Microbicidal [1,2,3]-triazole Schiff base complexes of Cu(II) and Zn(II) metal ions

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Abstract

Active carbonyl and imino groups (R-C=N-) are condensed to produce Schiff bases. Schiff bases are very proficient to stabilize several metals in different oxidation states. Since nitrogen contains lone pair electrons, the imines that are produced can combine with metal to create a complex. The Schiff bases ligand was produced and used in this work to construct the metal complexes of Cu (II) and Zn (II) ions. The Significance of metal (II) complexes has been thoroughly characterized using various physical, analytical and spectroscopic techniques and biological characteristics. The spectrum data obtained from the analytical characterization of FT-IR, UV-visible, ESR, and TGA procedures confirmed the postulated structure. The diamagnetic and paramagnetic properties of Cu(II) and Zn(II) complexes have been confirmed using ESR research. The chelation between ligands and the metal can be confirmed by analyzing FT-IR and UV-visible spectra. The coordination sphere of the complexes is devoid of ions, as indicated by the low conductance measurements. The TGA's thermal tests supported the complex's structural configuration, revealing its breakdown kinetics and the subsequent release of various ions at different temperatures. All of the spectrum evidence indicated the presence of complexes with a square pyramidal form. The bacteria, including Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, and Escherichia coli, exhibited a moderate susceptibility to the complexes.

Keywords: Schiff base, Copper ion complex, Zinc ion complex, Square pyramidal, Antibacterial

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1. Introduction

Heterocyclic compounds belong to the significant class of organic chemical compounds where the aromatic ring contains more than one heteroatom. The hetero atom functions as a ligand in transition metal complexes due to a single pair of electrons [1,2]. Triazoles are five-membered heterocyclic compounds used in the pharmacological and medicinal applications. Having three polarisable nitrogen atoms in a cyclopentenyl ring, triazoleshave gained considerable attention in organometallic chemistry[3-5].The triazole ring can coordinate with transition metals more effectively when multiple donor sites are present. Thus, these ligands are potentially adaptable for metal coordination. The stability of triazoles towards acid-base hydrolysis made their application in the medical field [6]. The [1,2,3]-triazoles and their derivatives bind well to various enzymes and receptors in biological systems [7].

An active carbonyl group and an imino group (R-C=N-) are condensed to create organic compounds with an azomethine group (-CH=N-), also called Schiff bases. Compared to the alkyl substituent, the Schiff base with aryl substituents is more stable and quickly produced.

Excellent chelate complexes with transition metals can be formed thanks to the C=N group's stability, synthetic flexibility, and relative ease of production [7]. The functional group of azamethine is essential for classifying compounds under "privileged ligands"[8]. In azomethine, nitrogen atoms' sp2 hybridized orbital, the lone pair of electrons is essential for biological activities such as Schiff base complexes' antibacterial, antiproliferative, anti-inflammatory, and antipyretic qualities [6,8].Therefore, Transition metal ions are preferred over other metal ions in forming chelated metal complexes primarily due to their ease of adopting several geometries and capacity to exist in various oxidation states. A vast and extensive body of literature has been developed regarding the coordination chemistry of Schiff-base ligands, Cu(II) and Zn(II). Cu(II) complexes exhibit blue, red, green, and brown colors due to significant absorption in the UV region, followed by charge transfer transitions [9, 10]. The Schiff base metal complexes have been essential in the study because of their multidisciplinary usefulness, including analytical science, biochemistry, and antimicrobial science. In this context, the antibacterial activity of copper and zinc ion Schiff base complexes against gram-negative and gram-positive bacteria is produced and investigated.

