



CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF NEW COBALT-NICKEL-COPPER (II) COMPLEXES WITH BENZOIN AND 3-AMINO BENZOIC ACID

M. R. Asabe¹, S. K. Sarsamkar^{2*} and N. S. Gramopadhye²

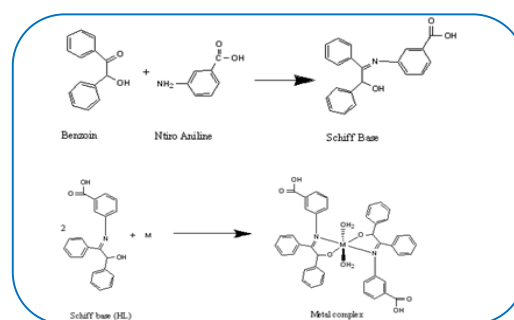
¹ Department of Chemistry, Walchand College of Arts and Science, Solapur, Maharashtra, India.

² Department of Chemistry, Walchand Institute of Technology, Solapur, Maharashtra, India.

*Corresponding author E-mail: sarsamkar30@yahoo.co.in¹

ABSTRACT :

Condensation of Benzoin and 3-Amino Phenol yields a novel Schiff base ligand of type HL. Also, we synthesized the Cobalt (Co⁺²)-Nickel (Ni⁺²)-Copper (Cu⁺²) metal complexes. The characterization study of the ligand and metal complexes were carried out by elemental analysis, UV-visible, IR, ¹H NMR spectroscopy, thermal studies and magnetic susceptibility measurement. On the basis of electronic spectral data and magnetic susceptibility measurement the octahedral geometry has been proposed for Co⁺², Ni⁺², and Cu⁺² complexes. In vitro antibacterial and antifungal activities assay were performed for the ligand and metal (II) complexes. The micro-organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the fungi *Candida albicans* and *Aspergillus Niger* were used. Our findings are very promising in the field of clinical research as the highest antimicrobial and antifungal activity is observed for copper complexes.



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KEYWORDS: 3-Amino Benzoic acid, Schiff base, Antifungal and Antibacterial activity, Transition metal complexes.

1. INTRODUCTION

The Schiff base complexes are important as medicine and show a variety of interesting biological activities such as antibacterial and antifungal activity [1-4]. Benzoin and its derivatives are used as intermediates for the synthesis of organic compounds and as a catalyst in photo polymerization. They are used in anticratering in powder coating. The benzoin can be used in skin diseases as an antibacterial and antifungal agent. Aryl amines are attractive targets for chemical synthesis because of their wide applications in fine chemicals, polymers and dyes. These are important components in many important biologically active natural products as well as medicinally important compounds. [5-6]. Transition metal complexes with various donor groups have been used in Organometallic chemistry [7]. A large number of Schiff base compounds have been synthesized and structurally characterized [6-8].

The various classes of Schiff bases that can be prepared by condensation of different types of amines and carbonyl compounds are very popular due to diverse chelating ability. Several transition metal complexes have been screened for their medicinal properties [8-10]. The first row transition metals have attracted much attention due to their biological importance [11]. These factors prompted us to carry out a study on synthesis of Schiff base and its complexes with Co (II), Ni (II) and Cu (II) metal ions.

Table 1: Analytical and Physical data of the compounds studied

Compound	Mole. Formula(Mol.wt)	Colour	M.P./ Decomposition temp. °C	Elemental analysis				Yield %
				% M Cal. (Found)	% C Cal. (Found)	% H Cal. (Found)	% N Cal. (Found)	
HL	C21H17N03 (331)	Yellow	115-118	-	77.06 (76.12)	4.83 (5.17)	4.73 (4.23)	66
HL-Co	C42H32N2O6Co.2H2O (755)	Yellowish green	>280	7.80 (7.66)	67.38 (66.70)	5.12 (4.80)	4.09 (3.70)	69
HL-Ni	C42H32N2O6Ni.2H2O (755)	Brownish yellow	>280	7.77 (7.33)	67.59 (66.78)	4.88 (4.80)	4.20 (3.71)	64
HL-Cu	C42H32N2O6Cu.2H2O (760)	Faint brown	>280	8.36 (8.56)	66.04 (66.30)	5.39 (4.74)	4.16 (3.68)	64

The ligand and the metal (II) complexes are soluble in common polar solvents like methanol, ethanol and chloroform. They are soluble even in DMF and acetone. The ligand and the metal (II) complexes synthesized were stable at room temperature. The synthesized ligand and the metal complexes were characterized by elemental and spectral analysis. Biological activity of the ligand and the metal complexes were also studied. The geometry of the synthesized compounds has been elucidated on the basis of the elemental and spectral studies.

