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Co(II), Ni(II), Cu(II) COMPLEXES OF SULPHUR CONTAINING SCHIFF BASE LIGAND-SYNTHESIS, SPECTROSCOPIC, MOLECULAR MODELLING AND ANTIMICROBIAL STUDIES

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Keywords:

Schiff base, Metal complexes, Octahedral geometry, Molecular modeling, Antimicrobial activity

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ABSTRACT: Metal (II) complexes of Co, Ni and Cu with Schiff base derived from 2, 4-Dihydroxy-5-acetylacetophenone and Aminothiophenol (DAAAP) were synthesized and characterized by elemental analysis, FTIR, ^{1}H NMR, electronic spectra, thermal analysis, magnetic susceptibility and conductometric measurements. On the basis of these studies, a six coordinated octahedral geometry has been proposed for all the complexes. The Schiff base and its complexes were tested for biological activity against various types of Gram-positive (*Bacillus subtilis* ATTC 6051 and *S. pyogones* ATTC 12600) and Gram – negative bacteria (*Escherichia coli* ATTC 11775 and *Proteus vulgaris* ATTC 13315) and *Fusarium solani* Martius and *Aspergillus niger* Fungus. It has been found that metal complexes are found to have sensitivity for inhibition of Gram-positive more than Gram-negative bacteria. All the metal complexes show antifungal activity and it is clear from the data that the complexes exhibit more antifungal activity compared to the parent ligand.

INTRODUCTION: Schiff bases and their bio-active complexes have been studied extensively over the past decade [1, 2]. Schiff bases are useful chelators because of their ease of preparation, structural varieties, varied denticities and subtle steric and electronic control on their framework. Many Schiff base ligands have been reported by their potential applications in industry, pharma, drug, and catalysis. Metal complexes of Schiff base ligands have shown a wide variety of applications in the medicinal [3, 4] and biological fields [5, 6] such as antibacterial, anti-proliferative, antimalarial, anticancer [7-9], antifungal, anti-inflammatory, antiviral and antipyretic properties[10].

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The development of new antibacterial drugs enriched by innovatory and more effective mechanisms of actions is an urgent medical need. Schiff bases are identified as promising antibacterial agents [11]. Exploration and development of more effective antifungal agents is a necessity, and the individual Schiff bases are considered to be promising antifungal medicines [12, 13]. Metal complexes containing sulfur, nitrogen chelating ligands have gained importance, due to their distinct biological activities and models of metalloenzyme active sites [14-16].

A class of multidentate ligands developed from symmetric aromatic diketones have received investigation in our laboratory in view of their potentiality to simultaneously bind at least two metal ions with the help of two chelating sequences. Such ligands have been designated as bis-chelating ligands [17, 18]. The metal complexes synthesized from such ligands have been observed to possess interesting structural features like

polymerism, antiferromagnetism and unusual geometries. 2, 4-Dihydroxy-5-acetylacetophenone (H_2 – DAAP) is a diketone which can bind to metal ions through two O O sequences. Variety of bis-chelating ligands can be synthesized by condensing H_2 – DAAP with different amines, which may behave as either symmetric or unsymmetric bis-chelants towards transition metal ions. Infact some of such ligands can be tailor made or designed to drive metal ions into suitable / preferred coordination geometries. Hence we describe here the synthesis, characterization and antimicrobial activity of Co(II), Ni(II), Cu(II) complexes of sulphur containing Schiff base ligand by condensation of 2, 4-Dihydroxy-5-acetylacetophenone with ortho Aminothiophenol.

MATERIALS AND METHODS: All the chemicals and solvents used were of AR grade ≥ 99.0% (Merck, Mumbai, India). The purity of the ligand and complexes was tested by thin layer chromatography (TLC). Suitable solvent mixtures of ethyl acetate, benzene, carbon tetrachloride, *etc*., were used to develop chromatograms. The spots were identified by iodine vapor. The antibacterial activities of the ligand were studied by disc diffusion method. Double distilled water was used for the preparation of buffer solutions. Melting points of all the compounds were determined in open glass capillaries and are uncorrected.

Elemental analysis (C, H, and N) was carried out on PERKIN ELMER, Series II, and 2400 CHNS/Analyser. Infrared spectra were recorded on a Fourier transform infrared (FTIR), GX FT-IR PERKIN ELMER, in the range 4000-400 cm^{-1} by making a KBr pellet of the compound. Conductivity measurements were done using an Elico Digital conductivity bridge (model: CM-180). The electronic spectra of samples were recorded on UV Lambda 19 PerkinElmer spectrophotometer.

