II) complexes. However, the electronic spectra of Zn(II) and Cd(II) complexes does not contain d-d transitions and was found to be diamagnetic.

#### 3.2 IR Spectral Study

A comparison of the IR spectra of the complexes and those of the free Schiff base ligand allows us to determine the coordination sites that could be involved in chelation process. The spectra of these complexes contain a broad band around 3000–3360  $\text{cm}^{-1}$  attributed to  $\nu(\text{OH})$ . Additionally, the band at 1657  $\text{cm}^{-1}$  and 1645  $\text{cm}^{-1}$  attributed to  $\nu(\text{HC=N})$  from the Schiff base is shifted to lower values (1620–1630  $\text{cm}^{-1}$ ) in the complexes, which suggests that the Schiff base ligand is coordinated to the metallic ion by the N atom in the azomethine group. The absorption band at 2800  $\text{cm}^{-1}$  attributed to the formation of an intramolecular hydrogen bonding does no longer appear in the complexes spectra, which proves the deprotonation of –OH group. This shift indicates the participation of the O atom of the deprotonated hydroxyl group in the formation of the M–O bonds. Accordingly, the ligands act as a tetradentate chelating agent, bonded to the metal ion through the four nitrogen atoms of the Schiff base. The metal complexes are also characterized by the appearance of some new bands at 510–520  $\text{cm}^{-1}$  and 419–423  $\text{cm}^{-1}$ , which are assigned to  $\nu(\text{M–O})$  and  $\nu(\text{M–N})$  stretching frequencies, respectively. The IR spectra of the synthesized Schiff base complexes are given in Fig 1-4.

