

Synthesis, Characterization and Antibacterial Activity of Cu(II), Zn(II) Ternary Complexes with Maltol and Glycylglycine

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Abstract: Ternary complexes of copper(II) and zinc(II) metal ions with the ligands maltol; 3-hydroxy-2-methyl-4*H*-pyrone (MA) and glycylglycine (GG) have been synthesized and characterized by IR, ¹H NMR, UV-Visible, mass spectra and TGA techniques. The complexes have been screened for antibacterial activity and the results are compared with the activity of free ligands. Increased activity of ternary complexes than corresponding free ligands is evident from these studies suggests more lipophilicity of metal complexes to act on bacterial cell.

Keywords: Synthesis, Spectral studies, Thermal analysis, Antibacterial studies

Introduction

Mixed ligand complexes derived from transition metal ions and designed ligands, having specific functional groups are useful in biomimetic studies for exploring the role of such metal ions in enzymatic processes. Since biomolecules play a key role in basic life processes, the study of mixed ligand complexes in biochemical systems is more extensive. The prominence of these complexes is evident from the fact that, a large number of biochemical reactions occur within the coordination sphere of metal ion. Essential metal ions such as copper and zinc¹⁻⁴ play consequential role in biological systems. Metal ligand interactions are very important since most of the biomolecules are all coordination complexes. Due to probability of bringing together two different kinds of ligands, the metal ligand complex formation can be applied to the biological systems^{5,6}.

Maltol is a non-toxic (LD50 1410 mg/kg) chelating ligand of natural origin, attracts an ever-increasing interest⁷. This fact is explained by the possible applications of 'MA' to control the level of metal content in an organism, either by excretion of their excess in the form of metal chelates through coordination with MA in the organism^{8,9} or by transportation of necessary metals as chelate complexes to the organism with therapeutic and diagnostic purposes¹⁰. The effectiveness MA to act as metal binding species is attributed to its capability to form both σ and π bonds with metal ions and availability of donor groups at physiological pH. Its stability arises from the easiness to deprotonate and to behave as anionic, bidentate metal chelators.

Studies on ternary complexes involving peptides are of immense biological interest¹¹ because of their role in the storage and transport of trace metals¹² in human body and in other biological systems. Small peptides containing 2-3 amino acid residues play very attractive role in organisms which are quite different from that of amino acids. Peptides with non coordinating side chains like 'GG' have only three or four types of donor centers available for a metal ion. These are N, O and N/O donor sites of amine, carboxylate and amide/carbonyl groups respectively.

In the present work, we have studied the formation of ternary complexes *i.e.* Cu(II)-MA-GG and Zn(II)-MA-GG as there are numerous metal-maltol complexes used in biomedical applications⁷⁻⁹.

Experimental

Cu(II) and Zn(II) metal salts were of analar grade from MERK. MA is obtained from Sigma-Aldrich and GG from SRL (India). Other reagents and solvents used were purified in the laboratory prior to use.

Synthesis of ternary complexes

[Cu(II)-MA-GG]

An aqueous solution of 5 mL of $\text{CuNO}_3 \cdot 3\text{H}_2\text{O}$ (1 m mole) was added to GG (1 m mole) dissolved in 5 mL of distilled water and stirred for 5 min. To this mixture, MA (1 m mole) dissolved in 10 mL of hot water was added and followed by the addition of methanolic ammonia to adjust the pH 7-8 to enable deprotonation of ligands. This solution was refluxed for 1 h. After 4-5 days green color Cu(II) complex was obtained and collected^{13,14}.

[Zn(II)-MA-GG]

An aqueous solution of 5 mL zinc chloride (1m mole) was added to GG (1 m mole) dissolved in 5 mL of distilled water and stirred for 5 min. To this mixture, MA (1 m mole) dissolved in 10 mL of methanol was added and followed by the addition of methanolic ammonia to adjust the solution pH 7-8 and deprotonate the ligand. Then the solution is refluxed for 1 h. After 2-3 days colorless Zn(II) complex was obtained and collected^{13,14}. These complexes were characterized by IR, ¹H NMR, UV-Visible, mass and TGA spectral data analysis.

