



**Structural Elucidation of Bio-Active Metal Complexes of Schiff Base
Derived from Condensation of 2,5-Dimethoxybenzaldehyde with
Substituted 1,2,4-Triazine Scaffold**

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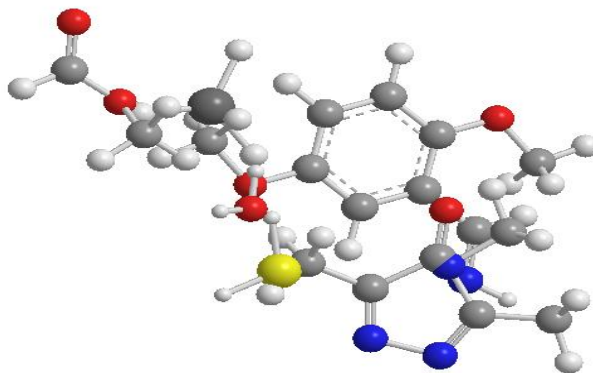
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ABSTRACT

Coordination complexes of Co(II), Ni(II), Cu(II) and Zn(II) metal ions have been reported with newly synthesized Schiff base derived from refluxing the equimolar mixture of 4-amino--3-mercapto-6-methyl-5-oxo-1,2,4-triazine with 2,5-dimethoxybenzaldehyde. Formation of Schiff base was confirmed by IR and ¹H-NMR spectroscopy. Electronic, Thermal, Fluorescence and Electrochemical studies have been used to study the different behavior of metal complexes. The general empirical formula proposed for 1:1 Co(II), Ni(II) and Zn(II) complexes are M(L)(OAc).3H₂O, for Cu(II) complex Cu(L)(OAc).H₂O, for 1:2 Co(II), Ni(II) and Zn(II) complexes are M(L)₂.2H₂O and for Cu(II) complex Cu(L)₂. Agar well diffusion method was used to evaluate Schiff base and all the metal complexes against various microbes named as: *B. subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Saccharomyces cerevisiae* and compared with standard drugs Ciprofloxacin and Amphotericin B. This study suggests that activity of Schiff base enhances upon complexation.

GRAPHICAL ABSTRACT



Optimized Structure of Cu (L)(OAc).H₂O

Keywords: Schiff base, Metal complexes, ESR, Electronic spectra, Antimicrobial activity.

INTRODUCTION

From the past few years, numerous Schiff bases and their metal complexes have been reported [1, 2] as Schiff bases have superior efficacy to form biologically active metal complexes. 1,2,4-triazine based Schiff bases represent an important class of compounds due to presence of nitrogen and sulphur atom in the backbone of these ligands. This exocyclic N and S atoms in Schiff base inspired the chemists as they possess diverse and fruitful activities viz. anticoagulant [3], antitubercular [4], anti-inflammatory [5], antimicrobial [6-7], anthelmintic [8], antidepressant [9], antimalarial [10], antioxidant [11] and further their metal complexes show more enhancements in all these activities [12, 13]. So, it has been found interesting to study the metal complexes containing Schiff base as ligand. Schiff base metal complexes are different in color with respect to their starting material i.e. Schiff base and transition metal salt. These metal complexes show various absorption bands differ in position as well as intensity and which also differ from their starting material [14-16]. So, we can say that colored property of complexes suggests the occurrence of coordination. All these achievements provided enormous impetus to synthesize and characterized Co(II), Ni(II), Cu(II) and Zn(II) complexes of Schiff base derived from condensation of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine with 2,5-dimethoxybenzaldehyde. Thus, Schiff base has good chelating ability with metal ions and these metal complexes confer good biological activity [17].

MATERIALS AND METHODS

All the reagents and various metal acetates were of analytical grade and are used as supplied. Solvents were distilled off and then used for synthesis. IR spectra were recorded on a MB-3000 ABB spectrometer in KBr pellets/Nujol mulls. Far IR spectra of metal complexes were recorded at SAIF, PU on RX1 Perkin Elmer spectrometer in the region 700-30 cm^{-1} . $^1\text{H-NMR}$ was recorded on Bruker ACF 300 spectrometer at 300 MHz by using $\text{CDCl}_3/\text{DMSO-d}_6$ solvents. Magnetic moment measurements were carried out on Vibrating Sample Magnetometer at Institute Instrumentation Centre, IIT Roorkee. Electronic spectra of metal complexes were recorded on T 90 (PG Instruments Ltd) UV/VIS spectrometer in the region 1100-200 nm by using DMF as solvent. TG analysis was carried out on the Perkin Elmer (Pyris Diamond) instrument at heating rate $10^\circ\text{C}/\text{Min}$ by using alumina as standard. Fluorescence spectra of Schiff base and metal complexes were carried out on SHIMADZU RF-5301 PC spectrophotometer. Cyclic Voltammogram of copper complexes were recorded on Ivium Stat Electrochemical Analyzer by using TBAP as supporting electrolyte. ESR spectra of Copper complexes were recorded on Varian E-112 ESR spectrometer at SAIF, IIT Bombay under the magnetic field 3000 Gauss and frequency 9.1 GHz.