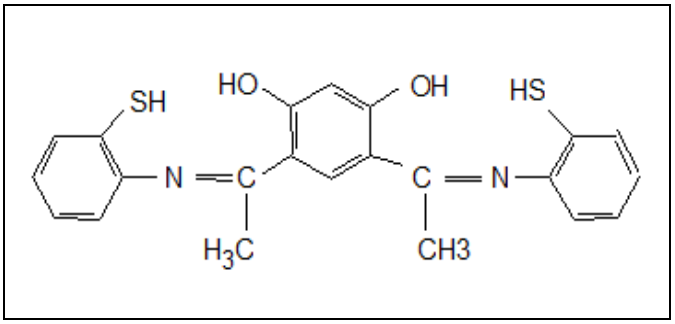


FIG. 1: PROPOSED STRUCTURE OF THE LIGAND

Synthesis of the Schiff Base Ligand: The Schiff base ligand (DAAAP) was synthesized by the condensation 2, 4-Dihydroxy-5-acetylacetophenone with ortho Aminothiophenol [19]. The ligand was prepared, characterized, and reported from our laboratory.

Preparation of the Schiff Base Metal (II) Complexes: An ethanolic (20 ml) solution of Schiff base ligand (40m mol) was added drop wise to 20 ml of the metal salts [20mmol, 0.04g of $CuCl_2.4H_2O$, 0.048g of $CoCl_2.6H_2O$, 0.047g of $NiCl_2.6H_2O$] in boiling ethanol (78.7 °C). The reactions took place in a 2:1 mole ratio of H_2L: metal. The pH of the reaction mixture was adjusted to 7 by adding aqueous ammonia in methanol, and the mixture was refluxed for 4 h. The colored product obtained was filtered in the hot condition and was washed thoroughly with small amounts of methanol, petroleum ether and dried in vacuum [20, 21]. The purity of the complexes was tested by TLC using different solvent mixtures.

Yields: Cobalt(II) 60%, Nickel(II) 50%, Copper(II) 60%.

Co(II) Complex: Yield: 65%; colour: brown; Melting point: > 300 ºC; solubility: DMSO; Anal. Data cal: C 60.41%, H 5.03%, N 6.40%, M 13.50%; Found: C 59.91%, H 5.01%, N 6.35%, M 13.46%; µeff B.M: 4.74; ΩM (mho cm^2 mol^{-1}) 12; Selected FT-IR data (KBrcm^{-1} 1590 (υ C=N), 1254(υ C-O), 2648(υ SH), 3378 (υ OH), 532 & 495 (νM-O), 448 & 312 (νM-N); UV-Vis (solid) λmax(cm^{-1}): 8890 ($4T_{1g}(F) \rightarrow 4T_{2g}$) 17,249 ($4T_{1g}(F) \rightarrow 4A_{2g}(F)$) and 28,000 ($4T_{1g}(F) \rightarrow$ 4T1g(P)).

Ni(II) Complex: Yield: 70%; colour: black; Melting point: >300 ºC; solubility: DMSO; Anal. Data cal: C 60.45%, H 5.03%, N 6.41%, M 13.50%; Found: C 60.37%, H 4.99%, N 6.32%, M 13.31%; µeff B.M: 2.88; ΩM (mho cm^2 mol^{-1}) 14; Selected FT-IR data (KBr cm^{-1}: 1591 (υ C=N), 1225(υ C-O), 2645(υ SH), 3400 (υ OH), 582 & 549 (υ M-O), 416 & 372 (υ M-N); UV-Vis (solid) λmax (cm^{-1}): 9250 ($^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$), 15518 ($^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, and 26315 ($^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$).

Cu(II) Complex: Yield: 72%; colour: black; Melting point: >300 ºC; solubility: DMSO; Anal. Data cal: C 59.79%, H 4.98%, N 6.34%, M

14.38%; Found: C 59.68%, H 4.92%, N 6.28%, M 14.32%; μeff B.M: 1.74; ΩM (mho cm^2 mol^{-1}) 22; Selected FT-IR data (KBr cm^{-1}): 1574 (υ C=N), 1246 (υ C-O), 2647 (υ SH), 3400 (υ OH), 533 & 524 (υ M-O), 425 & 362 (υ M-N); UV-Vis (solid) λmax (cm^{-1}): 12,000 ($^2B_{1g} \rightarrow {}^2A_{1g}$), 17241 ($^2B_{1g} \rightarrow {}^2B_{2g}$), 20000 ($^2B_{1g} \rightarrow {}^2E_{1g}$).