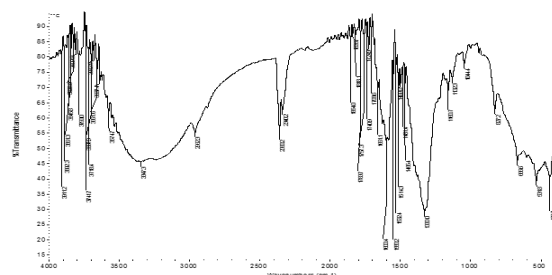


Fig. 1: IR Spectra of ZnGlu(glu)<sub>2</sub>.2H<sub>2</sub>O complex

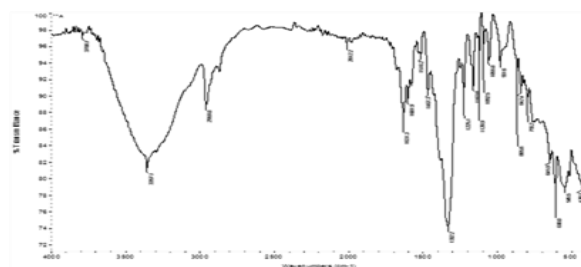


Fig. 2: IR Spectra of CdGlu(glu)<sub>2</sub>.2H<sub>2</sub>O complex

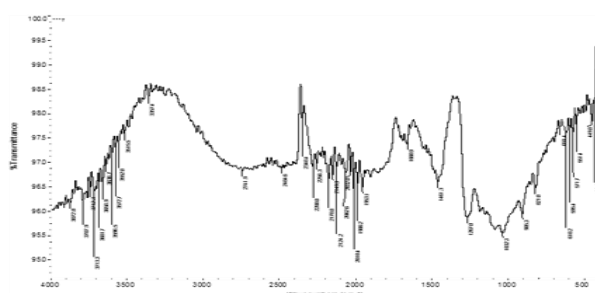


Fig. 3: IR Spectra of ZnGlu(leu)<sub>2</sub>.2H<sub>2</sub>O complex

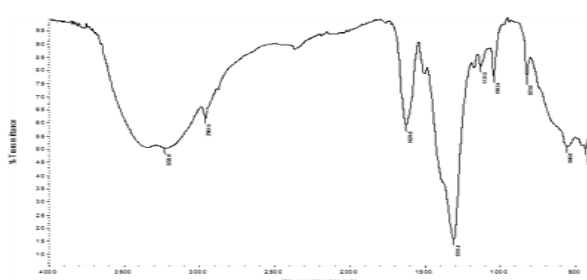
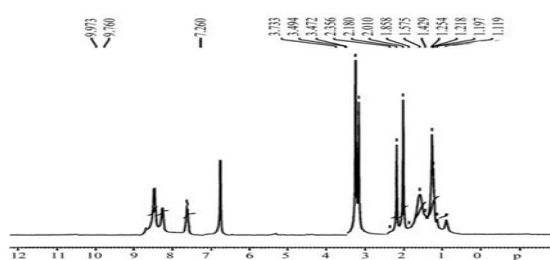
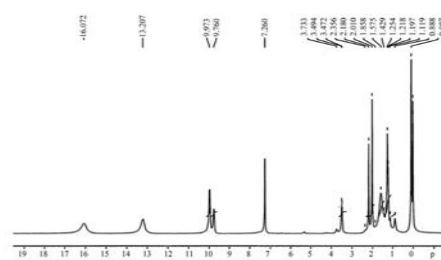
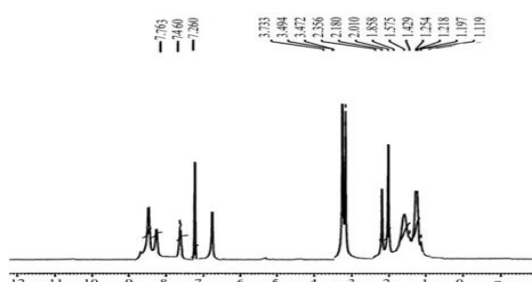
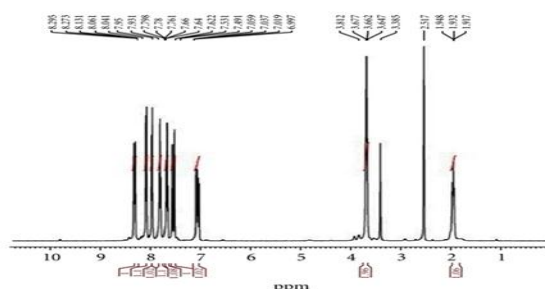


Fig. 4: IR Spectra of CdGlu(leu)<sub>2</sub>.2H<sub>2</sub>O complex

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

The <sup>1</sup>H NMR Spectrum of the ligand H Glu(glu)<sub>2</sub> and H Glu(glu)<sub>2</sub> recorded in DMSO solution shows a multiplet at 2.5, 2.3, 1.6 and 1.8 ppm due to the methyl protons. Furthermore, the <sup>1</sup>H NMR Spectrum of the Schiff base complexes ZnGlu(glu)<sub>2</sub>.2H<sub>2</sub>O, CdGlu(glu)<sub>2</sub>.2H<sub>2</sub>O, ZnGlu(leu)<sub>2</sub>.2H<sub>2</sub>O, and CdGlu(leu)<sub>2</sub>.2H<sub>2</sub>O exhibited signals at 8.4, 8.8, 8.1, 8.7 ppm and 7.8, 7.9, 7.5, 7.4 ppm, that is attributable to CH=N- and -NH protons respectively **Fig. 5-8**. The azomethine proton signal in the spectrum of the corresponding complexes are shifted downfield compared to the free ligands, suggesting the deshielding of the azomethine group due to the coordination with the metal ions. There are no appreciable change in all other signals of the complexes.