Synthesis of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (AMMOT): It was synthesized according to literature procedure [18].

Synthesis of Schiff base: Ethanolic solution of 2,5-dimethoxybenzaldehyde (1.05 g, 6.32 mmol) was added slowly to the ethanolic solution of 4-amino-3-mercapto-6-methyl-5-oxo-1,2,4-triazine (1.00 g, 6.32 mmol) and the reaction mixture were refluxed for 7 h. The progress of the reaction was checked with TLC. The solid separated, filtered, washed with ethanol, dried and recrystallized from the same solvent. M.P. :200-204 $^\circ\text{C}$, For $\text{C}_{13}\text{H}_{14}\text{N}_4\text{O}_3\text{S}$, Calcd. C, 50.97; H, 4.61; N, 18.29; found: C, 50.74; H, 4.56; N, 18.09%.

Synthesis of 1:1 Metal complexes: 1:1 metal complexes were synthesized by mixing the hot ethanolic solutions of ligand (0.20 g, 0.65 mmol) in hot ethanolic solutions of acetates of Co(II) (0.163 g, 0.65 mmol), Ni(II) (0.163 g, 0.65 mmol), Cu(II) (0.131 g, 0.65 mmol) and Zn(II) (0.144 g, 0.65 mmol). The solid separated out immediately filtered, washed with warm water, aqueous ethanol and finally with acetone and then dried. The purity of the complexes was checked with TLC. Co(L)(OAc).3H₂O: $\text{C}_{15}\text{H}_{22}\text{CoN}_4\text{O}_8\text{S}$, Calcd. C, 37.74; H, 4.65; N, 11.74; Co, 12.35; found: C, 37.65; H, 4.58; N, 11.61; Co, 12.26%. Ni(L)(OAc).3H₂O: $\text{C}_{15}\text{H}_{22}\text{N}_4\text{NiO}_8\text{S}$, Calcd. C, 37.76; H, 4.65; N, 11.74; Ni, 12.30; found: C, 37.64; H, 4.60; N, 11.60; Ni, 12.22%. Cu(L)(OAc).H₂O: $\text{C}_{15}\text{H}_{18}\text{CuN}_4\text{O}_6\text{S}$, Calcd. C, 40.40; H, 4.07; N,

12.56; Cu, 14.25; found: C, 40.32; H, 4.00; N, 12.44; Cu, 14.18%. Zn(L)(OAc).3H₂O: C₁₅H₂₂N₄O₈SZn, Calcd. C, 37.24; H, 4.58; N, 11.58; Zn, 13.52; found: C, 37.18; H, 4.50; N, 11.50; Zn, 13.40%.

Synthesis of 1:2 Metal complexes: Treated the hot ethanolic solutions of acetates of Co (II) (0.163 g, 0.65 mmol), Ni(II) (0.163 g, 0.65 mmol), Cu(II) (0.131 g, 0.65 mmol) and Zn(II) (0.144 g, 0.65 mmol) with hot ethanolic solutions of ligand (0.40 g, 1.30 mmol). The solid product precipitates out, filtered, washed with warm water, aqueous ethanol and finally with acetone and then dried. Co(L)₂.2H₂O: C₂₆H₃₀CoN₈O₈S₂, Calcd. C, 44.26; H, 4.29; N, 15.88; Co, 8.35; found: C, 44.16; H, 4.18; N, 15.80; Co, 8.30%. Ni(L)₂.2H₂O: C₂₆H₃₀N₈NiO₈S₂, Calcd. C, 44.27; H, 4.29; N, 15.89; Ni, 8.32; found: C, 44.20; H, 4.20; N, 15.85; Ni, 8.26%. Cu(L)₂: C₂₆H₂₆CuN₈O₆S₂, Calcd. C, 46.32; H, 3.89; N, 16.62; Cu, 9.43; found: C, 46.20; H, 3.70; N, 16.56; Cu, 9.38%. Zn(L)₂.2H₂O: C₂₆H₃₀N₈O₈S₂Zn, Calcd. C, 43.85; H, 4.25; N, 15.74; Zn, 9.18; found: C, 43.78; H, 4.16; N, 15.68; Zn, 9.15%.