Anti-Microbial Activity: The Schiff base and its complexes were tested for biological activity against various types of Gram-positive (*Bacillus subtilis* ATTC 6051 and *S. pyogones* ATTC 12600) and Gram – negative bacteria (*Escherichia coli* ATTC 11775 and *Proteus vulgaris* ATTC 13315) and *Fusarium solani* Martius and *Aspergillus niger* Fungus. The sensitivity of a microorganism to antibiotics and other antimicrobial agents was determined by the assay plates which incubated at 28 °C for two days for yeasts and at 37 °C for one day for bacteria [22-24].

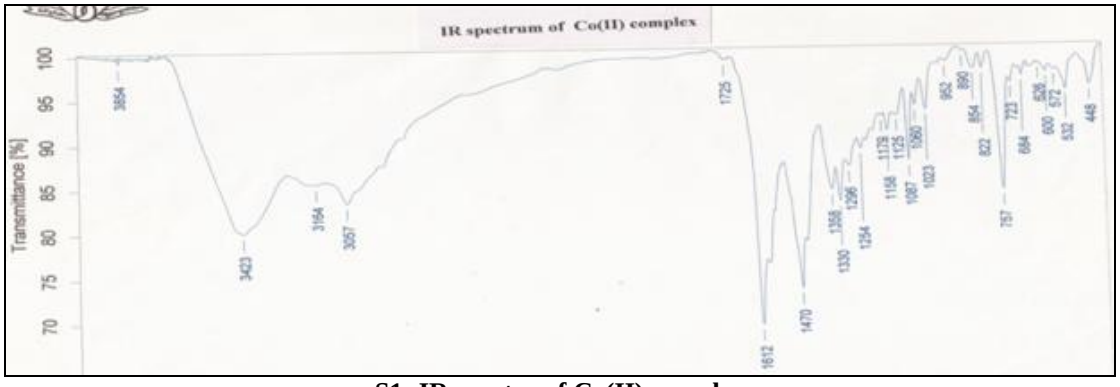
RESULTS AND DISCUSSION: All the metal complexes are colored solids, stable towards air, and have high melting points. The complexes are insoluble in water and common organic solvents but soluble in DMF and DMSO. The analytical data for the ligand and their complexes, together with some physical properties are summarized in **Table 1**. The conductivity measurements were carried out in freshly prepared 1×10^{-3} M dimethyl sulphoxide (DMSO) solutions. The molar conductivity values are given in **Table 1**.

Molar conductance values of the complexes (12 to 22 mho cm^2 $mole^{-1}$) indicate that all the complexes are non-electrolytes.

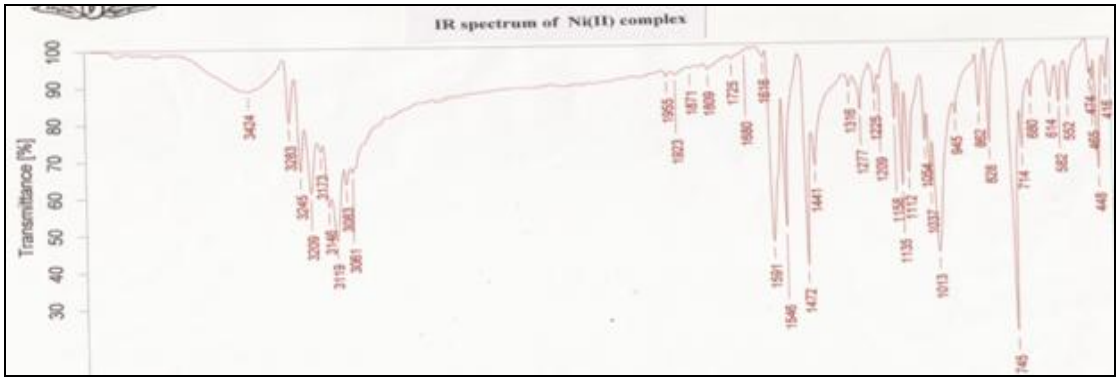
TABLE 1: ANALYTICAL DATA AND PHYSICAL PROPERTIES OF COMPLEXES

Compound	M. Pt	Yield	Elements (found) calcd %				ΩM (mho cm^2mol^{-1})
			carbon	hydrogen	nitrogen	metal	
Co(II) complex	>300	65%	(59.91)	(5.01)	(6.35)	(13.46)	12
			60.41	5.03	6.40	13.50	
Ni (II) complex	>300	70%	(60.37)	(4.99)	(6.32)	(13.31)	14
			60.45	5.03	6.41	13.50	
Cu (II) complex	>300	72%	(59.68)	(4.92)	(6.28)	(14.32)	22
			59.79	4.98	6.34	14.38	

