Synthesis, characterization and anti-microbial activity of tetazolium salt containing 8-hydroxy quinoline moiety in ligand and its metal chelates

R. T. Vashi* and N. M. Sheth

Department of Chemistry, Navyug Science College, Surat, India

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ABSTRACT

Novel ligand containing 8-hydroxyquinoline (HQ) moiety was prepared and characterized. For synthesis of ligands a hydroxyquinoline-5-carbaldehyde was treated with phenyl hydrazine to synthesis corresponding hydrazone. The hydrazone was condensed with diazonium salt of sulfanilamide to synthesis formazan. The formazan was converted to corresponding tetrazolium sals by using hydrogen peroxide, hydrobromic acid and ferrous sulphate. The isolated tetrazolium salt of 8-hydroxyquinoline derivative was designated as L_1. The novel ligand L_1 undergo the chelating reaction with Cu (II), Ni (II), Co (II), Mn (II), Zn (II) and Fe (III) salts to prepare transition metal chelates. These chelates were characterized by physicochemical methods such as elemental analysis, magnetic susceptibility, IR and electronic spectral data. The stoichiometry of the complex has been found to be 1: 2 (Metal: ligand) for divalent and 1:3 for trivalent ions. An octahedral geometry around Co (II), Ni (II),Fe (III) and Mn (II), distorted octahedral geometry around Cu (II) and tetrahedral geometry around Zn (II) have been proposed. The antimicrobial activity of ligand and its metal chelates was conducted against various bacteria.

Key words: 8-Hydroxyquinoline, Tetrazolium salt , Chelates, Electronic spectra, Antimicrobial activity.

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INTRODUCTION

The heterocyclic nitrogen compounds especially quinoline derivatives play a vital role in many biological processes and as synthetic drugs [1]. 8-Hydroxy quinoline or oxine have buff color crystalline compound almost insoluble in water , but readily soluble in organic solvents, such as alcohol, acetone and acetic acid. It is a versatile metal chelating agent in inorganic chemistry, it acts as bidentate anionic ligand and form chelate rings with wide range of metal ions. Chelate rings are formed by covalent bond and coordinate bond. The acidic group (-OH) form covalent bond by replacement of H$^+$ ions with metallic cation and tertiary amine (-N=) form coordinate bond by donation of electron pair [2] . Formazans and tetrazolium salts are known for their antiviral activity [3] . Anti-microbial activity of 8-Hydroxy quinoline derivatives of Cu (II) chelates were also studied [4] **.** Synthesis of some Co (II), Ni (II) and Cu (II) mixed ligand chelates of 8-hydroxy quinoline synthesis,anthranilic acid and o-aminophenol [5] was studied.. Mn (II) complex [6] and Zn (II) complex of 8-hydroxyquinoline-5-sulphonic acid was studied [7]**.** The present communication comprises the synthesis, characterization and chelating properties of novel tetrazolium salts containing 8-hydroxyquinoline merged molecules as ligand L_1 and its complexes with Cu (II), Ni (II), Co (II), Mn (II), Fe (III) and Zn (II). The ligand and metal ions were also studied for antimicrobial activities.

The reaction scheme for synthesis of ligand L_1, formazan, tetrazolium salts and its metal chelates have been presented in scheme-I.