2.Material and methods

Sigma-Aldrich is the source of Dichloromethane (DCM), Ethanol (EtOH), Chloroform ($CHCl_3$), Dimethyl formamide (DMF), Tetrahydrofuran (THF), Zinc chloride ($ZnCl_2$), and Copper chloride (CuCl2). Using a SHIMADZU spectrophotometer, the UV spectra of each sample are acquired and scanned between 200 and 800 nm. FT-IR spectra were obtained in the 500–4000 cm−1 wave range using a BRUKER FTIR spectrophotometer, which could do 24 scans per minute at a resolution of 4 cm−1. TG 220 modules are used to create TGA analyses. A sample of around 2 mg is obtained for examination. Every run is conducted air-conditioned using reference material on platinum pans. ά-Alumina showed a straight calorimetric trace in the ambient temperature range of -100°C to 700°C, without any thermal transition.Calcium sulfate is used as a standard material for calibrating the thermal analyzer. With a conductivity range of 0 µ to 200 mS and a cell constant of 1 cm, the molar conductance is measured in DCM as solvent at room temperature using a Systronics digital conductivity meter 304. The source frequency can be set to 100 Hz or 1 KHz. Using an ESR spectrometer, the classic varian approach is used to conduct ESR examinations of the complexes. With a sensitivity of 5 x 1010 ΔH spins and a frequency modulation range of 100 KHz to 35 Hz, the Varian E-line Century Series ESR spectrometer operates at X-band microwave frequencies [9.5GHz]. Simple, easy to use, and qualitative is the agar diffusion test.Nutrient agar Petri dishes are inoculated with bacterial cells, then test samples are layered on top. Subsequently, the plates are incubated at 37°C for 18–24 hours, and the growth of bacteria is recorded below the zone of inhibition. The lack of bacterial proliferation directly beneath the test sample shows the antimicrobial activity. The agar diffusion method was utilized to evaluate the antibacterial effectiveness of the complex against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa.

The Schiff base ligand (L) is synthesized from the ethanolic solution of 1,4-di-substituted [1,2,3]-triazole aldehyde and 4-hexyloxy aniline. The synthesis procedure and structural characterization of the ligand are reported in our earlier works[11].

2.1 Synthesis of Cu-Schiff base complex

The metal salt and the Schiff base ligand are taken in a 1:1 ratio. To the ethanolic solution of Schiff base ligand (0.130mmol) taken in a 100mL round bottom flask, $CuCl_2$ (0.146mmol) dissolved in ethanol is added in terms of drop-by-drop addition and refluxed for 3 hours. Once the reaction is finished, the mixture is filtered and refrigerated for the entire night. The solid obtained after recrystallization from DCM is used for further characterization.

$$L + CuCl.2H_2O \xrightarrow{\text{Ethanol/reflux}} ML + 2H_2O$$

2.2Synthesis of Zn-Schiff base complex

The metal salt and Schiff base ligand are taken in a 1:1 ratio. To the ethanolic solution of Schiff base ligand (0.165mmol) taken in a 100mL round bottom flask, $ZnCl_2$ (0.183mmol) dissolved in ethanol is added drop by drop and refluxed for 3 hours. Once the reaction is finished, the mixture is filtered and refrigerated for the entire night and filtered.The solid obtained after recrystallization from DCM is used for further characterization.

$$L + ZnCl_2(anhyd) \xrightarrow{\text{Ethanol/reflux}} M_2L$$

3. Results and discussions

3.1 Physical methods

3.1.1 Colour

The ligand is colorless, whereas the Schiff base metal complexes of copper and zinc are brownish red and pale yellow, respectively. This color difference may suggest the formation of metal complexes. The color of the metal complex is due to the strong absorption followed by charge transfer transitions in the UV region.

3.2 Chemical methods

3.2.1 Melting point

Generally,metal complexes possess higher melting points than respective ligands. The melting points of the ligand, Cu,and Zn-Schiff base complex are 113℃, 128.67 ℃, and 138.37 ℃, respectively. This change inthe melting point of metal complexes from ligands suggests the formation of complexes.

3.2.2 Solubility

Thesolubility of the ligand and metal complexes is checked in most organic solvents. The ligands are soluble in almost all organic solvents like DCM, DMF, hexane, chloroform, and THF, but metal complexes are soluble only in DCM.