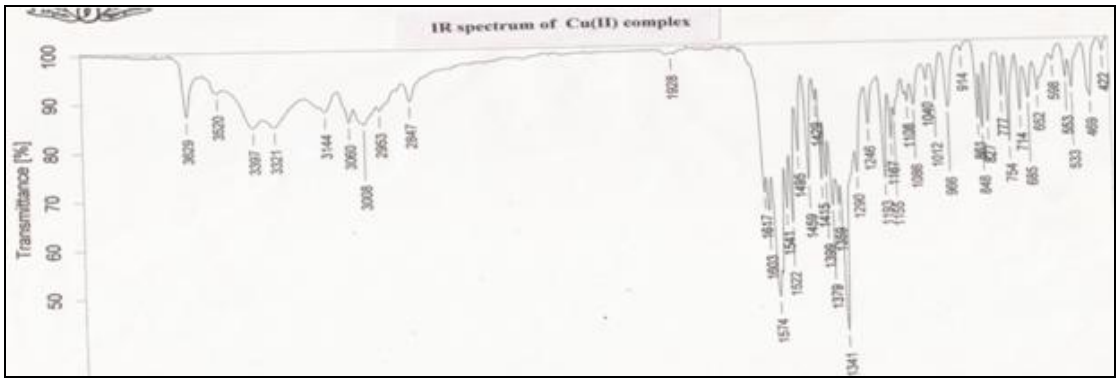
IR Spectral Studies: The characteristic vibrational stretching frequencies of the complexes were shifted when compared to that of the ligand. The very strong band at 1607 cm^{-1} is characteristics of the azomethine nitrogen present in the ligand H_2-DAAAP [25]. This was shifted to 1574-1590 cm^{-1} in the complexes, which indicates the coordination of the metal to the azomethine nitrogen [26, 27]. υ(C-O) observed at 1206 cm^{-1} in the ligand spectra, has shown a positive shift of 20-30 cm^{-1} indicating coordination through phenolic oxygen [28]. The ligand showed a strong absorption band at 3378 cm^{-1} which is assigned to the phenolic O-H and band at 2648 cm^{-1} can be assigned to υS-H. The strong absorption bands shown around 3400 cm^{-1} in all the complexes can be assigned to υ(OH) of coordinated water. New bands observed at474-594 cm^{-1} and 291-435 cm^{-1} in the far infrared region are assignable to υ(M-O) and υ(M-N) modes. This occurrence indicates that there is coordination between the metal and the lone pair of electron on the nitrogen atom of the ligand and formation of M-O bond in the complexes.



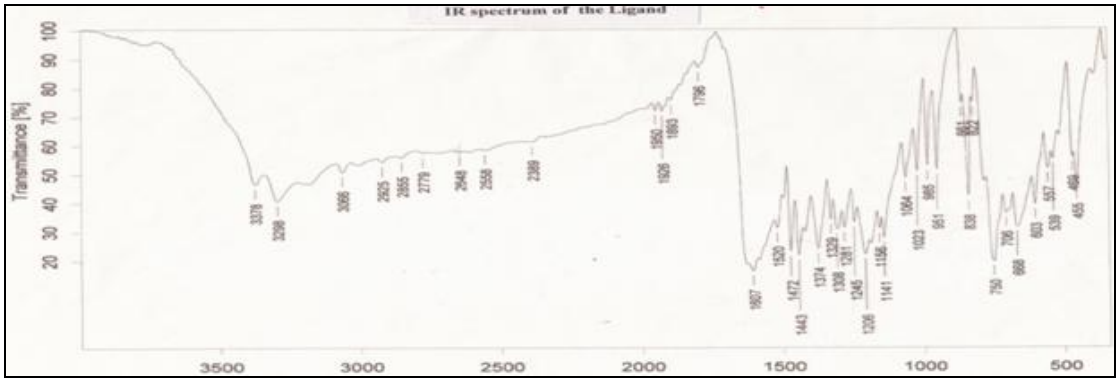
S1: IR spectra of Co(II) complex



S2: IR spectra of Ni(II) complex



S3: IR spectra of Cu(II) complex



S4: IR spectra of the ligand

TABLE 2: CHARACTERISTIC INFRARED FREQUENCIES OF DAAAP COMPLEXES (cm^{-1})