Antibacterial activity

In vivo antibacterial activities of the ligands and metal complexes were determined by agar well diffusion method. The bacteria species used for this test include clinical sample of gram-positive *Bacillus subtilis* [MTCC 2063], *Staphylococcus aureus* [MTCC 2901] and Gram-negative *Escherichia coli* [MTCC 1652].

Results and Discussion

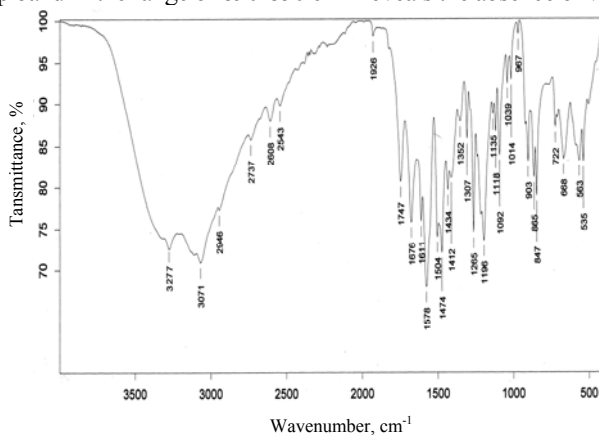
IR spectral analysis

Infrared spectral values of free ligands GG, MA and ternary metal complexes are compiled in Table 1. The IR spectra of GG exhibited significant features in $\nu(\text{NH}_3^+)$, $\nu(\text{COO}^-)$ regions^{13,14}, shows it exists as zwitter ion like a simple amino acid. The peaks at 3288 cm^{-1} , 3058 cm^{-1} were assigned to $\nu(\text{NH})_{\text{peptide}}$ and $\nu(\text{NH})\text{NH}_3^+$ stretching frequencies respectively, CH group frequency is observed at 2927 cm^{-1} while 1676 and 1657 cm^{-1} were assigned to amide group stretching frequencies. The broad band observed at 3260 cm^{-1} in the IR spectrum of MA, is attributed to the OH stretching. The bands at 3062 cm^{-1} and 2921 cm^{-1} are assigned for aromatic and aliphatic CH vibrations respectively. The band at 1660 cm^{-1} is assigned to $\nu(\text{C=O})$ while the combination bands of the $\nu(\text{C=O})$ and $\nu(\text{C=C})$ are observed at 1629 and 1562 cm^{-1} .

Table 1. Frequencies and the assignment of absorption bands (cm^{-1}) in the Infrared spectrum of free ligands and ternary complexes

GG	$\nu(\text{NH})_{\text{Peptide}}$	$\nu(\text{NH}_3)$	$\nu(\text{CH}_2)$	$\nu(\text{C=O})_{\text{Peptide}}$
Free ligand	3288	3058	2927	1676
[Zn(II)-(MA)-(GG)]	-	3244 _(\text{NH}_2)	2851	1664
[Cu(II)-(MA)-(GG)]	-	3277 _(\text{NH}_2)	2737	1676
MA	$\nu(\text{OH})$	$\nu(\text{CH}_3)$	$\nu(\text{C=O})$	$\nu(\text{C=O})$ and $\nu(\text{C=C})_{\text{ring}}$
Free ligand	3260	2995	1656	1629 and 1562
[Zn(II)-(MA)-(GG)]	-	2937	1610	1581 and 1514
[Cu(II)-(MA)-(GG)]	-	2946	1611	1578 and 1504