3.3 Analytical characterization

3.3.1 FT-IR Spectral Analysis

Comparison is made between the ligand's and complexes' FT-IR bands. The Schiff base's imine linkage is shown by a prominent band in the spectra of the ligand around 1638 cm^{-1}, characteristic of the v(CH=N) (azomethine) stretching mode for ligands. According to Figure 3.1, chelation with the metal is indicated by these bands being pushed towards distinct frequencies in the spectra of copper and zinc complexes, at 1608 and 1606 cm^{-1}, respectively. The lone pair electrons of nitrogen atoms from the triazole ring, which forms bonds with metal ions, are involved in moving to a higher location. There is a shift to lower wave numbers of 1513 cm^{-1} and 1517 cm^{-1} from the band at 1511 cm^{-1} designated for N=N stretching in the free ligand of [1,2,3]-triazole ring.The lower wave number of 1513 cm^{-1} and 1517 cm^{-1} in the Zn-Schiff base complex and Cu-Schiff base complex, respectively, shifts the band at 1511 cm^{-1} designated for N=N stretching in the free ligand of [1,2,3]-triazole ring [12, 13]. This shows that the triazole nitrogen atom can coordinate with the metal ions. Additionally, the emergence of FT-IR bands resulting from v(Zn-N) at 415 cm^{-1} and v(Cu-N) vibrations in the range of 525 cm^{-1} further supports the coordination behavior of the ligand with the metal. The coordination sphere is devoid of water molecules, as indicated by the absence of a –OH signal at around 3400 cm^{-1}. The coordination of metal to chloride ions inside both complexes is demonstrated by the existence of v(M-Cl) at around 500-530 cm^{-1}.

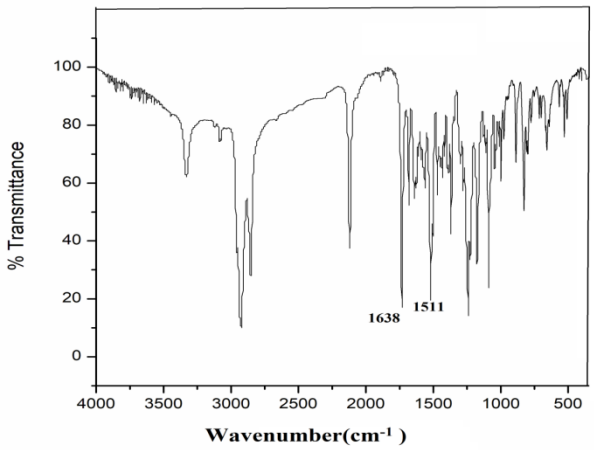


Fig. 3.1 a

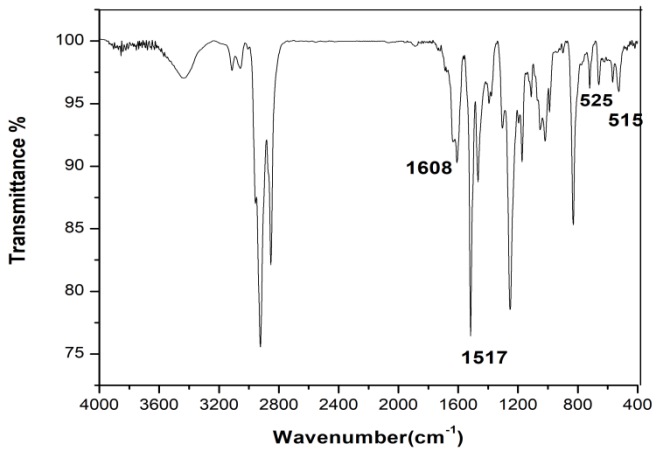


Fig. 3.1 b

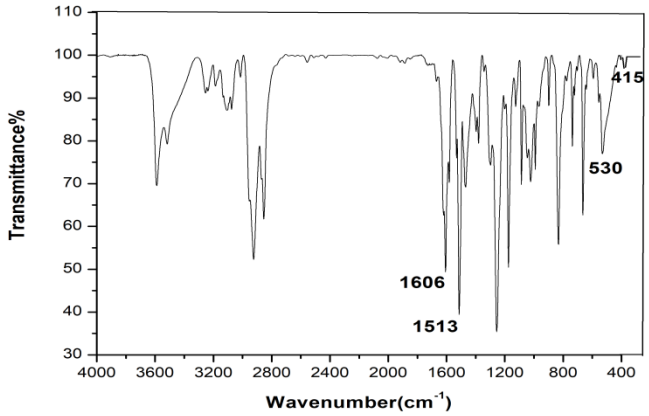


Fig. 3.1 c

Fig 3.1 FTIR spectra of a) ligand b) Schiff base copper ion complex c) Schiff base zinc ion complex

3.3.2 Electronic Spectral Analysis

The synthesized ligands and complexes' electronic spectra are measured in room temperature DCM solutions (10-4M), and the results are displayed in Figure 3.2. The ligand's UV-visible spectrum displays three bands at 237, 280, and 337 nm, which are caused by the nitrogen non-bonding electrons of the triazole and azomethine group as well as the π-π* and n-π* of the benzene (Fig. 3.2). Similar benzene absorption occurs in both ligand and complex forms. However, the band shifts to a larger wavelength due to transitions of the lone pair of electrons of nitrogen in the azomethine group and triazole ring, indicating the coordination of the ligand to the metal center.