Compound	υ (OH) phenolic/H_2O	υ C=N	υ C-O	υ (SH)	New bands
DAAAP	3378	1607	1206	2648	
Co(II) complex	3423	1590	1254	2648	572,532,495,448,312
Ni (II) complex	3424	1591	1225	2645	582,549,448,416,372
Cu (II) complex	3397	1574	1246	2647	598,533,524,425,362

Thermo Gravimetric Analysis: Thermal study of complexes show that there is a loss of two coordinated water molecules [29, 30] with weight loss of about 5-6%. The complexes exhibit thermal stability up to 350 ℃.

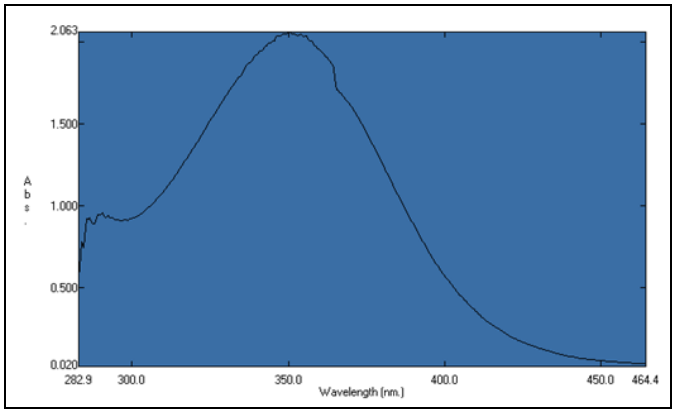
Electronic Spectral Studies: The electronic transition study of the free ligand exhibited three distinct bands at 35714, 29940, 28571, 22,222, 23,752 cm^{-1}. The first two bands correspond to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of the azomethine chromophore respectively.

The electronic spectra of Co(II) complex showed multiple bands at 8890,17,249 and 28,000 cm^{-1}. These bands are ascribed to $^4T_{1g}(F) \rightarrow {}^4T_{2g}$; $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ transitions, respectively, consistent with the octahedral geometry. υ_2 / υ_1 ratio is found to be 1.94, which further supports octahedral geometry for Co(II)

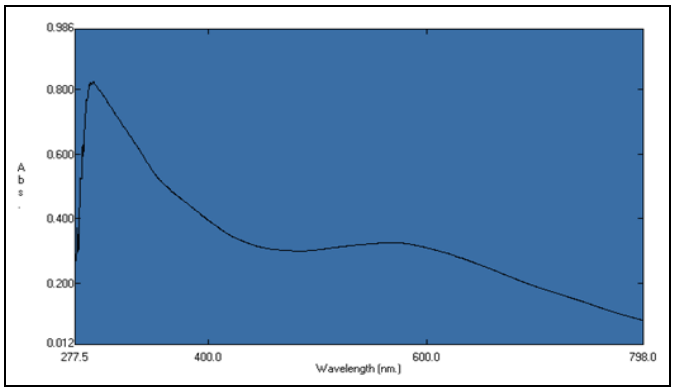
complex [31]. The magnetic moment value for the Co(II) complex has been used as a criterion to determine the type of coordination around the metal ion. Due to the intrinsic orbital angular momentum in the ground state, there is consistently a considerable orbital contribution and the effective magnetic moment lies between 4.7 and 5.2 B.M. at room temperature. The magnetic moment value of 4.74 B.M. suggests an octahedral geometry for the Co(II) complex in the high-spin state. The Ni(II) complexes have three spin-allowed transitions at 9250, 15518, 22,222 and 26315 cm^{-1}. These bands are correlated to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$; $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ transitions, respectively in an octahedral stereochemistry [32]. υ_2 / υ_1 ratio is found to be 1.66 which further supports octahedral geometry. The magnetic moment value (2.88 B.M.) is within the range expected for similar octahedral Ni(II) ions. The electronic spectra of Cu(II) complex displays bands at 12,000,12987, 17241 and 20000 cm^{-1} attributed to $^2B_{1g} \rightarrow {}^2A_{1g}$; $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2E_{1g}$ transitions expected for an octahedral geometry of Cu(II) complex [33]. The magnetic moment value of the Cu(II) complex (1.74 B.M.) is very close to the spin value (1.73 B.M.) expected for one unpaired electron which offers the possibility of an octahedral geometry.