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General reaction sequence has been shown in scheme I:
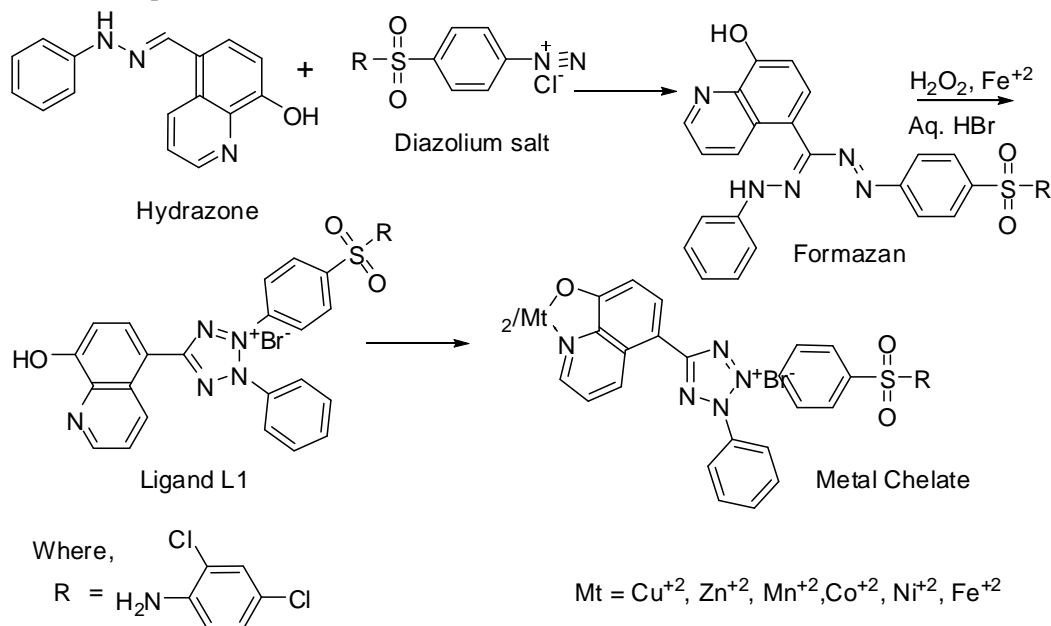
MATERIALS AND METHODS

All the chemicals used were of pure grade (Merck and B.D.H). Double distilled water was used throughout the experiment.

Synthesis of ligand L₁: The novel ligand   1-{4'-{[(2''',6'''-dichlorophenyl)amino] sulfonyl}phenyl}3-(8-hydroxyquinolin-5-yl)5-phenyl tetrazolium bromide was prepared from formazan.

Synthesis of Formazan :
To stir solution of 4-amino-N-(2,4-dichlorophenyl)benzenesulfonamide (1.72 gm, 0.01 mole) in mixture of acetic acid and concentrate hydrochloric acid (50:50 v/v, 20 ml), a solution of sodium nitrite (0.79 gm, 0.01 mole) in 10 ml water was added by maintaining the temperature between 0-5^{0}C. The diazotised compound was gradually added to stir solution of phenyl hydrazone derivative in pyridine (20 ml) by maintaining temperature below 10°C. The reaction mixture was allowed to stand at room temperature overnight and then quenched by pouring it in crushed ice. The separated solid product was washed with petroleum ether (40-60^{0}C) and then purified by re-crystallization in methanol to furnish formazan with 48 % yield.

Synthesis of Tetrazolium salt:
A solution of formazan (0.01 mole) in methanol was added to aqueous 2 N H_2SO_4 (5 ml) containing traces of ferrous sulphate. 20% Hydrogen peroxide solution (2 ml) was added in it at room temperature. The reaction mixture was heated on water bath for 5 h. The completion of oxidation was indicated by disappearance of color.

Excess methanol was distilled off and obtained syrup was treated with excess of aqueous sodium bromide to precipitate out the crude tetrazolium bromide, the solid product was filtered, washed with a little water. The product was suck dry and then triturated with petroleum ether (60-80 ^{0}C) to furnish tetrazolium bromide (ligand) with 50 % yield. The air dried product was quantitative. Melting point for L₁ was >300 °C.

Synthesis of Chelates:
Ligand L₁ solution preparation: Ligand L₁ (0.01 mol) was dissolved in 85 % (v/v) formic acid, diluted with 100 ml. distilled water and warm on water bath for 20 minutes. This clear solution was designated as reagent solution. This solution was used for preparation of chelates with metal ions by addition of transition metal ions.

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Trans metal ion solution: A solution of cupric acetate hydrate (0.011 mole) was prepared in warm 50 % formic acid (25 ml).The Trans metal ion solution was added drop wise to the solution of ligand under stirring. The resulting solution gave green coloration. The pH of reaction mass was adjusted to pH 5.0 by addition of liquor ammonia and stirred further for 1 h on water bath at 70 ºC. The solid mass was filtered and washed with distilled water followed by alcohol and dried at 100^0C for 24 h . Other metal chelates of ligand L_1 with Ni (II) ,Co (II) ,Zn (II), Mn (II) and Fe (III) ions were prepared in the same manner. Percentage yield of chelates was between 58-70 %.