The infrared spectra of ternary complexes involving the above ligands have shown shift in characteristic band positions of ligands which can be correlated to ligand chelation with metal ions. The IR spectra of ternary complexes (Figure 1 and Figure 2) exhibited significant features in $\nu(\text{NH}_2)$, $\nu(\text{COO}^-)$ regions. The IR spectrum of free GG exhibited a peak at 3058 cm^{-1} which is attributed to NH_3^+ stretching frequency (the region of $3130\text{-}3030 \text{ cm}^{-1}$). But the same peak at isoelectric point observed in the region of $3500\text{-}3300 \text{ cm}^{-1}$ is due to free NH_2 group. In ternary complexes of Cu(II) and Zn(II) the IR spectra (Table 1) showed characteristic bands of $\nu(\text{NH})$ at 3288 and 3244 cm^{-1} respectively, which are lower compared to uncoordinated NH_2 ($3300\text{-}3200 \text{ cm}^{-1}$) and high compared to νNH_3^+ . In the complexes NH_3^+ get deprotonated and binds to metal through the neutral NH_2 group. Hence it can be concluded that the nitrogen of the amine group is in coordination with metal ion. No considerable shift was observed in the asymmetric stretching vibration of carboxylate groups signifying the non-coordination of carboxylate groups with metal ion¹⁵. These ternary complexes have shown a band in the $1770\text{-}1700$ regions indicates that the carboxylate group is not involving in the binding. This assignment is based on the fact that the unionized and uncoordinated COO^- stretching vibration band occurs in the $1750\text{-}1700 \text{ cm}^{-1}$ range where as the ionized and coordinated COO^- stretching vibration band occurs in the $1650\text{-}1580 \text{ cm}^{-1}$ range¹⁶⁻¹⁸. Hence it can be concluded that dipeptides acting as bidentate in presence of MA. The peak at 3288 cm^{-1} due to amide NH in the free GG is lost in both Zn(II) and Cu(II) metal complexes. This can be attributed to the coordination of amide nitrogen to metal ion by deprotonation. In the IR spectra of both complexes, the broad band is not in the range of $3550\text{-}3350 \text{ cm}^{-1}$ and the absence of a sharp band in the range of $890\text{-}850 \text{ cm}^{-1}$ reveals the absence of water molecules^{14,16}.

**Figure 1.** IR Spectrum of [Cu(II)-MA-GG]

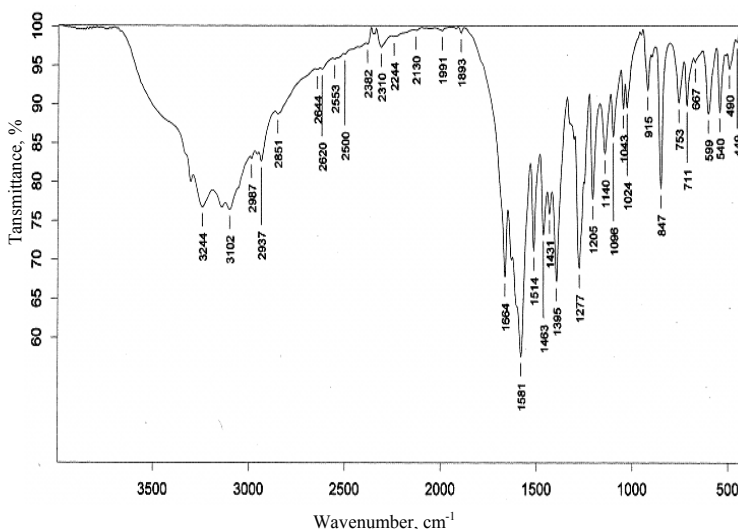


Figure 2. IR Spectrum of [Zn(II)-MA-GG]

The $\nu(\text{C}=\text{O})$ observed at 1656 cm^{-1} in MA is decreased^{19,20} to 1611 and 1610 cm^{-1} in Cu(II) and Zn(II) ternary complexes respectively, confirming participation of the oxygen atom of carbonyl group in bonding. In turn, the disappearance of the peak corresponding to the hydroxyl group of the free ligand $\nu(\text{OH})$ at 3260 cm^{-1} in the IR spectra of ternary complexes is the evidence that MA binds to metal ion through the oxygen atom of the deprotonated hydroxyl group. Thus it is stated that the complexes formed have the chelate structure. The comparison of spectra of ternary complexes with ligands also reveals that there is a shift in $\nu(\text{C}=\text{C})$ of conjugated double bonds^{20,21} which is due to redistribution of the electron density in chelates resulting a decrease in the frequencies of stretching vibrations. The observed IR characteristics are in good agreement with literature data of ternary metal complexes of MA reported earlier²²⁻²⁴.