TABLE 3: MAGNETIC & ELECTRONIC SPECTRAL DATA OF DAAAP COMPLEXES

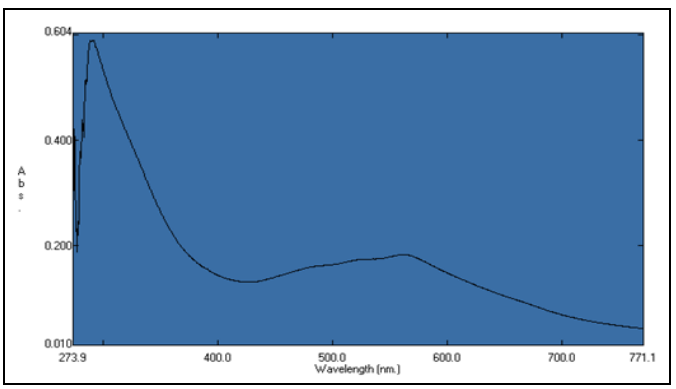
Complex	μeff B.M (observed)	UV-Vis bands (cm^{-1})	Assignment	υ_2 / υ_1
Co(II) complex	4.74	8890	$^4T_{1g}(F) \rightarrow {}^4T_{2g}$	1.94
		17,249 28,000	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$	
			$^4T_{1g}(F) \rightarrow {}^4T1g(P)$	
Ni (II) complex	2.88	9250,	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$	1.66
		15518,	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$	
		22,222	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	
		26315		
Cu (II) complex	1.74	12,000,	$^2B_{1g} \rightarrow {}^2A_{1g}$	1.82
		21840,	$^2B_{1g} \rightarrow {}^2B_{2g}$	
		23424	$^2B_{1g} \rightarrow {}^2E_{1g}$	
		25072		



S5: UV-Vis-NIR SPECTRA of Co(II) COMPLEX



S6: UV-Vis-NIR SPECTRA of Ni(II) COMPLEX



S7: UV-Vis-NIR SPECTRA of Cu(II) COMPLEX

Based on the above analytical and spectral studies, the following structure has been proposed for the complexes.

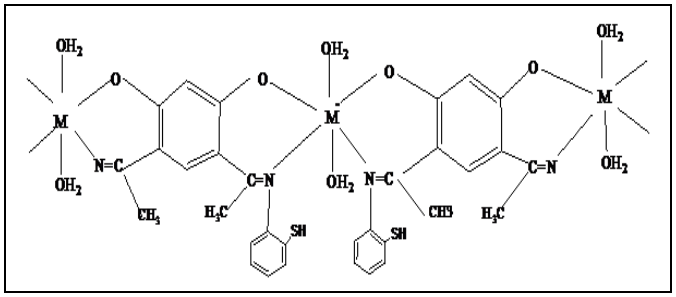


FIG. 2: PROPOSED STRUCTURE OF THE COMPLEXES. M= Co(II), Ni(II), Cu(II)

Molecular Modeling: The possible geometry of the Schiff base and its metal complexes were evaluated using molecular calculation with Argus Lab software [34] **Fig. 1**. The molecule was built, and geometry optimization was done using quantum mechanics, molecular orbital calculations were performed with AM1 (Austin Model 1) approximation for the synthesized ligand. The Self-consistent field (SCF) energy value and heat of formation ΔHf for the optimized geometry of the ligand are reported as -96791.36 kcal/mol and -21.45 kcal/mol respectively. The electron density surfaces of highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO) are also generated for the ligand from the calculations for the ground state of the molecule. The 3-dimensional structures of the metal complexes with possible configuration were evaluated using molecular Mechanics (UFF) calculations. The most stable structure with minimum energy among the possible ones is judged as the possible structure for all the complexes, as shown in **Table 3**.