The C, H and N contents of metal chelates were determined by elemental analyzer   Thermofiniggan 1101 Flash EA. The metal contents were estimated using standard methods [8]. The IR spectra (KBr) were recorded in the range 4000-400 cm^{-1} on a Perkin-Elmer-983 spectrophotometer. ^{1}H NMR spectra of ligand was recorded on Bruker NMR spectrophotometer (300 MHz). PMR chemical shifts were recorded in δ–value using TMS as an internal standard in DMSO d_6 solvent. The reflectance spectra of solid metal chelates were recorded on a Beckman DU spectrophotometer using MgO as reference. Magnetic susceptibility ' χ_m' was measured by Gouy's method [9] at room temperature (300 K) using Hg [Co(CNS)$_4$] as calibrant [10], and the effective magnetic moment from relation [11] , $\mu_{eff} = 2.84 \sqrt{\chi_m \times T}$, where T is the absolute temperature. Diamagnetic corrections were made by using Pascal's constants.

The ligand and its metal chelates were screen at 1000 ppm concentration in vitro for their antibacterial activity against five bacteria viz. *Staphylococcus aureus*, *E.Coli*, *P.Vulgarius* , *Pseudomonas auriginsa* and *Klebsiella* using DMF as solvent. The activity was compared with the known antibiotics viz. Ampicillin, Tetracycline, Gentamicin and Chloramphenicol at same concentration. The antibacterial activity of the compounds was measured by cup plate method [12]. The activity of standards and inhibition due to DMF (solvent) are given in Table-2. The results shown by compounds and standards were corrected for DMF.

The percentage inhibition of bacteria was calculated using the formula given below:

Percentage of inhibition =   100 (X-Y) / X

Where X= area of colony in control plate (without sample) and Y = area of colony in test plate.

RESULTS AND DISCUSSION

The complexes are microcrystalline colored powders having melting points higher than the ligand. They are stable in air at room temperature. All compounds gave satisfactory elemental analysis, suggesting 1:2 (metal: ligand ratio) for divalent and 1:3 for trivalent ions. The estimated values were consistent with those calculated on the basis of each repeated unit participating in the reaction. Elemental analysis data was shown in Table-1.

Infrared spectra of ligand L$_1$: IR spectrum of ligand L_1 (Fig.1) show a broad band extended from 3600 to 2500 cm^{-1} which might be responsible to phenolic group bonded to N atom of 8-hydroxy quinoline moiety [13-14]. The inflextious at 2923, 2852 and 1470 cm^{-1} are due to aromatic CH and methylene group of bridge and piperazine ring [15].Several bands appeared between 1500-1600 cm^{-1} region may arise from aromatic breathing. The IR band at ~ 1635 cm^{-1} might be due to -C=N- group of 8-quinolinol system of L_1 ligand shifted to higher frequency side ~1622 in the spectra of the metal chelates indicating involvement of nitrogen in the chelate formation [13-16]. The band around 1333 cm^{-1} is responsible to –SO$_2$ group.

IR Specra of Chelates: Most of bands appeared in the spectra of corresponding ligand are observed at their metal chelates. The –OH stretching band in the chelate is much less broad than that of the parent ligand. Only a new band at 1095 cm^{-1} had appeared in the spectra of metal chelates. This may be assigned to υc-o of C-O-M bond formation.The band at 614-682 cm^{-1} and 500-514 cm^{-1} can possibly be assigned to Mt-O and Mt-N bonds respectively [17]. IR spectra of Cu (II) and Co(II) chelates were shown in Fig.2 as well as in Fig. 3 respectively.
As the ligand was insoluble in common organic solvents its NMR spectra have not been scanned.

Magnetic moment and Electronic spectra: Analytical and magnetic moment data of ligand and chelates were shown in Table-1. At room temperature, the magnetic moment 'μ_{eff}' value for the Co (II) complex is 4.85 B.M. suggest high spin octahedral geometry [18], which is further supported by the electronic spectral data. The electronic

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spectra of the Co(II) chelate display three absorption bands in the range 9520,17240 and 21740 cm^{-1}, assigned to the transitions $^4T_{1g}$ (F) ----> $^4T_{2g}$ (F) (υ_1), $^4T_{1g}$ (F)---> $^4A_{2g}$ (F) (υ_2) and $^4T_{1g}$ (F) ----> $^4T_{1g}$ (P) (υ_3) respectively. These transition suggest an octahedral environment around the cobalt ion [19]. The values of transition ratio υ_2/υ_1 is 1.81 providing further evidences for octahedral geometry for the Co (II) complexes.