Antimicrobial Assay: 2.3.1 Newly synthesized Schiff base and its metal complexes were screened *in vitro* for antimicrobial activity. They were screened against six microbial strains, four bacteria (*Staphylococcus aureus* MTCC 96, *Bacillus subtilis* MTCC 121, *Pseudomonas aeruginosa* MTCC 741 and *Escherichia coli* MTCC 1652) and two yeast (*Candida albicans* MTCC 227 and *Saccharomyces cerevisiae* MTCC 170). All the bacterial cultures were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh.

In vitro antimicrobial activity: Agar well-diffusion method was used to screen the Schiff base and its metal complexes for biological activity. All the microbial culture were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5x10⁸ cfu/ml. 20 mL of Muller Hinton agar medium was poured into each Petri plate and plates were swabbed with 100 µL inocula of the test microorganisms and kept for 15 min for adsorption using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with 100 µL volume with concentration 4.0 mg mL⁻¹ of each compound reconstituted in dimethyl sulphoxide (DMSO). All the plates were incubated at 37°C for 24 h. Antimicrobial activity of each compound was evaluated by measuring the growth of inhibition zone against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas Ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism [19, 20].

Minimum Inhibitory Concentration (MIC) MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. MIC of the Schiff base and metal complexes were tested against bacterial strains through a modified agar well-diffusion method [21].

RESULTS AND DISCUSSION

Synthesis of bidentate Schiff base is outlined in figure 1. Schiff base are found to be colored, non-hygroscopic and stable. Schiff base metal complexes are soluble in organic solvents such as DMF, DMSO etc. The molar conductivity observed in the range 16-24 ohm⁻¹ cm mol⁻¹ suggests their non-electrolytic behavior. IR and thermal data suggest the presence of coordinated water molecules in the complexes.

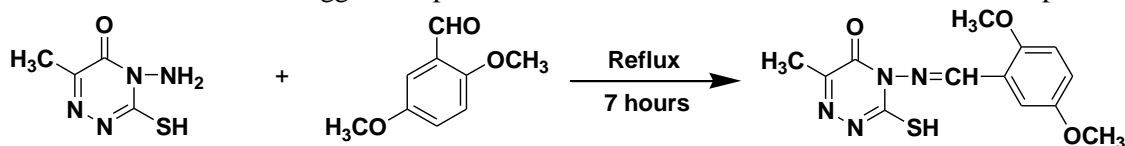


Figure 1 Synthesis of Schiff base

IR Spectra The tentative assignments of IR spectra of Schiff base and metal complexes have been listed in table 1. IR spectrum of Schiff base shows characteristic bands at 1612 and 2854 cm⁻¹ which is assigned to

imine (-CH=N-) group and thiol group respectively. The imine group vibration changes to lower frequency in the spectra of metal complexes indicate the coordination through N atom [22]. Band due to thiol group disappeared in the spectra of metal complexes indicates the complexation through S atom [23]. Further, in Schiff base a band observed at 1705 cm^{-1} due to $\nu(\text{C}=\text{O})$ group which remained same in the spectra of metal complexes indicates the non-participation of keto group in chelation. In metal complexes, bands observed in the region $3495\text{-}3742\text{ cm}^{-1}$ is assigned to coordinated water molecules [24]. Two new bands observed in metal complexes in the region $312\text{-}347\text{ cm}^{-1}$ and $459\text{-}570\text{ cm}^{-1}$ assigned to $\nu(\text{M-S})$ and $\nu(\text{M-N})$ respectively. In addition to this, bands observed at ~ 1651 and $\sim 1443\text{ cm}^{-1}$ due to asymmetric and symmetric stretch of acetate group which confirms the monodentate nature of acetate ion. In 1:1 metal complexes, band observed in the region $1740\text{-}1744\text{ cm}^{-1}$ due to $\nu(\text{OCOCH}_3)$ group.