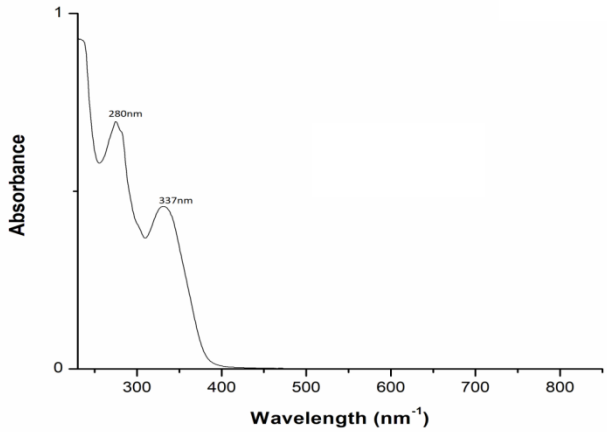


Fig 3.2 a

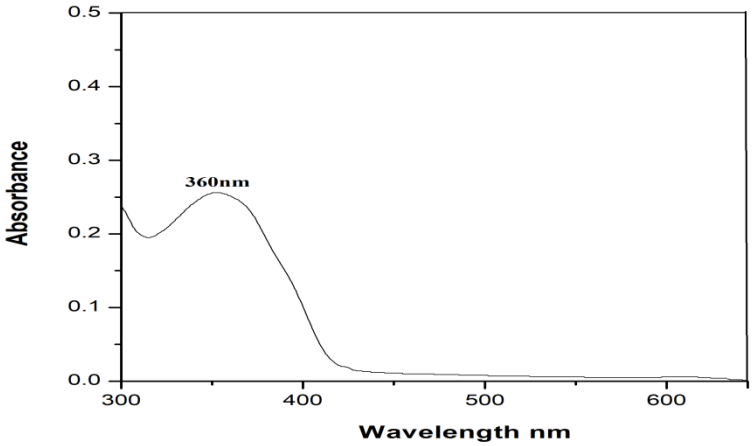


Fig 3.2 b

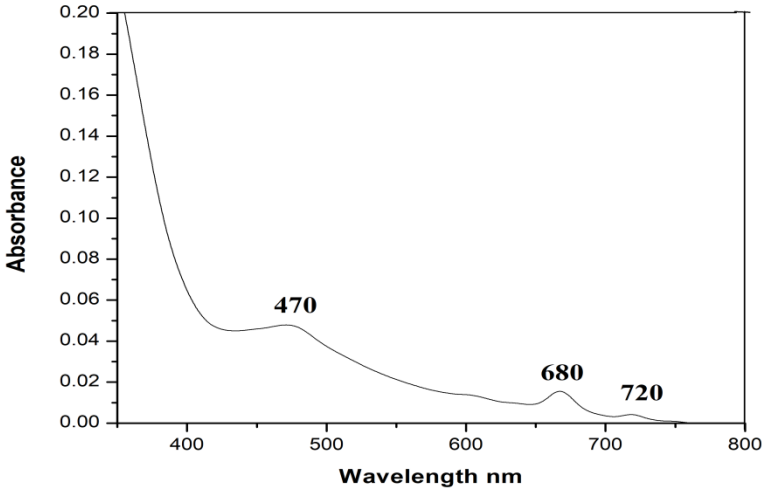


Fig 3.2 c

Fig 3.2 UV spectra of a) ligand b) Schiff base copper ion complex, and c) Schiff base zinc ion complex

Fig 3.2 a, b, and cshows the UV spectra of ligand Schiff basecopper ion and Schiff base zinc ion complexes.The observed peak in the UV-visible spectrum of the Schiff base copper ion complex is around 14,705, 13,888,and 21,000cm^{-1}. These peaks are due to the transition from

$^2B_1$ to $^2B_2$ and $^2B_1$ to ^2E, corresponding to the square pyramidal geometry.(Fig 3.2 b)[13-19].