In the Ni (II) complex, μ_{eff} value at room temperature is observed to be 3.17 B.M. as expected for six coordinated spin free Ni (II) species. The reflectance spectra of the Ni (II) complex, exhibit two strong bands at 9710, 14390 and 21740 cm^{-1}, assignable to $^3A_{2g}$ (F) ----> $^3T_{1g}$ (F) and $^3A_{2g}$ (F) ----> $^3T_{1g}$ (P) respectively [20]. The υ_2/υ_1 ratio for the chelate is 1.57 occurs in the usual range (1.6 –1.82) for octahedral Ni (II) chelates [20]. The spectral bands are well within the range observed for hexa-coordinate octahedral complexes reported earlier [21].

The Cu (II) complex exhibit normal magnetic moments (1.98 B.M.) indicating the distorted octahedral geometry, which is in agreement with data reported by several research workers [22-23]. This complex show broad asymmetric bands in the region 10870, 14710 cm^{-1} and at 20830 cm^{-1} assignable to $^2B_{1g}$ ----> $^2A_{1g}$ and charge transfer transition respectively [24-25]. These results reveal the distorted octahedral geometry for this complex. The former band may be due to ^2E_g ----> $^2T_{2g}$ accounted due to Jahn Teller effect suggesting thereby a distorted octahedral geometry for these complexes [26].The values of transition ratio v_2/v_1 is 1.50.

The electronic spectra of the Mn (II) complex exhibited three spin allowed bands in the region 20200, 22470 cm^{-1} and 23260 cm^{-1} assigned to the transitions $^6A_{1g}$ ----> $^4T_{1g}$ (^4G), $^6A_{1g}$----> $^4T_{2g}$ (^4G) and $^6A_{1g}$ ----> ^4E_g, $^4T_{1g}$ (^4G) respectively, indicating octahedral geometry. The observed magnetic moment (5.60 B.M.) of the complex indicates high spin octahedral environment [27].

Zinc (II) chelate is diamagnetic in nature as expected for d^{10} systems and may have tetrahedral geometry [28-29]. There is no evidence for the characteristic bands of coordinated water in IR spectra.

In the Fe (III) chelate, μ_{eff} value at room temperature is observed to be 4.93 B.M. which is lower than the theoretical value (5.92 B.M.). The reduction of magnetic moment may be due to a distortion from regular symmetry [30]. The reflectance spectra of the Fe (III) complex, exhibit two strong bands at 10420, 15630 and 19230 cm^{-1}, assignable to $^6A_{1g}$ (F) ---->$^4T_{1g}$ (F) and $^6A_{1g}$ (F) ---->$^4T_{2g}$ (P) and $^6A_{1g}$ (F) ---->$^4T_{1g}$ (^4Eg) respectively [31] . The υ_2/υ_1 ratio for the chelate is 1.50 occurs in the usual range for octahedral Fe (II) chelates.

Table-1 Analytical and physical data of ligand L$_1$ and its metal chelates.

Ligand / Mol. Formula	M. W. (g/mole)	Yield (%)	Elemental Analysis (%) Found (Calc.)				Observed μ_{eff} (B.M)
			C	**H**	**N**	**M**	
L$_1$ C$_{28}$H$_{19}$Cl$_2$N$_6$O$_3$S	590.0	72	60.20 (60.21)	3.3 (3.4)	15.00 (15.05)	-	-
Cu[L$_1$(H$_2$O)]$_2$ C$_{56}$H$_{40}$Cl$_4$N$_{12}$O$_8$S$_2$Cu	1277.54	68	52.50 (52.60)	3.10 (3.13)	13.10 (13.15)	4.90 (4.97)	1.98 (1.7-2.2)
Co[L$_1$(H$_2$O)]$_2$ C$_{56}$H$_{40}$Cl$_4$N$_{12}$O$_8$S$_2$Co	1272.93	70	52.70 (52.79)	3.00 (3.14)	13.10 (13.19)	4.60 (4.62)	4.85 (4.4-5.2)
Ni[L$_1$(H$_2$O)]$_2$ C$_{56}$H$_{40}$Cl$_4$N$_{12}$O$_8$S$_2$Ni	1272.69	69	52.70 (52.80)	3..00 (3.14)	13.00 (13.20)	4.60 (4.61)	3.17 (2.9-3.4)
Mn[L$_1$(H$_2$O)]$_2$ C$_{56}$H$_{40}$Cl$_4$N$_{12}$O$_8$S$_2$Mn	1268.93	58	52.90 (52.95)	3.10 (3.15)	13.20 (13.23)	4.30 (4.32)	5.60 (5.2-6.0)
Zn[L$_1$(H$_2$O)]$_2$ C$_{56}$H$_{40}$Cl$_4$N$_{12}$O$_8$S$_2$Zn	1279.39	60	52.50 (52.52)	3.00 (3.12)	13.00 (13.13)	5.10 (5.11)	-
Fe[L$_1$(H$_2$O)]$_3$ C$_{84}$H$_{60}$Cl$_6$N$_{18}$O$_{12}$S$_3$Fe	1876.84	62	53.60 (53.70)	3.10 (3.19)	11.40 (11.50)	2.95 (2.97)	5.9 (5.2-6.0)