Table 1 IR frequencies (cm^{-1}) of Schiff base and its metal complexes

Compound	ν (N=CH)	ν (C-S)	ν (S-H)	ν (OCOCH ₃)	ν (H ₂ O/OH)	ν (M-S)	ν (M-N)
HL	1612	-	2854	-	-	-	-
Co(L)(OAc).3H ₂ O	1520	752	-	1744	3742	335	459
Co(L) ₂ .2H ₂ O	1551	760	-	-	3618	347	461
Ni(L)(OAc).3H ₂ O	1589	750	-	1740	3502	312	484
Ni(L) ₂ .2H ₂ O	1589	756	-	-	3540	339	475
Cu(L)(OAc).H ₂ O	1597	756	-	1744	3595	343	524
Cu(L) ₂	1590	768	-	-	-	320	570
Zn(L)(OAc).3H ₂ O	1589	764	-	1744	3564	332	500
Zn(L) ₂ .2H ₂ O	1605	752	-	-	3495	341	494

¹H-NMR Spectra ¹H-NMR spectra of Schiff base and metal complexes have been recorded along with possible assignments and shown in table 2. In Schiff base spectrum, peak due to azomethine proton appeared at δ 8.78 ppm which observed at higher δ in spectra of Zn (II) complexes again confirms the coordination of Schiff base with metal through azomethine N atom [25]. Peak appeared at δ 13.60 ppm in Schiff base due to thiol proton disappeared in the spectra of metal complexes unambiguously indicates the deprotonation and complexation through S atom of thiol group [26]. In 1:1 spectrum of Zn (II) complex, new signal found at δ 1.80 ppm due to $-\text{CH}_3$ proton of $-\text{OCOCH}_3$ group.

Table 2 ¹H NMR spectral data of Schiff base and Zn(II) metal complexes

Compound	¹ H NMR (DMSO-d ₆) (ppm)
HL [C ₁₃ H ₁₄ N ₄ O ₃ S]	2.15 (s, 3H, -CH ₃), 3.78 (s, 6H, -OCH ₃), 7.15 (d, 1H, Ar-H), 7.23 (dd, 1H, Ar-H), 7.50 (d, 1H, Ar-H), 8.78 (s, 1H, -CH=N-), 13.60 (s, 1H, -SH)
Zn(L)(OAc).3H ₂ O [C ₁₅ H ₂₂ N ₄ O ₈ SZn]	2.19 (s, 3H, -CH ₃), 3.78 (s, 6H, -OCH ₃), 7.13 (d, 1H, Ar-H), 7.22 (dd, 1H, Ar-H), 7.51 (d, 1H, Ar-H), 9.84 (s, 1H, -CH=N-), 1.80 (s, 3H, -OCOCH ₃), 4.77 (s, 6H, -OH ₂)
Zn(L) ₂ .2H ₂ O [C ₂₆ H ₃₀ N ₈ O ₈ S ₂ Zn]	2.18 (s, 6H, -CH ₃), 3.77 (s, 12H, -OCH ₃), 7.14 (d, 2H, Ar-H), 7.21 (dd, 2H, Ar-H), 7.50 (d, 2H, Ar-H), 9.83 (s, 2H, -CH=N-), 4.79 (s, 4H, -OH ₂)

ESR Spectra: ESR spectra of Cu (L) OAc.H₂O and Cu(L)₂ have been recorded on X band at frequency 9.1 GHz under the magnetic field strength of 3000 G. The calculated electronic g factor for 1:1 and 1:2 Cu(II) complexes, g_{||} values observed as: 2.09, 2.17, g_⊥ = 2.05, 2.06, g_{av} as: 2.06, 2.09. The g value is less

than 2.3 indicate the partial covalent character in Cu (II) complexes. The g_{\parallel} and g_{\perp} values indicate the unpaired electron localized in $d_{x^2-y^2}$ orbital and suggests the square planar geometry for Cu (II) complexes [27, 28]. It was further evident from the value of G (Axial symmetry parameter) which is found less than 4 and also suggests the considerable exchange interaction present in Cu (II) complexes [29].

Electronic Spectra and Magnetic Moment Measurements: The electronic absorption spectra of Schiff base and metal complexes have been recorded by using DMF solvent in 10^{-3} M concentration (Table 3). The absorption spectra of Co(II) complexes show two absorption bands in the region $10012-10987\text{ cm}^{-1}$ (ν_1) and $19585-20110\text{ cm}^{-1}$ (ν_3) assigned to ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1) and ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3) transitions respectively. The ligand field parameters (D_q , B, β , $\beta\%$ [30], support octahedral geometry of Co (II) complexes. The value of D_q found in the range $1110-1220\text{ cm}^{-1}$ (within expected range of octahedral). The values of Racah interelectronic repulsion parameter (B) were less than free ion value, suggest the orbital overlap and delocalization of d-electrons. The values of β were less than unity; support the partial covalent nature of M-L bond. For Co (II) complexes the magnetic moment values are observed in the range 4.7-4.8 which lies in the expected range (4.3-5.2 BM) of octahedral complexes suggests the octahedral geometry for Co(II) complexes [31].