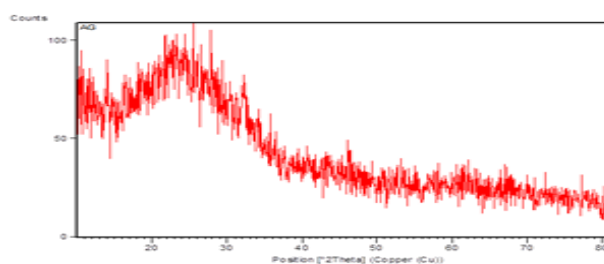
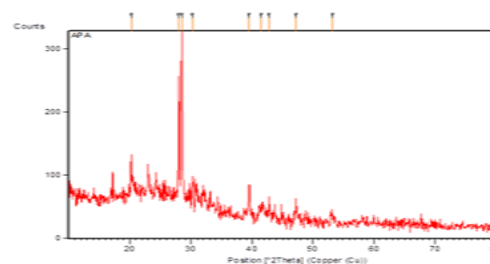
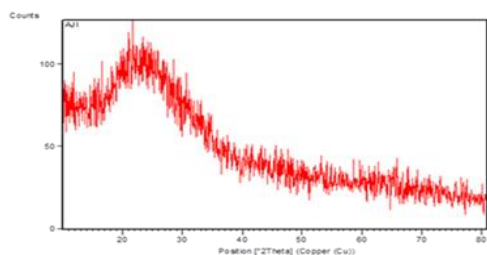
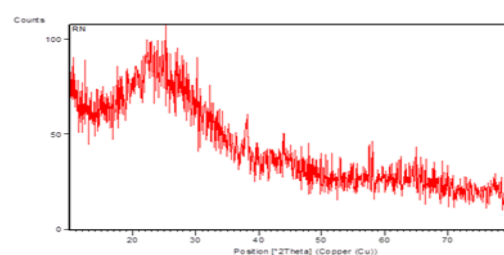
Fig. 5: <sup>1</sup>H NMR Spectra of ZnGlu(glu)<sub>2</sub>.2H<sub>2</sub>OFig. 6: <sup>1</sup>H NMR Spectra of CdGlu(glu)<sub>2</sub>.2H<sub>2</sub>OFig. 7: <sup>1</sup>H NMR Spectra of ZnGlu(leu)<sub>2</sub>.2H<sub>2</sub>OFig. 8: <sup>1</sup>H NMR Spectra of CdGlu(leu)<sub>2</sub>.2H<sub>2</sub>O

### 3.5 Powder XRD Study

X-ray diffraction data was recorded by using Cu K $\alpha$  radiation (1.5406 Angstrom). The intensity data was collected over a  $2\theta$  range of 4-60°. The mean grain size of the particles was determined from the XRD line broadening measurement using Scherrer's equation(1)

$$d_{\text{XRD}} = 0.9 \lambda / \beta \cos \theta \quad (1)$$

Where  $\lambda$  is the wavelength (Cu K $\alpha$ ),  $\beta$  is the full width at half maxima (FWHM) and  $\theta$  is the diffraction angle. All the diffraction peaks in the X-ray diffraction pattern of the Schiff base metal complexes agreed with the reported standard data and no characteristic peaks were obtained. The lattice parameters calculated were also in agreement with the reported values. The powder XRD shows that ZnGlu(glu)<sub>2</sub>.2H<sub>2</sub>O and CdGlu(glu)<sub>2</sub>.2H<sub>2</sub>O complexes has the crystallite size of 45 nm and 48 nm. XRD diffractogram also shows ZnGlu(leu)<sub>2</sub>.2H<sub>2</sub>O and CdGlu(ala)<sub>2</sub>.2H<sub>2</sub>O complexes has the crystallite size of 41 nm and 47 nm suggesting the microcrystalline nature. The powder XRD pattern of the Zn(II) and Cd(II) Schiff base complexes are given in Fig 9-12.

Fig. 9: XRD pattern of ZnGlu(glu)<sub>2</sub>.2H<sub>2</sub>OFig. 10: XRD pattern of CdGlu(glu)<sub>2</sub>.2H<sub>2</sub>OFig. 11: XRD pattern of ZnGlu(leu)<sub>2</sub>.2H<sub>2</sub>OFig.12: XRD pattern of CdGlu(leu)<sub>2</sub>.2H<sub>2</sub>O



### 3.6 Scanning Electron Microscope

The scanning electron micrographs revealed the morphology of Schiff base ligands and its corresponding metal complexes. In SEM image macroscopic phase separation in dense layer was noticed. The domain size of about 100 $\mu\text{m}$  with void spaces were observed in all the systems. The void spaces are certainly due to the result of macroscopic phase separation. In scanning electron micrograph of the ligands, the morphology showed that the surface was spongy and soft with large macroscopic phase separation. This phase separation was minimized after the formation of complexes. Also the spongy and softness was reduced due to introduction of metal ion. The similar observations were reported for the reduction in phase separation and softness of the surface on complexation. From SEM images, it was clear that there was a strong change in morphology of Schiff base on complexation. The SEM images of Schiff base complexes are given in Fig.13- 16.