Antibacterial activity:
The ligand and chelates have shown good to moderate activity against bacteria, such as *Staphylococcus aureus (Gram positive)*, *E.Coli*, *Proteus Vulgaris* , *Pseudomonas aeruginosa* and *Klebsiella Pnemoniae (gram negative)* as compared to known standard drugs. Ligand was found to be less active than the standard drugs (Table-2). It is observed that the Cu(II) and Ni (II) complexes show enhanced antibacterial activity as compared to the ligand. This is because of of the chelation, which reduces the polarity of metal ion due to partial sharing of its positive charge with donor groups and also due to the delcalisation of pi-electrons over whole ring. This chelation increases

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lipophilic character in the complexes and results in enhancement of activity **[32].** Complexes of ligand show inhibition of bacterial growth in following decreasing order: Cu (II) > Ni (II) > Fe (III) > Co (II) > Zn (II) > Mn (II).The substitutions of phenyl rings by chlorine have much more effect on the bacterial activity. The results suggest that variation in structure on coordination affects the growth of micro organisms and may result in to inhibitory or reduction in toxicology of metal ions towards some organisms **[33].**

Table-2 Antibacterial activity of ligand L_1 and its metal chelates.

Samples	Zone of inhibition at 1000 ppm (%)				
	Staphylococcus aureus	*E. Coli*	*Proteus Vulgaris*	*Pseudomonas aeruginosa*	*Klebsiella pnemoniae*
L_1	73	71	75	72	68
$(L_1)_2 Cu^{2+}$	74	78	80	76	75
$(L_1)_2 Ni^{2+}$	75	77	79	78	76
$(L_1)_2 Co^{2+}$	70	74	76	73	72
$(L_1)_2 Mn^{2+}$	67	70	72	69	68
$(L_1)_2 Zn^{2+}$	68	72	74	71	70
$(L_1)_2 Fe^{2+}$	73	76	78	75	74
DMF	5	5	5	5	5
Ampicillin	80	83	79	84	81
Tetracycline	72	86	81	71	69
Gentamycine	80	75	74	80	82

Fig.1. IR Spectrum of ligand L_1 .

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Type	Vibration Mode	Frequency cm⁻¹
N=C-N	C=N	1628
Aromatic	ring skeleton	1595 1521
sulphonamide	O=S=O	1333 1145
Aromatic	-C=H	3006