¹H-NMR spectra

¹H NMR spectrum of Zn(II) ternary metal complex is presented in Figure 3. The data given in Table 2 reveals the presence of two methylene groups. The peak at chemical shift 3.69 ppm is assigned to β -methylene near C-terminal and the other with 3.65 ppm value to the α -methylene near the N-terminal²⁵. The peaks of amine and amide protons are not observed in spectrum of GG, as the spectrum was recorded in D₂O. While the peak in the region $2.6\text{--}4.0\text{ ppm}$ (2H , NH_2) in the zinc complex spectrum clearly confirms the presence of amine group as the spectrum was recorded in DMSO-*d*₆. The chemical shift values of α -methylene and β -methylene groups in ternary complex at 2.4 and 2.55 ppm respectively indicate upward shift due to complexation of ligand. In the ¹H-NMR spectra of MA, the chemical shifts at 6.45 ppm , 7.74 ppm , 2.39 ppm and 7.12 ppm have been assigned to H(5), H(6), CH₃ and OH groups respectively²¹. The absence of OH signal at 7.12 ppm in ¹H-NMR data of zinc complex (Table 2) confirm the participation of the oxygen of hydroxyl group of MA in complexation through dissociation of its proton. The shift in resonance signals of other protons of both the ligand moieties provides an evidence of redistribution of the electron density in the new metal chelate molecule formed.

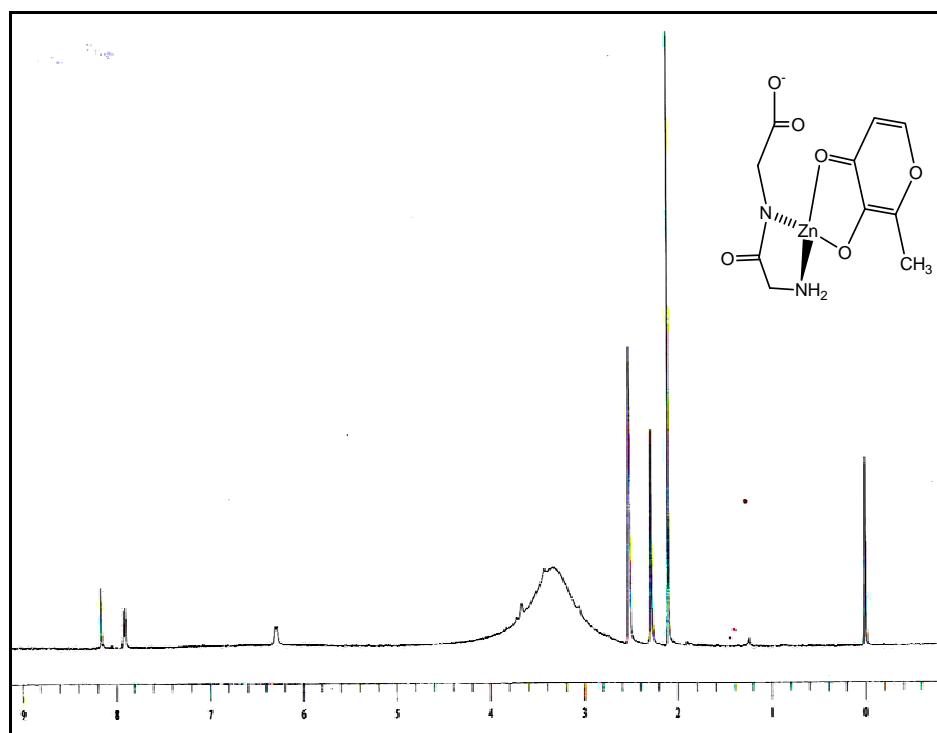


Figure 3. ^1H NMR spectrum of $[\text{Zn}(\text{II})\text{-MA-GG}]$

Table 1. ^1H NMR chemical shifts (in ppm) of MA, GG and $[\text{Zn}(\text{II})\text{-MA-GG}]$