The stoichiometry of ligand and their metal complexes is confirmed on the basis of their elemental analysis. The elemental analysis of ligand and the metal complexes are found in agreement with the proposed structure of ligand and the metal complexes are listed in **Table 1**.

The electronic spectra and magnetic moment studies helps us in the evaluation of results obtained by other methods of structural investigation. Information regarding the geometry of the complexes around the Co (II), Ni (II) and Cu (II) ions was obtained from electronic spectral studies and magnetic moments. The electronic spectra of ligand and their metal complexes were recorded at room temperature using methanol as a solvent.

The electronic spectra of ligand show bands in the region of 204-212 nm and 247-258 nm but in the complexes, they are slightly shifted to higher frequencies. The band between 325-335 nm can be assigned to $n \rightarrow \pi^*$ of transition of azomethine group. In the spectra of complexes the bands of azomethine chromophore $n \rightarrow \pi^*$ transition are shifted to lower frequencies indicates that imine nitrogen is involved in the co-ordination of metal ion. A very weak low intensity absorption band associated with d-d transition for Cu(II) complexes at 465, 532 nm (typical octahedral transition) Co(II) complexes at 460 nm [4 T_{1g}(F) → 4 T_{1g}(P)], 570 nm [4 T_{1g} → 4 T_{2g}] and 734 nm [4 T_{1g} → 4 T_{2g}] and for Ni(II) complexes at 515 nm (Charge transfer), 695nm[3 A_{2g}(F) → 3 T_{1g}(P)] 996 nm [3 A_{2g} → 3 T_{1g}(F)] respectively supports the octahedral geometry of metal complexes [26].

The magnetic moment obtained at room temperature is as shown in **Table 2**.

Table 2: IR spectral data and Magnetic moment data

Compounds	ν (C=N)	ν (C-O)	ν (M-O)	ν (M-N)	ν (C-O-H)	ν (H ₂ O) (Coordinated)	Magnetic Susceptibility. eff. B.M.
HL	1620	1090	-	-	1390	3378	-
HL-Co	1600	1112	470	588	1283	3385	4.96
HL-Ni	1595	1112	462	542	1287	3383	3.10
HL-Cu	1603	1111	466	560	1292	3385	1.78

The magnetic moment obtained at room temperature indicates paramagnetism. The Co(II) complex shows magnetic moment of 4.96 B.M., the spin free octahedral complex of Co(II) are reported

to exhibit magnetic moment in the range of 4.46 -5.53 B.M. [12-14]. Hence, the observed magnetic moment for the Co (II) complex indicates that it has an octahedral configuration. The Ni (II) complex shows magnetic moment of 3.10 B.M. The magnetic moment of octahedral Ni (II) complexes are reported to exhibit magnetic moment in the range of 2.80 -3.40 B.M. [13-14] indicating spin orbital coupling contribution from $^3A_{2g}$ and higher $^3T_{2g}$ states. Hence, the observed magnetic moment for the Ni (II) complex suggests that it may have octahedral geometry. The observed magnetic moment of Cu (II) complex is 1.78B.M. and it indicates octahedral geometry [8,14-16]. Thus the electronic spectral data and magnetic moment data support the octahedral geometry of the complexes.

The Infra Red (IR) spectral data of Schiff base [HL] and their metal complexes are presented in **Table 2**. In order to determine the coordination sites the, IR spectra of complexes are compared with those of ligand. A strong band observed at 1622 cm^{-1} in a ligand [HL] is a characteristic band of (HC=N) azomethine group. The shifting of this band towards lower frequency region by $15\text{ -}20\text{ cm}^{-1}$ in complexes indicates involvement of azomethine nitrogen in coordination with metal ion [17-19]. The assignment of the proposed coordination sites is further supported by appearance of band at $462\text{ -}470\text{ cm}^{-1}$ suggesting the ν (M-N) bond. The presence of ν (M-O) stretching vibration at $542\text{ -}588\text{ cm}^{-1}$ supports the involvement of oxygen atom in complexation with metal ions. The ligand and metal complexes were characterized mainly using the azomethine and -OH bands. In the complexes, the broad band in the range of $3383\text{ -}3385\text{ cm}^{-1}$ is attributed to the presence of water molecules. Presence of water molecules was also confirmed by thermal analysis. The presence of band in the range of $1314\text{ -}1393\text{ cm}^{-1}$ (OH in-plane bending vibration of carboxylic group) in ligand and metal complexes confirms the carboxylic functional group remains unchanged. Therefore, from the IR spectra, it is concluded, that the ligand coordinated to the metal ions via azomethine nitrogen and deprotonated oxygen atom from benzoin.