Fig.- 6.1: IR Spectrum of Cu-L₁ Metal Chelate
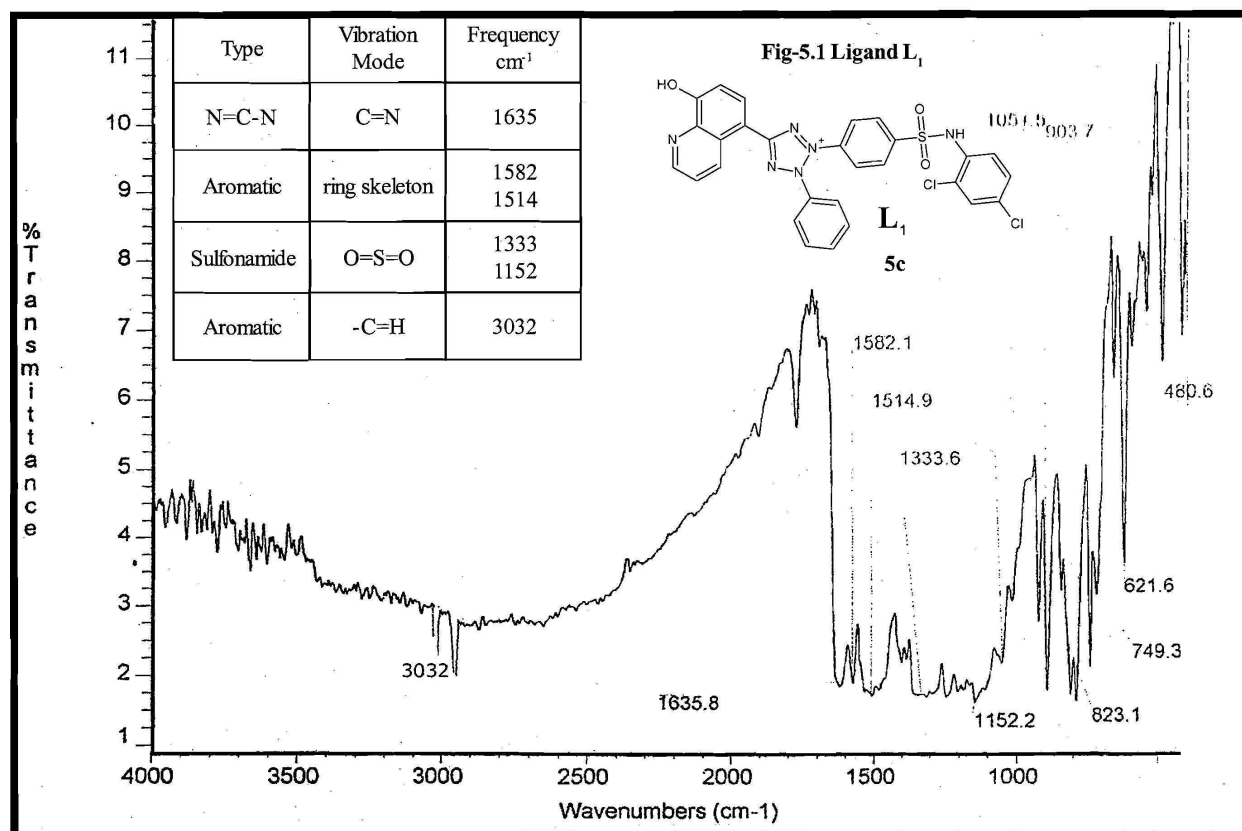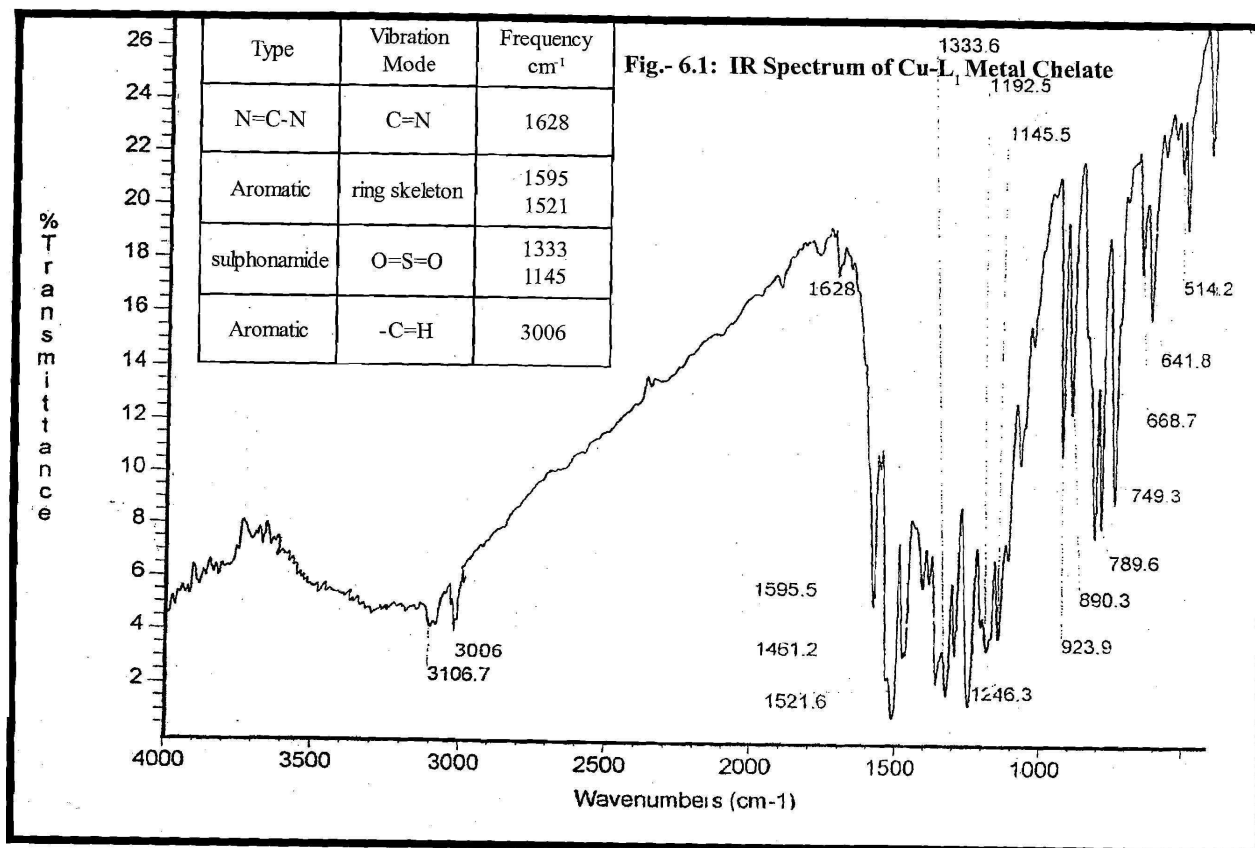
Fig.2.   IR Spectrum of Cu (II) chelate of Ligand L₁.