3.3.3 ESR Spectral Analysis

The structure and metal-ligand bonding in the Schiff base copper ion complex is obtained through the ESR spectrum. The absence of awell-defined ESR spectrum confirms the diamagnetic nature of Zinc ions in the Schiff base zinc ion complex. ESR spectrum of the Schiff base copper ion complex exhibited a single broad signal due to spin-latticerelaxation. The EPR spectrum of the Schiff base copper ion complex (Fig 3.3) shows axial spectra with well-defined $g_{||}$ (2.19163) and $g_{\perp}$ (2.0684) as Cu(II) is paramagnetic of d^9 configuration. Since $g_{||}>g_{\perp}$, the Schiff base copper ion complex may exhibit square planar geometry [17]. The observed measurements $g_{||}$(2.19163) > $g_{\perp}$(2.0684) > 2.0023 may indicate that the complex has a $d_{x^2-y^2}$ ground state [19]. The metal-ligand bond nature is shown by the $g_{||}$ value. The presence of ionic character in the metal-ligand bond is shown by $g_{||}>2.3$, whereas covalent character is shown when $g_{||}<2.3$. The complex's $g_{||}$ value is smaller than 2.3 in this instance, indicating that the metal-ligand link is covalent. Equating $g_{avg}= (1/3)(2g_{\perp}+ g_{||})$,one can determine the averaged "g" value for total distortion, which comes out to be 2.10.

The geometric parameter (G) can be computed using the formula $G = g_{||}-2.0023/g_{\perp}- 2.0023$, quantifying the exchange interaction value. The exchange contact between the copper centers can be disregarded if the G value is higher. In the current observations, the computed G value for the Schiff base copper ion complex is 2.8, indicating the exchange coupling effects.

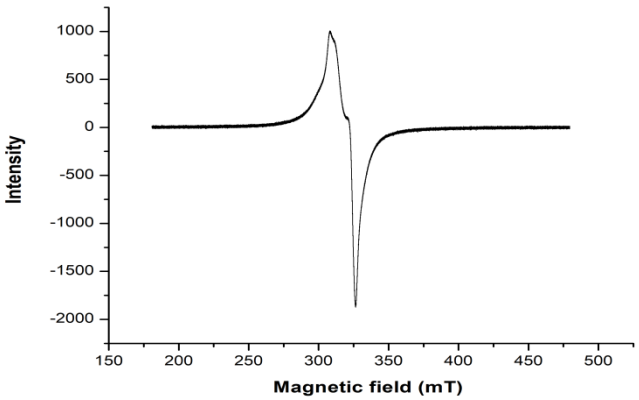


Fig 3.3 ESR spectra of Schiff base Copper ion complex

3.3.4 Molar conductivity

A DCM solution containing 10-4 M concentrated solution is used to assess the complexes' molar conductivity at room temperature. Table 3.1 displays the complexes' molar conductance. This value suggests that none of the complexes have any particular nature that would be considered electrolytic, probably because the coordination sphere of the metal complexes contains no chloride ions. [21]

Table 3.1 Molar conductance of metal complexes

Compound	Conductance(µS)
Zn(II) complex	2.3
Cu(II) complex	11.5

3
.3.5 Thermal studies

TGA is a practical technique to determine a material's thermal behavior. The breakdown caused by the metal complexes' steady weight loss as temperature rises. Figure 3.4 displays both complexes' TGA. Three stages of breakdown, ranging from 25 to 500°C, are depicted on the graph. The lack of temperature inflection up to 100 °C indicates that there are no water molecules in the coordination sphere.The first decomposition step of both the complexes around 189 °C indicates the loss of two chloride ions (Cu ion complex:10%$_{(exp)}$; 11%$_{(cal)}$ and Zn ion complex: 11% $_{(exp)}$;10.93%$_{(cal)}$). The loss of chloride ions is calculated from the weight loss percentage. The second decomposition step around 269 °C indicates the loss of flexible side chain containing alkyl moiety (–O-C_8H_{17}) from the ligand (Cu ion complex:37%$_{(exp)}$; 41.09%$_{(cal)}$ and Zn ion complex: 41%$_{(exp)}$; 40.93%$_{(cal)}$) The third decomposition step around 387 °C indicates the loss of benzene ring from the ligand (Cu ion complex: 20%$_{(exp)}$; 23.84%$_{(cal)}$ and Zn ion complex: 20%$_{(exp)}$; 23.75%$_{(cal)}$). From these data, the central metal ion may coordinate with the chloride ion and ligand. The weight loss also predicts the 1:1 stoichiometry of ligand and metal ions [22].