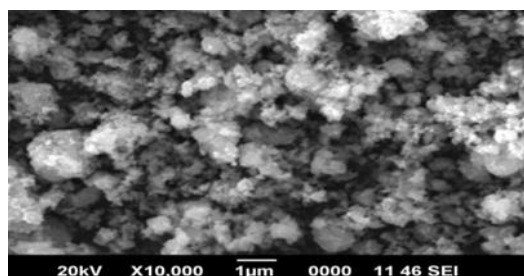


Fig. 13: SEM image of ZnGlu(glu)<sub>2</sub>·2H<sub>2</sub>O

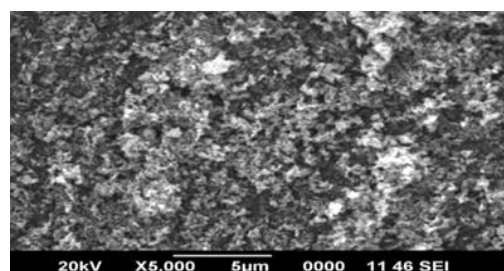


Fig. 14: SEM image of CdGlu(glu)<sub>2</sub>·2H<sub>2</sub>O

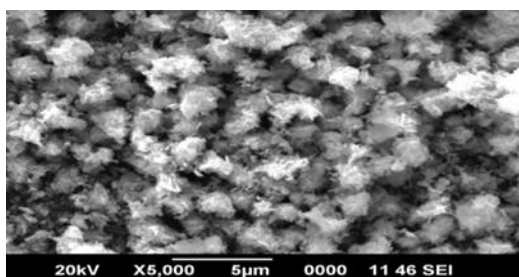


Fig. 15: SEM image of ZnGlu(leu)<sub>2</sub>·2H<sub>2</sub>O

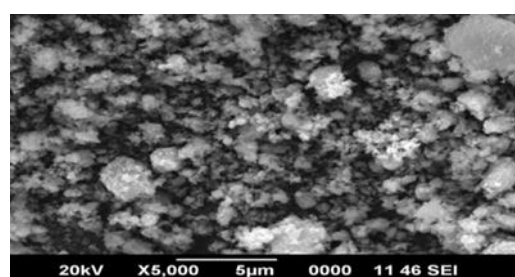


Fig. 16: SEM image of CdGlu(leu)<sub>2</sub>·2H<sub>2</sub>O

### 3.7 Thermal analysis

Thermal analyses data of the Schiff base and its complexes were used to obtain information about the thermal stability of these species as well as to verify the presence and status of water molecules. In the present investigation, the following thermo analytical methods have been used: (1) thermogravimetric analysis (TG) and (2) differential thermal analysis (DTA). Thermogravimetric analyses for the Zn(II) and Cd(II) complexes were carried out from room temperature to 900 $^{\circ}\text{C}$  and show nearly the same pattern. Calculated and experimental mass losses are comparable. The weight loss in the range 180–240 $^{\circ}\text{C}$  with experimental mass loss of 2.55-2.75% in all the complexes indicates the loss of two coordinated water molecules (calculated value, 2.86%). This temperature required for water loss indicates that water molecules are strongly bonded to the metal ion and this type of thermal behaviour is characteristic of coordinated water molecules. From 220 $^{\circ}\text{C}$  to 463 $^{\circ}\text{C}$ , a sharp decrease in weight indicated the loss of fragments from two Schiff base molecules from the complexes with experimental mass loss of 47.32-49.32% for all the complexes, respectively. In the final stage, which occurs in the 480-650 $^{\circ}\text{C}$  temperature range, both decomposition products with experimental mass loss of 32.75–33.75% for the complexes and black residue were eliminated. Chemical analysis of the black final residue corresponds to the metallic oxide. In conclusion, TG experiments revealed the nature of complex species as anhydrous and confirmed their compositions suggested by the analytical data.