The absorption spectra of Ni(II) complexes shows three absorption bands in the region $10112-10977\text{ cm}^{-1}$ (ν_1), $17500-17646\text{ cm}^{-1}$ (ν_2) and $23995-24346\text{ cm}^{-1}$ (ν_3) attributed to ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1), ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3) transitions respectively. The values of D_q were found in the range $1010-1100\text{ cm}^{-1}$ (lies in octahedral range) support the octahedral geometry of Ni (II) complexes. The value of B was less than free ion value indicates the orbital overlap and delocalization of d-electrons. The values of β were less than unity suggests the partial covalent nature of metal-ligand bond. The magnetic moment values for Ni(II) complexes observed in the range 3.2-3.8 BM which correlate well with the range of octahedral Ni(II) complexes [32].

The absorption spectra of Cu (II) complexes shows one absorption band in the region $18449-19687\text{ cm}^{-1}$ assignable to ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transition which suggest the square planar geometry for Cu (II) complexes [33]. Again magnetic moment value for Cu (II) complexes observed in the range 1.8-2.0 BM which is well within the range of square planar complexes.

Table 3 Electronic spectral data and Ligand field parameters of metal complexes

Compound	ν (cm^{-1})	Band Assignment	D_q (cm^{-1})	B (cm^{-1})	ν_2/ν_1	β	$\beta\%$
Co(L)(OAc).3H ₂ O	10012	${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1)	1116.7	715	2.12	0.736	26.4
	21179*						
	19585	${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3)					
Co(L) ₂ .2H ₂ O	10987	${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ (ν_1)	1216.4	686.6	2.11	0.707	29.3
	23151*						
	20110	${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ (ν_3)					
Ni(L)(OAc).3H ₂ O	10112	${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1)	1011.2	767	1.73	0.736	26.4
	17500	${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2)					
	24346	${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3)					
Ni(L) ₂ .2H ₂ O	10977	${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1)	1097.7	580.6	1.60	0.558	44.2
	17646	${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2)					
	23995	${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3)					

* Calculated Values

Thermal Analysis: The TG analysis of Co(L)(OAc).3H₂O, Ni(L)(OAc).3H₂O, Cu(L)(OAc).H₂O and Zn(L)(OAc).3H₂O have been carried out in temperature range 50-700°C by using $\alpha\text{-Al}_2\text{O}_3$ as reference [34]. The thermo grams of the compounds are presented in figure 2 and data given in table 4.

The thermo gram of Co (L) (OAc).3H₂O shows decomposition in three steps. First decomposition step has been observed in the temperature range 90-240 °C with mass loss 11.00% (Calcd. 11.32%) corresponds to the loss of three water molecules. Second step results in mass loss 45.19% (Calcd. 46.76%) in the temperature range 241-395 °C due to removal of organic as well as acetate moieties. Third step corresponds to the loss of triazine ring with mass loss of 28.34% (Calcd. 29.56%) observed in the temperature range 396-550 °C. The remaining residue was estimated as CoO.

In case of Ni(L)(OAc).3H₂O, the first mass loss 10.53% (Calcd. 11.33%) was noticed in the temperature range 95-225 °C attributed to removal of three water molecules. Second step took place in the temperature range 226-450 °C with mass loss 45.61% (Calcd. 46.78 %) corresponds to the loss of organic and acetate moieties. Third step (451-600 °C) exhibits mass loss 28.34% (Calcd. 29.58%) assigned to removal of triazine ring leaving NiO as residue.

The TG curve of Cu (L)(OAc).H₂O decomposed in three steps. First step found in temperature range 85-200 °C with mass loss 3.24% (Calcd. 4.04%) consistent with the elimination of one water molecule. Second step (201-410 °C) involved mass loss 49.21% (Calcd. 50.05%) corresponds to removal of organic and acetate moieties. Third step has been found in the temperature range 411-640 °C with mass loss 30.79% (Calcd. 31.65%) due to loss of triazine ring leaving behind CuO as residue.

In Zn(L)(OAc).3H₂O, first decomposition step observed in the temperature range 100-230 °C with mass loss 10.24% (Calcd. 11.17%) attributed to the loss of three water molecules. Second step corresponds to the removal of organic and acetate moieties with mass loss 46.02% (Calcd. 46.13%) in the temperature range 231-400 °C. Third decomposition step (401-560 °C) results in mass loss 28.78% (Calcd. 29.17%) corresponds to the loss of triazine ring. The remaining residual estimated as ZnO.