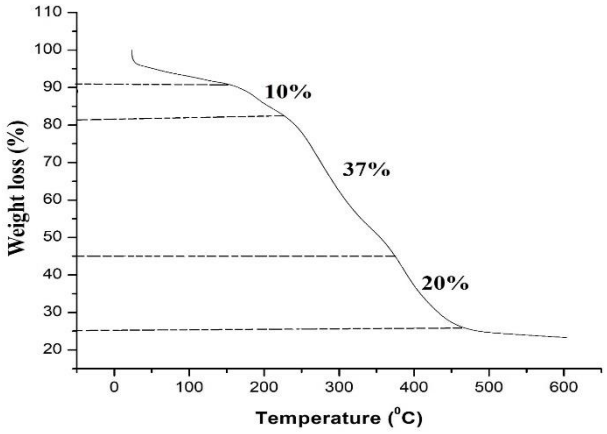


Fig 3.4 a

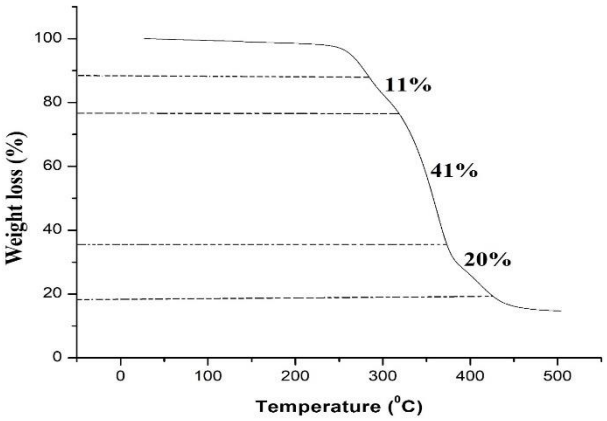
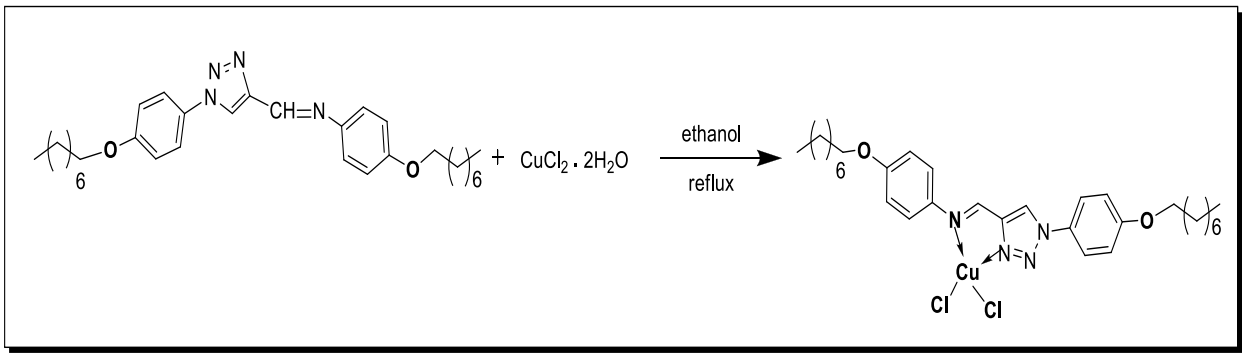
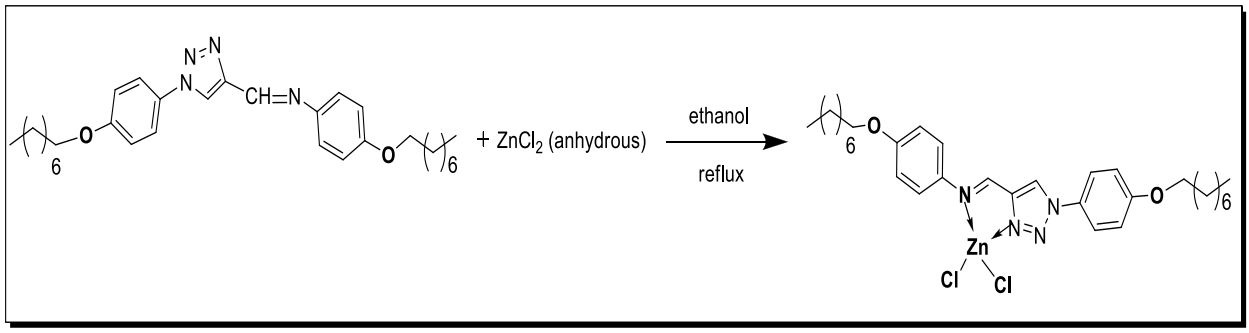