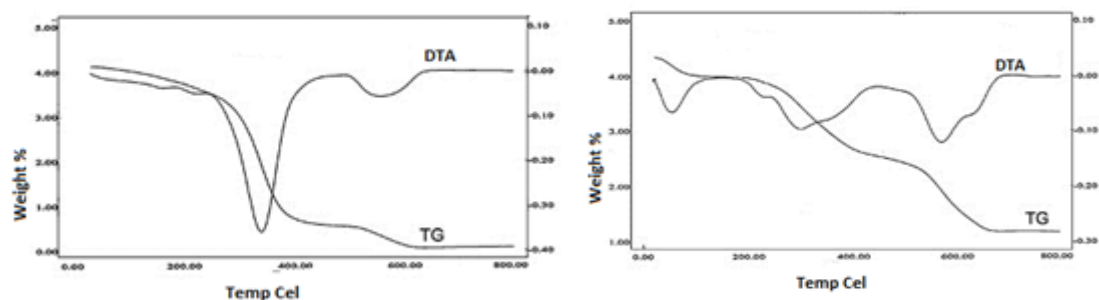


Fig. 17: TG/DTA pattern of  $\text{ZnGlu(glu)}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{CdGlu(glu)}_2 \cdot 2\text{H}_2\text{O}$  complexes

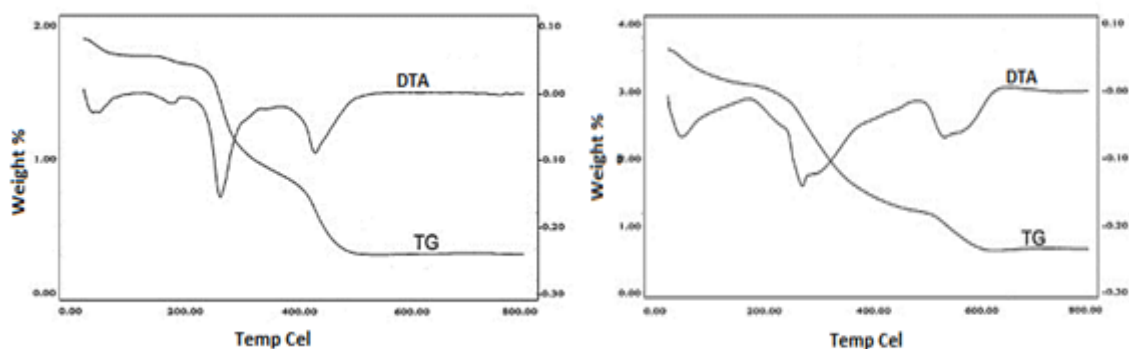
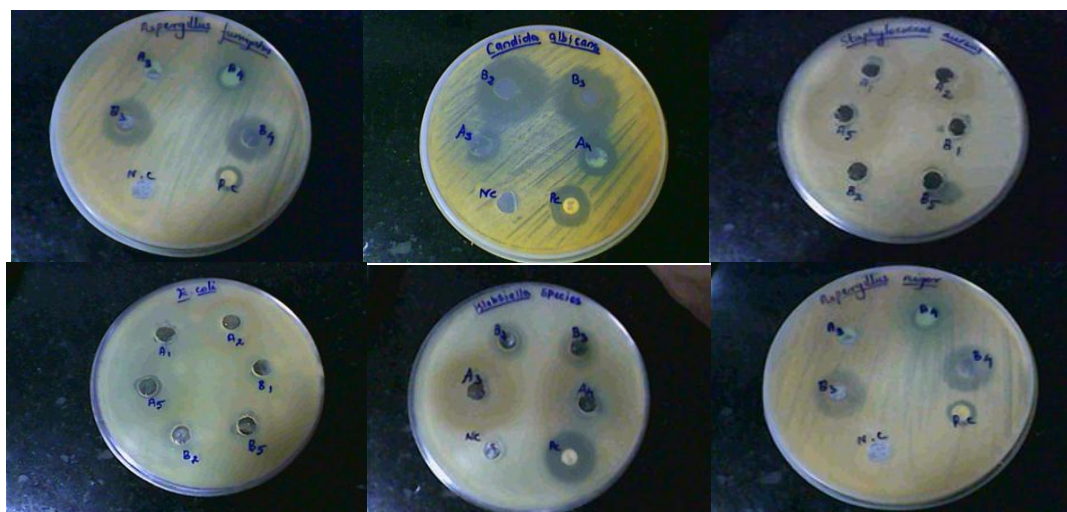