System	H(5)	H(6)	CH_3	OH
MA (DMSO-d_6)	6.463-6.45(d)	7.74-7.73(d)	2.395(s)	7.12
$[\text{Zn}(\text{II})\text{-(MA)-(GG)}]$ (DMSO-d_6)	6.38(s)	7.9(d)	2.2(s)	-
System	$\alpha\text{-CH}_2$		$\beta\text{-CH}_2$	NH_2
GG (in D_2O)	3.65(s)		3.694(s)	-
$[\text{Zn}(\text{II})\text{-(MA)-(GG)}]$ (DMSO-d_6)	(DMSO- d_6)		2.55 (s)	2.6-4(broad)

s: singlet, *d*: doublet

Spectrophotometric measurements

The UV spectrum of GG free ligand has shown a charge transfer band at 203 nm^{26,27}, upon complexation this band has shifted to 332 and 310 nm with Cu(II) and Zn(II) respectively. This can be assigned to ligand to metal charge transfer^{17,28,29}. Spectrophotometry of free MA exhibits maximum absorbance at $\lambda = 276$ nm. An increase in alkalinity (pH 7-10) resulted the ionization of OH groups, which in turn developed a bathochromic shift of $\pi \rightarrow \pi^*$ electron transition, thus the absorbance maximum shifted to around 300 nm^{7,30}. The absorption band at wavelength 274 in MA ternary complex can be assignable to undissociated maltol, and at 299 can be assignable to dissociated maltol which provides the information about $\pi \rightarrow t_{2g}(\pi^*)$ transition of MA at anion bonded with metal through both hydroxyl and carbonyl groups.

The spectrum in the visible region shown the $d \rightarrow d^*$ transitions, which apparently have low intensity than the charge transfer transitions. Hence the visible spectrum Cu(II) ternary complex (Figure 4) is recorded at high concentrations. Zn(II) ternary complex is colorless, hence it is not possible to record Visible spectra. The observed peak at 662.50 nm suggesting a tetrahedral geometry^{15,31}. Visible spectrum of MA ternary complex shown a peak at 662.50 nm, assignable to spin allowed ${}^3T_1(P) \leftarrow {}^3T_1(F)$ transition and two weak bands at 893, 935 nm represents transition of ${}^3T_2(F) \leftarrow {}^3T_1(F)$ and ${}^3A_2(F) \leftarrow {}^3T_1(F)$ respectively.

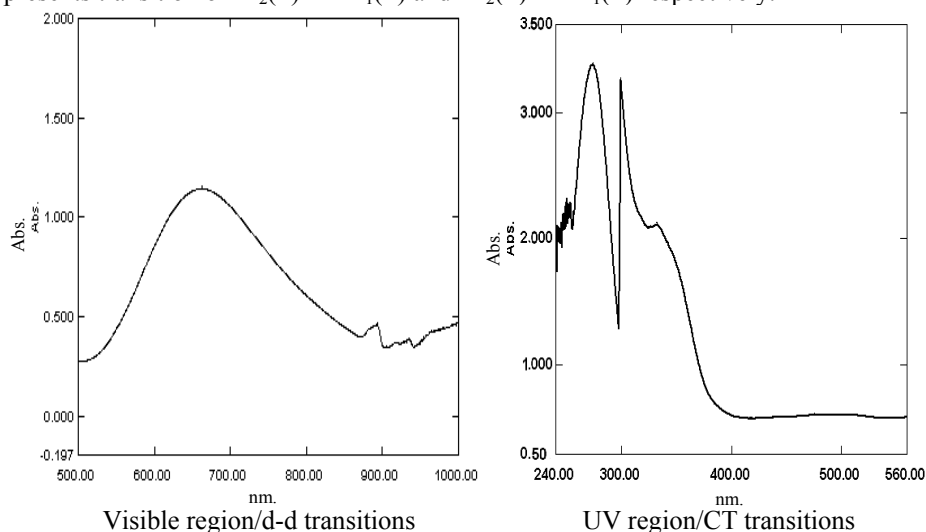


Figure 4. UV-Visible spectra of [Cu(II)-MA-GG]

Mass spectra

In the ESI mass spectra of ternary metal complexes, the molecular ion peak was observed at m/z 320 which is in agreement with the molecular weight 320 of the proposed structures for both Cu(II) and Zn(II) metal complexes. The mass spectra of [Zn(II)-MA-GG] and [Cu(II)-MA-GG] given in Figure 5 and Figure 6 showed a peak at m/z 277 and 276 respectively, which is assigned as fragment peak by the loss of COO^- group. The remaining are adduct peaks. Thus mass spectra further strengthen the proposed structures.