Fig 3.4 b

Fig 3.4 TGA graph of a) Schiff base copper ion complex b) Schiff base zinc ion complex





Scheme 3.1 Propsed structure of Schiff base copper complex and Schiff base zinc complex from the spectral analysis

3.4 Applications

This disc diffusion method was used to study the inhibition of bacterial growth in the presence of these compounds. The zone inhibition approach was used to evaluate the aforementioned four microorganisms [23, 24]: Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, and Staphylococcus aureus. Zinc and copper metal complexes' relative zones of inhibition are displayed in Table 3.2. Zinc Schiff base complexes are superior to copper Schiff base complexes when combating *Escherichia coli, Klebsiellapneumonia*, and*Staphylococcus aureus*. There's no bacterial death going on with the control (deionized sterile water). The antibacterial activity is due to the sufficient binding ability of the triazole ring heteroatom, copper ion, and zinc ion with bacteria.

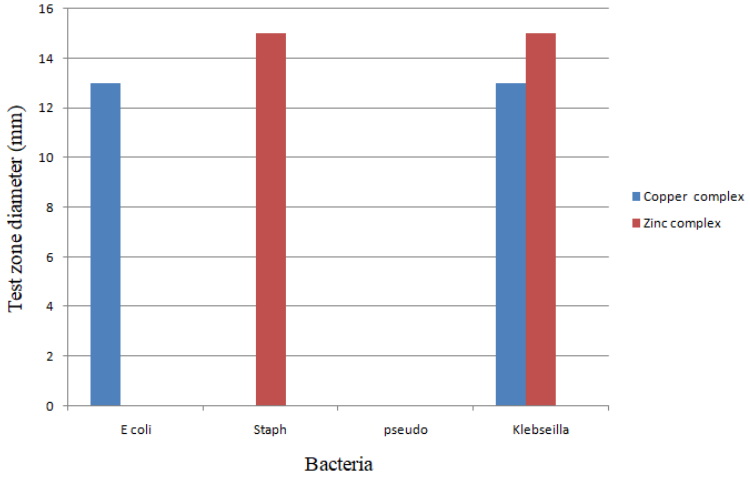


Fig 3.5 Graphical representation of the zone of inhibition ofSchiff base copper ion complex and Schiff base Zinc ion complex

Bacteria	Test zone diameter (mm)	
	Copper ion complex	Zinc ion complex
Escherichia coli	**0**	**15**
Staphylococcus aureus	**13**	**0**
Pseudomonas aeruginosa	**0**	**0**
Klebsiella pneumonia	**13**	**15**

Table 3.2 Zone inhibition of Schiff base copper ion and zinc ion complex

4. Conclusion

The applications of [1,2,3] triazole and Schiff bases are chemically important in metalloorganic chemistry as they have high coordination ability with metals. The presence of three N atoms on [1,2,3]-triazole generates dipoles and polarisability to the molecule. This presence of heteroatom in [1,2,3]-triazole finds applications in pharmaceuticals and industries.

The development of metal complexes may causecolor differences, high melting point variance from ligands, and solubility discrepancies. The coordination of nitrogen with metal in azomethine and triazole is suggested by peak shifts in the FT-IR spectra of metal complexes derived from ligands. The coordination of ligand to the metal center is indicated by a shift in the n-π* of nitrogen in the triazole ring and azomethine group, as shown in the electronic spectra of metal complexes. The line peak in EPR suggests the Cu(II) metal complex's paramagnetic character. The EPR further suggests the zinc complex's diamagnetic character. The low molar conductivity values show the absence of counter ions, which points to the non-electrolytic nature of metal complexes.TGA indicates that there is no temperature inflection up to 100°C, indicating that there are no water molecules in the coordination sphere. It also provides ligands and chloride ions with information on metal coordination. All of the above investigations suggest the square planar shape for the Schiff base zinc ion and Schiff base copper ion combination. The antibacterial results of both complexes indicate that they are only somewhat susceptible to gram-positive and gram-negative bacteria.

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