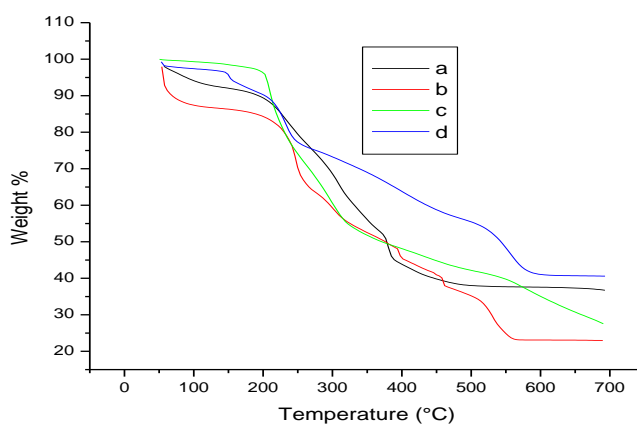


Figure 2 TG curve of complexes a) Co(L)(OAc).3H₂O b) Ni(L)(OAc).3H₂O c) Cu(L)(OAc).H₂O d) Zn(L)(OAc).3H₂O

Table 4 TG degradation of 1:1 metal complexes

Complexes	Stages	TG temperature range (°C)	Loss of mass percentage from TG (Theoretical)	Probable Assignment	Metallic Residue
Co(L)(OAc).3H ₂ O [C ₁₅ H ₂₂ CoN ₄ O ₈ S]	1 st	90-240	11.00 (11.32)	H ₂ O molecules OAc, Organic moiety Triazine ring	CoO
	2 nd	241-395	45.19 (46.76)		
	3 rd	396-550	28.34 (29.56)		

Ni(L)(OAc).3H ₂ O [C ₁₅ H ₂₂ N ₄ NiO ₈ S]	1 st	95-225	10.53 (11.33)	H ₂ O molecules OAc, Organic moiety Triazine ring	NiO
	2 nd	226-450	45.61 (46.78)		
	3 rd	451-600	28.34 (29.58)		
Cu(L)(OAc).H ₂ O [C ₁₅ H ₁₈ CuN ₄ O ₆ S]	1 st	85-200	3.24 (4.04)	H ₂ O molecules OAc, Organic moiety Triazine ring	CuO
	2 nd	201-410	49.21 (50.05)		
	3 rd	411-640	30.79 (31.65)		
Zn(L)(OAc).3H ₂ O [C ₁₅ H ₂₂ N ₄ O ₈ SZn]	1 st	100-230	10.24 (11.17)	H ₂ O molecules OAc, Organic moiety Triazine ring	ZnO
	2 nd	231-400	46.02 (46.13)		
	3 rd	401-560	28.78 (29.17)		

Cyclic Voltammogram: Electrochemical technique has been used to study the redox behavior of copper (II) complexes. Cyclic voltammogram of copper (II) complexes were studied in DMF solution at scan rate 100 mV/s with potential range 1.5 to -1.5 V by using TBAP as supporting electrolyte. 1:1 copper complex show reduction peak at $E_{pc} = -0.90$ V and oxidation peak at $E_{pa} = -1.30$ V. 1:2 copper complex show reduction peak at $E_{pc} = -0.85$ V and oxidation peak at $E_{pa} = -1.35$ V (Figure 3). The reduction peak associated with Cu(II)/Cu(I) couple and oxidation peak associated with Cu(I)/Cu(II) couple. Cyclic voltammogram of copper complexes reveal the one electron quasireversible behavior of copper complexes [35, 36].