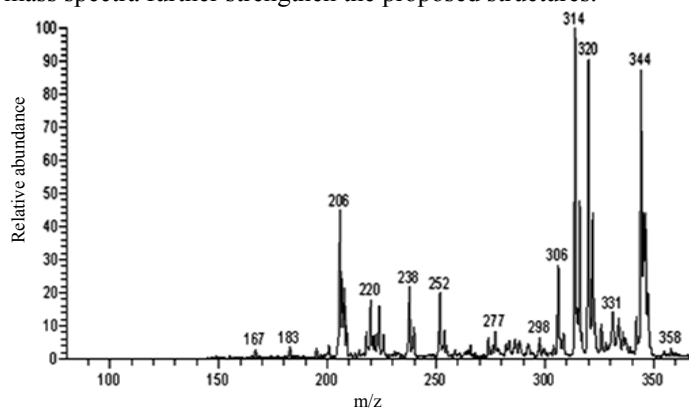


Figure 5. Mass spectrum of [Cu(II)-MA-GG]

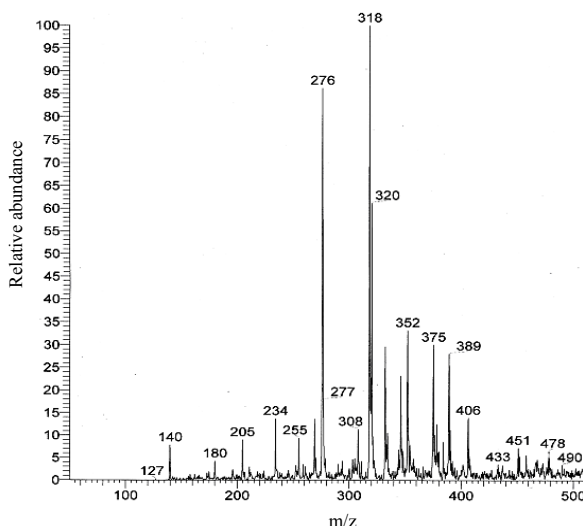


Figure 6. Mass spectrum of [Zn(II)-MA-GG]

Thermal analysis

The TGA – DTA curves of Cu(II)-MA-GG and Zn(II)-MA-GG (Figure 7 and Figure 8) complexes showed sudden weight loss is at 240 and 320 °C respectively and reveals the absence of water molecules in both complexes. The weight loss at nearly 250 °C in Cu(II) ternary complex is 30% which corresponds to a weight loss of molecular weight 98 units ($C_3O_3NH_2$) from GG ligand. The Zn(II) ternary complex TGA curve shows the weight loss 18% at nearly 320 °C corresponds the loss of molecular weight 58 units ($C_2H_3O_2$) from GG ligand and 45% weight loss at nearly 420 °C indicates the loss of molecular weight 145 units, $C_4H_7N_2O_3$ from GG and CH_3 from MA.

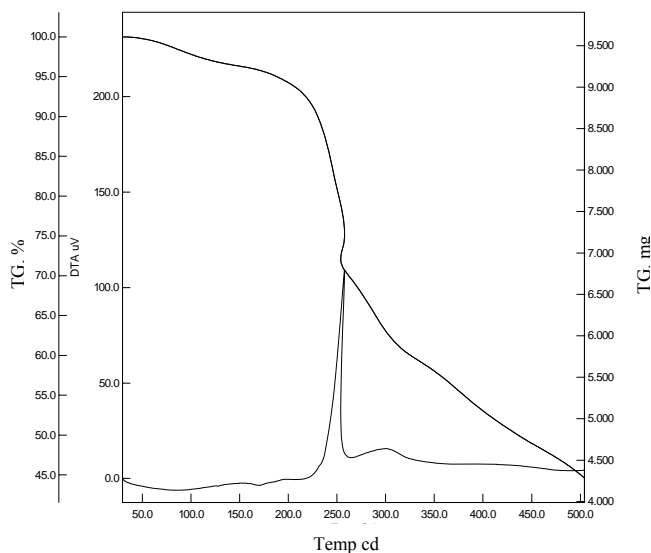


Figure 7. TG and DTA of [Cu(II)-MA-GG]