Type	Vibration Mode	Frequency cm⁻¹
N=C-N	C=N	1622
Aromatic	ring skeleton	1582 1501
sulphonamide	O=S=O	1326 1152
Aromatic	-C=H	3002

Fig.- 6.2: IR Spectrum of Co-L₁ Metal Chelate
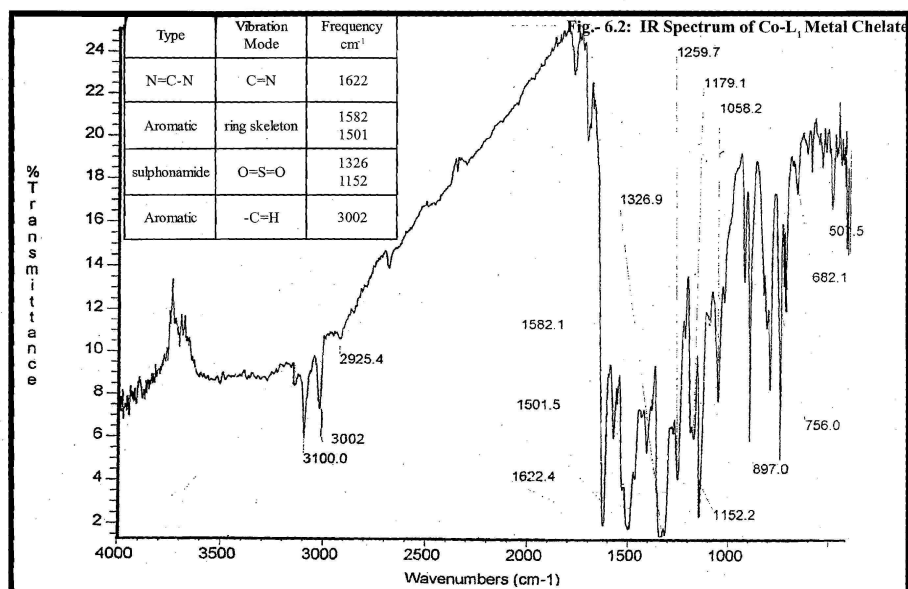
Fig 3 . IR Spectrum of Co (II) Chelate of Ligand L₁.

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CONCLUSION

Octahedral structures for Ni (II), Co(II) and Mn (II) complexes, tetrahedral polymeric structure for Zn(II), and distorted octahedral for Cu(II) complex have been tentatively proposed. Copper (II) chelate was found to have the maximum inhibition capacity against all the bacterial species under study. Present study will contribute in the field of new antibacterial for some plant pathogenic organisms.

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