TABLE 4: SELECTED BOND LENGTHS AND BOND ANGLES OF 1, 2, 3, COMPLEXES FROM MOLECULAR MODELLING STUDIES

Complex	Bond length		Bond angles		Geometry optimization Final geometry energy
Co	Co_1-O_3	1.96	O_2-Co_1-O_3	90.0	
complex	Co_1-O_2	1.93	O_2-Co_1-O_4	90.0	
	Co_1-N_{35}	1.97	O_2-Co_1-N_{13}	90.0	
	Co_1-N_{13}	1.97	O_2-Co_1-O_{35}	90.0	
	Co_1-O_4	1.93	O_2-Co_1-O_{45}	90.0	
	Co_1-O_{45}	1.96	O_3-Co_1-N_{13}	90.0	152.18kcal/mol
			O_3-Co_1-N_{35}	90.0	
			O_3-Co_1-N_{45}	90.0	
			N_{35}-Co_1-N_{45}	90.0	
			O_4-Co_1-N_{13}	90.0	
			O_4-Co_1-N_{35}	90.0	
			O_4-Co_1-O_{45}	90.0	
Ni	Ni_1-O_2	1.84	O_2-Ni_1-O_3	90.0	
complex	Ni_1-O_3	1.87	O_2-Ni_1-O_4	90.0	
	Ni_1-O_4	1.84	O_2-Ni_1-N_{13}	90.0	
	Ni_1-N_{13}	1.88	O_2-Ni_1-N_{35}	90.0	
	Ni_1-N_{35}	1.88	O_2-Ni_1-O_{45}	90.0	
	Ni_1-O_{45}	1.87	O_3-Ni_1-O_4	90.0	167.27kcal/mol
			O_3-Ni_1-N_{13}	90.0	
			O_3-Ni_1-N_{35}	90.0	
			O_3-Ni_1-O_{45}	90.0	
			O_4-Ni_1-N_{13}	90.0	
			O_4-Ni_1-N_{35}	90.0	
			O_4-Ni_1-O_{45}	90.0	
Cu	Cu_1-O_2	1.99	O_2-Cu_1-O_3	109.47	
complex	Cu_1-O_3	2.02	O_2-Cu_1-O_4	109.47	
	Cu_1-O_4	1.99	O_2-Cu_1-N_{13}	109.47	
	Cu_1-N_{13}	2.03	O_2-Cu_1-N_{35}	109.47	
	Cu_1-N_{35}	2.03	O_2-Cu_1-O_{45}	109.47	
	Cu_1-N_{45}	2.02	O_3-Cu_1-O_4	109.47	340.65kcal/mol
			O_3-Cu_1-N_{13}	109.47	
			O_3-Cu_1-N_{35}	109.47	
			O_3-Cu_1-O_{45}	109.47	
			O_4-Cu_1-N_{13}	109.47	
			O_4-Cu_1-O_{35}	109.47	
			O_4-Cu_1-O_{45}	109.47	