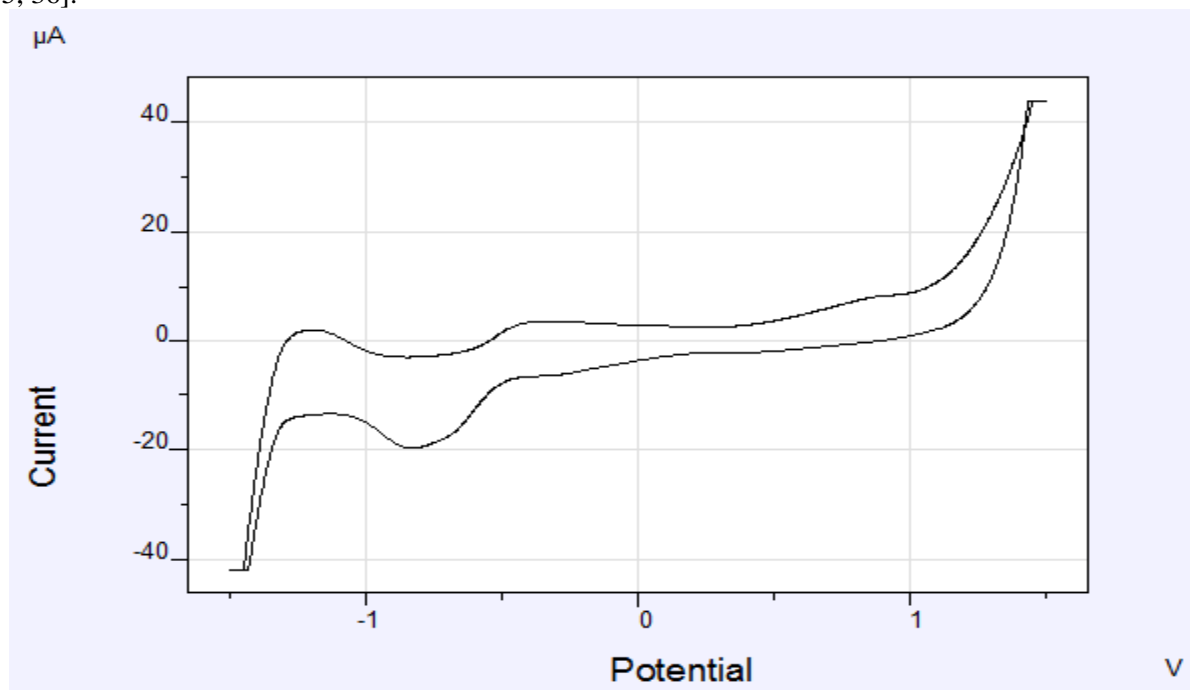


Figure 3 Cyclic voltammetry graph of Cu(L)₂

Fluorescence Spectra: Fluorescence property of Schiff base and its 1:1 metal complexes were studied in 10⁻³M solutions in DMF with excitation wavelength 265 nm. Schiff base display the emission band at 548 nm whereas Co(II), Ni(II), Cu(II), Zn(II) complexes characterized by emission band at 451 nm, 416 nm, 470 nm, 471 nm respectively (Figure 4). On the other hand, Zn (II) complex shows high intensity emission band over other metal complexes. Thus, Zn (II) complexes can be used as selective chemosensor [37, 38]. The enhancement of fluorescent intensity can be explain due to isomerization of C=N double bond. Schiff base show weak fluorescent intensity due to C=N isomerization in excited state and complex formation with metals prevent the process of C=N isomerization which enhances the fluorescent intensity [39]. The enhancement order of metal complexes are Schiff base < Cu(II) < Ni(II) < Co(II) < Zn(II).

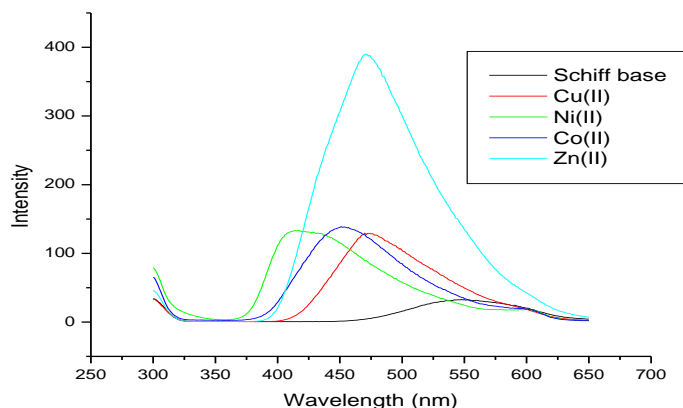


Figure 4 Fluorescence emission spectra of Schiff base and its 1:1 metal complexes