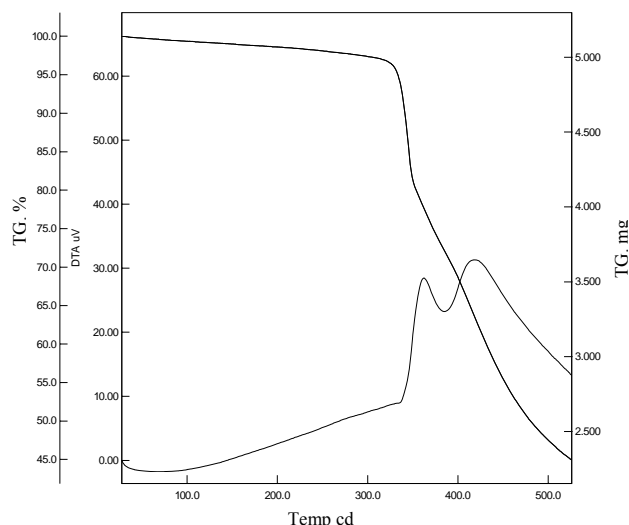


Figure 8. TG and DTA of [Zn(II)-MA-GG]

Biological activity

The complexation of biologically important Cu(II) and Zn(II) metal ion with ligands *i.e.*, MA and GG were further explored with the evaluation of their antibacterial activity against gram-positive *Bacillus subtilis* [MTCC 2063] and gram-negative *Escherichia coli* [MTCC 1652]. MA and complexes exhibited activity against the tested strains. The ligand possess moderate antibacterial activity against two bacterial species, ternary metal complexes of Cu(II) and Zn(II) have been shown activity against gram-negative *Escherichia coli*, but/however insignificant increase in activity has been observed (Figure 9) for Cu(II) complexes for gram-positive *Bacillus subtilis*. The studies demonstrated that metallation can increase the antibacterial activity than free ligand. It has been suggested that³⁰ metal chelation reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups and possibly the π -electron delocalization occurring within the whole chelate ring system formed during coordination. This process of chelation thus increases the lipophilic nature of the central metal ion, which in turn favors its permeation through the lipid layer of the membrane³² and causing the metal complex to cross the membrane of the microorganism cell wall more effectively and increasing the activity.

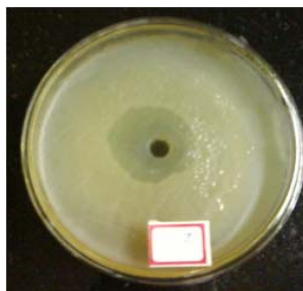


Figure 9. Inhibitory action of ternary Cu(II) complex on the growth of *Bacillus subtilis* organism

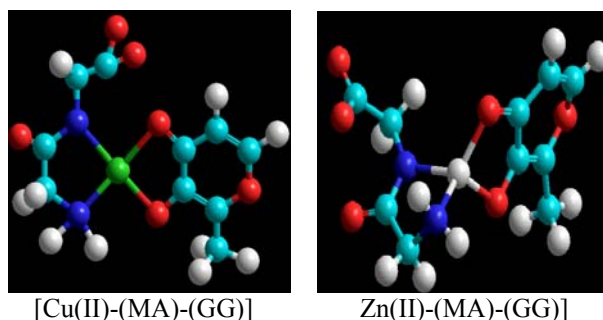


Figure 10. Energy minimized structures of the most stable isomers of ternary complexes

Conclusion

Ternary complex formation is determined by synthesis and characterization *viz*; IR, ^1H NMR, UV-Visible, mass spectral data and TGA analysis. The spectral characterization infers the mode of bonding and structural changes in ligand upon coordination with metal ion. The results unraveled the information about the availability and involvement of coordination sites in both the ligands. It can be concluded that COO^- is not involved in bonding as its participation increases the steric hindrance in ternary complex. The formation of ternary complexes studied keeping in the biological importance of MA indicated enhanced antibacterial activity. Based on the results obtained, the tetrahedral geometry was assigned for the ternary complexes under present study. The most stable isomers with minimum energy are obtained by using hyperchem software and represented in Figure 10.

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