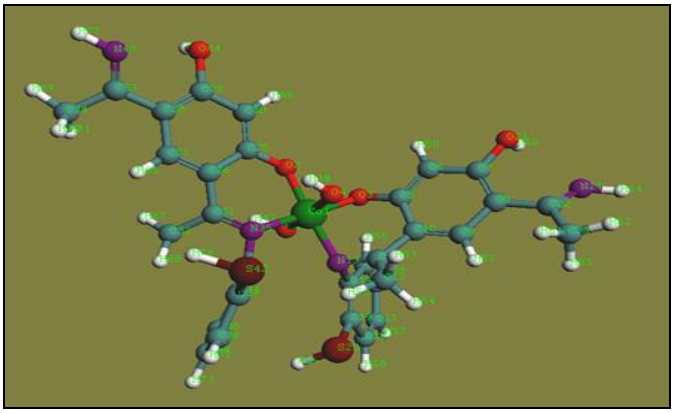


FIG. 3: Co(II) COMPLEX

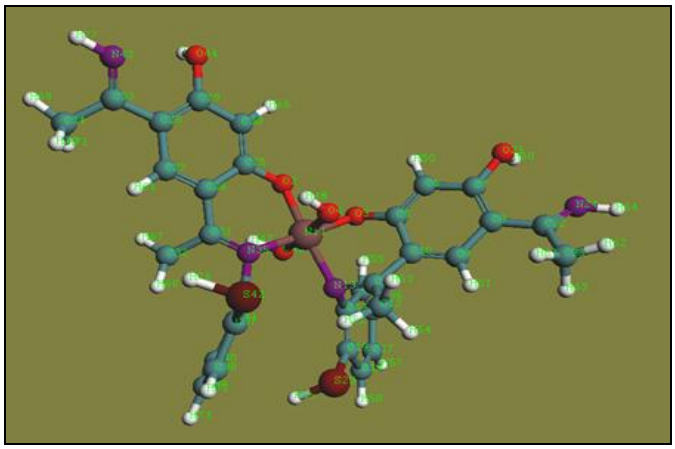


FIG. 4: Ni(II) COMPLEX

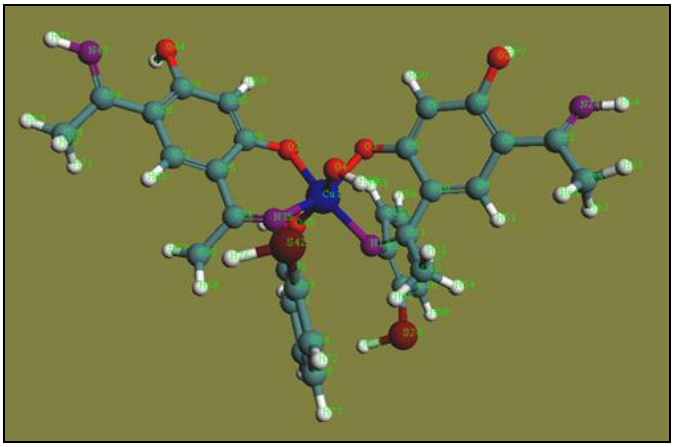


FIG. 5: Cu(II) COMPLEX

Biological Activity: The antibacterial and antifungal activity of these compounds is tested by using more than one test organism to increase the chance of detecting antibiotic principles in tested materials. The sensitivity of a microorganism to antibiotics and other antimicrobial agents was determined by the assay plates which incubated at 28 ºC for two days for yeasts and at 37 ºC for one day for bacteria. All of the tested compounds showed remarkable biological activity against different types of Gram-positive (*Bacillus subtilis* ATTC 6051 and *S. pyogones* ATTC 12600) and Gram-negative bacteria (*Escherichia coli* ATTC 11775 and *Proteus vulgaris* ATTC 13315) and *Fusarium solani* Martius and *Aspergillus niger* Fungus. It has been found that the ligand has no biological activity against all tested bacteria. But metal complexes are found to have sensitivity for inhibition of Gram-positive more than Gram-negative bacteria.

The results obtained also reveal that the ligand shows antifungal activity against *F. solani* and *A. niger* and is found to have high sensitivity against *F. solani* than *A. niger*. All the metal complexes show antifungal activity. It is also clear from **Table 5** that the complexes exhibit more antifungal activity compared to the parent ligand.

TABLE 5: BIOLOGICAL ACTIVITY AND MIC_{50} OF LIGAND AND METAL COMPLEXES

Sample	Fungus		Bacteria			
			G. -ve		G. +ve	
	F. solani	***A. niger***	***E. coli***	***P. vulgaris***	***B. subtilis***	***S. pyogones***
Ligand	1.2	1.0	-ve	-ve	-ve	-ve
MIC_{50}	100µg/mL	>100µg/mL	-ve	-ve	-ve	-ve
Co(II) complex	2.1	2.4	2.7	2.9	3.9	3.8
MIC_{50}	>100µg/mL	>100 µg/mL	50 µg/mL	50 µg/mL	25 µg/mL	25 µg/mL
Ni(II) complex	2.2	2.3	3.0	3.2	3.3	3.4
MIC_{50}	>100µg/mL	>100µg/mL	50 µg/mL	50 µg/mL	50 µg/mL	50 µg/mL
Cu(II) complex	3.1	3.1	-ve	-ve	-ve	-ve
MIC_{50}	50 µg/mL	50 µg/mL	-ve	-ve	-ve	-ve

It can be concluded, from the data obtained that the presence of metal ions as the result of complexation enhance the biological activity of the parent Schiff base. The enhancement of the activity of complexes over the ligand can be explained by Overtone's Concept and Chelation Theory [35]. The mode of action of the complexes may involve the formation of a hydrogen bond through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes.

CONCLUSION: In this paper, the synthesis of a Schiff base ligand DAAAP derived from the condensation of 2,4-Dihydroxy-5-acetylaceto-phenone and Aminothiophenol, and its complexes have been described. The ligand is of significant synthetic interest from chelation point of view. As the chelating functions are present on the opposite sides of the benzene ring, they are likely to produce polynuclear complexes. The molar conductivity data of the complexes in DMSO indicated that they are non-electrolytes. The structural features of the complexes were characterized by analytical and spectral data. Proposed structures of the complexes are given in **Fig. 4**. *In-vitro* antimicrobial results show that the complexes have higher activities compared to the free ligand.

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