Fig. 18: TG/DTA pattern of  $\text{ZnGlu(leu)}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{CdGlu(leu)}_2 \cdot 2\text{H}_2\text{O}$  complexes

### 3.8. In Vitro Antimicrobial activity

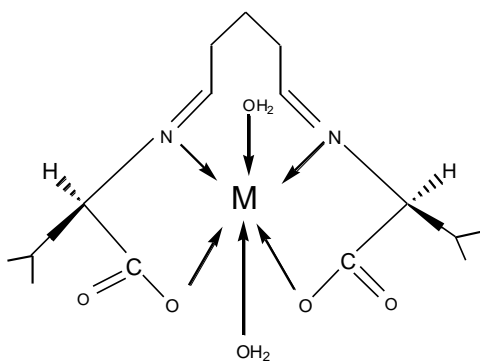
The qualitative screening of the susceptibility spectra of various microbial strains to newly synthesized compounds showed that all tested compounds exhibited antimicrobial effect quantified by the occurrence of a growth inhibition zone. For all tested complexes, the diameters of the inhibition zones were superior to those exhibited by DMSO alone and, also, those exhibited by the ligand, suggesting that the antimicrobial activity of the obtained complexes is clearly superior to that of the ligand. The antimicrobial activities of some of the complexes  $\text{CdGlu(glu)}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{CdGlu(leu)}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{ZnGlu(glu)}_2 \cdot 2\text{H}_2\text{O}$  against *Klebsiella sps*, *Aspergillus fumigates*, *Candida albicans* and *Aspergillus niger* were significantly higher than the standard drug, indicating their potentials as antimicrobial agents against these microbes. The lowest antimicrobial spectrum was noticed for the  $\text{ZnGlu(leu)}_2 \cdot 2\text{H}_2\text{O}$  complex, while the largest inhibitions zones were exhibited by the  $\text{ZnGlu(glu)}_2 \cdot 2\text{H}_2\text{O}$  complex and it was proved to be active against all tested microbial strains. Tweedy's chelation theory offers an explanation for the increased antimicrobial activity of the metal complexes. The increased lipophilic character of these coordinated compounds, with the resultant enhanced ability to permeate the cell membrane of the microbes, have been suggested as reasons for their improved activity over their parent ligands. Chelation, which has been reported to reduce the polarity of the metal ion by partial sharing of its positive charge with the donor group of ligands, also supports this theory. In the chelated complex, the positive charge of the metal ion is partially shared with the donor atoms of the ligand and electron delocalization occurs over the whole chelate ring. In this way, the lipophilic character of the metal chelate is increasing and favouring its permeation through the lipid layers of the bacterial membranes and blocking the metal binding sites in the microorganism.



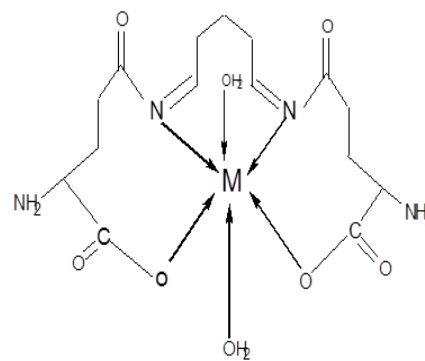
**Fig. 19: Antimicrobial activities of Schiff base ligands and its complexes by disc diffusion method against various microbial species (Zone inhibition)**

#### 4. CONCLUSION

Four metal (II) complexes with the Schiff base derived by the condensation of Glutaraldehyde with L-L-Glutamine and L-Leucine have been synthesized and characterized. Data from IR spectra concluded that the ligand behaves as a tetradentate ligand coordinated in all the complexes. Electronic spectra and magnetic measurements indicate an octahedral geometry for Zn(II) and Cd(II) complexes. The results from the biological activity demonstrated that the newly synthesized complexes could exhibit, in some cases, improved antimicrobial activity against both bacteria and fungi, superior to that of the ligand and the included antibiotic. Hence, chelation may serve as a useful tool in the design of potential antimicrobial agents.



Where M is Cd or Zn



**Fig. 20: Structure of HGlu(leu)<sub>2</sub> complexes**

**Fig. 21: Structure of HGlu(glu)<sub>2</sub> complexes**

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