APPLICATIONS

Antimicrobial Screening: Agar well diffusion method has been used to investigate the antimicrobial activity of synthesized Schiff base and their complexes. The investigations have been carried out against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and *S. cerevisiae* microbes. Diameter of growth of inhibition zone for gram positive bacteria was observed in the range 10-25 mm, gram negative bacteria observed in 10-21 mm and yeast was found in between 12-20 mm. The data are listed in table 5 and figure 5. A comparative study of ligand and metal complexes indicates that metal complexes show enhancement in the activity as compared to parent ligand. This enhancement can be explained on the basis of Overtone's concept [40] and Tweedy's chelation theory [41]. According to the Overtone's concept of cell permeability, the lipid membrane surrounding the cell favors the passage of only lipid-soluble material; therefore, liposolubility is an important factor which controls the antimicrobial activity. Chelation enhances the lipophilic character of the central metal ion and reduces the polarity of the metal ion to a greater extent because of overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. So chelation favors the permeation of metal ion through the lipid membranes and blocks the metal binding sites in the enzymes of microorganisms. $\text{Co(L)(OAc)} \cdot 3\text{H}_2\text{O}$ show highest diameter of inhibition zone i.e. 22 mm, 20 mm against *B. subtilis* and *E. coli* respectively, $\text{Co(L)}_2 \cdot 2\text{H}_2\text{O}$ show highest diameter of inhibition zone i.e. 25 mm, 21 mm, 20 mm against *S. aureus*, *P. aeruginosa*, *S. cerevisiae* respectively. $\text{Cu(L)(OAc)} \cdot \text{H}_2\text{O}$ show highest diameter of inhibition zone (20 mm) against *C. albicans*.

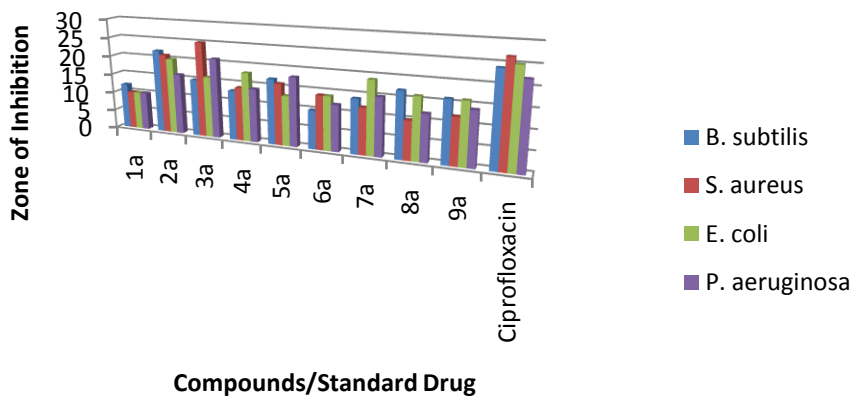


Figure 5 Antimicrobial activities of compounds

Table 5 Biological Activity of synthesized compounds

Compounds	Diameter of growth of inhibition zone (mm) ^a					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
HL	12	10	10	10	14	12
Co(L)(OAc).3H ₂ O	22	21	20	16	14	15
Co(L) ₂ .2H ₂ O	15	25	16	21	13	20
Ni(L)(OAc).3H ₂ O	13	14	18	14	19	10
Ni(L) ₂ .2H ₂ O	17	16	13	18	18	13
Cu(L)(OAc).H ₂ O	10	14	14	12	20	12
Cu(L) ₂	14	12	19	15	17	10
Zn(L)(OAc).3H ₂ O	17	10	16	12	15	13
Zn(L) ₂ .2H ₂ O	16	12	16	14	13	15
Ciprofloxacin	24.0	26.6	25.0	22	-	-
Amphotericin-B	-	-	-	-	16.6	19.3

^a Values including diameter of the well (8 mm), are means of three replicates.

Minimum Inhibitory Concentration of selected compounds compared with standard drugs Ciprofloxacin and Amphotericin B and listed in table 6.

Table 6 MIC values of synthesized compounds

Compounds	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>S. cerevisiae</i>
HL	-	-	-	-	-	-
Co(L)(OAc).3H ₂ O	6.25	12.5	12.5	-	-	-
Co(L) ₂ .2H ₂ O	-	6.25	-	12.5	-	25
Ni(L)(OAc).3H ₂ O	-	-	-	-	25	-
Ni(L) ₂ .2H ₂ O	25	-	-	50	25	-
Cu(L)(OAc).H ₂ O	-	-	-	-	12.5	-
Cu(L) ₂	-	-	25	-	50	-
Zn(L)(OAc).3H ₂ O	25	-	-	-	-	25
Zn(L) ₂ .2H ₂ O	-	-	-	-	-	25
Ciprofloxacin	6.25	6.25	6.25	12.5	-	-
Amphotericin-B	-	-	-	-	12.5	12.5

“-” not tested.

CONCLUSIONS

On the basis of above results and discussion, it is worth to mention that Schiff base ligand is of bidentate in nature. 1:1 and 1:2 metal complexes of Co (II), Ni (II) and Zn (II) possess octahedral geometry and 1:1 and 1:2 Cu(II) complexes possess square planar geometry. Proposed structures are presented in figure 6. Biological evaluation explain in the article help us to say that some metal complexes possess better biological activity as compared to ligand and standard drugs so that they can be used in medicine.

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