The ^1H NMR spectra of Schiff base is recorded in CDCl_3 , using Tetramethylsilane (TMS) as an internal standard at IIT, Mumbai. The ^1H NMR spectra of benzoin show signals at δ 6.03 ppm assignable to -OH group, δ 6.09 ppm assignable to -CH- group and δ 7.06-8.01 ppm assignable to aromatic protons. In the NMR spectra of ligand, the signal due to -OH of benzoin shifts to δ 5.97 ppm [20]. The signal at 11 ppm is assignable to -OH of carboxylic acid. Since, Co^{2+} , Ni^{2+} and Cu^{2+} complexes are paramagnetic; their ^1H NMR spectra could not be obtained [15]. The typical thermogram of nickel complex is shown in Figure 1.

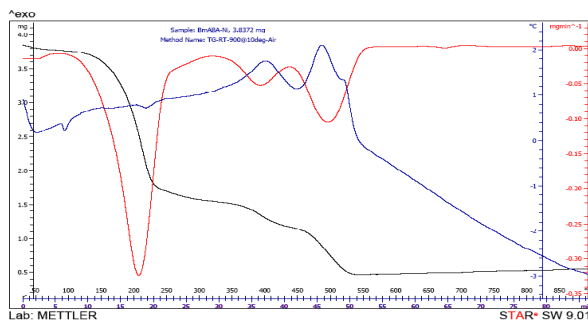


Fig. 1: Thermal analysis of nickel complex

Thermal decomposition of Co (II), Ni (II) and Cu (II) metal complexes has been studied as a function of temperature by TGA and DTG. It exhibited three step decomposition for the Schiff base metal (II) complexes. The first decomposition takes place around $200\text{ }^{\circ}\text{C}$ due to total cleavage of the base metal complex along with hydroxide to oxide transformation followed by concomitant release of water molecules corresponding to loss of coordinated water molecules (calc. 4.76% ;found 5.2%). An endothermic peak of DTA confirmed the loss of water molecules. The second decomposition takes place around 400°C , attributed to the release of intermediate organic moiety. The third slow and broad decomposition around $510\text{ -}530^{\circ}\text{C}$ can be attributed to total decomposition of organic moiety into

carbon dioxide and other gases. The remaining 11 % weight is totally due to presence of inorganic metal oxide [12, 19].

The antibacterial and antifungal activity of the Schiff bases and their metal complexes was tested on Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus Niger and Candida albicans. The method used for antibacterial activity was Agar Well-Diffusion method [8,20] and for antifungal activity was Agar-Ditch method [8-21]. The stock solution 1mg/ml was prepared and was used to prepare concentrations of 0.8, 0.6, 0.4 and 0.2 mg/l. The bacteria and fungi were inoculated on the surface of Nutrient agar and Sabouraud’s agar, respectively. The various concentrations of the compounds were inoculated in the wells prepared on the agar plates. The plates were incubated at room temperature for 24h. In order to clarify the effect of DMF on the biological screening, separate studies were carried out with DMF and showed no activity against any bacteria and fungi. The results are as summarized in the Table 3.

Table 3: Biological activity data of complexes

Name	Conc. µg / mL	Staphylococcus Aureus	Pseudomonas Aeruginosa	Aspergillus Niger	Candida albicans
HL	2	+	+	+	++
	4	+	+	+	+++
	6	+	++	++	+++
	8	++	++	++	+++
	10	++	++	+++	++++
HL-Co	2	++	++	++	++
	4	++	++	++	++
	6	++	++	++	++
	8	++	++	+++	++
	10	+++	++	+++	+++
HL-Ni	2	++	++	++	++
	4	++	++	++	++
	6	++	++	++	++
	8	+++	++	+++	+++
	10	+++	++	+++	+++
HL-Cu	2	++	++	++	++
	4	++	++	++	++
	6	+++	+++	+++	+++
	8	++++	++++	++++	++++
	10	++++	++++	++++	++++

Inhibition:

Less than 5mm = “+” (Inactive)

6-10 mm = “++” (weakly active)

11-14mm = “+++” (moderately active)

More than 15 mm = “++++” (Highly active)

The ligand shows less activity against the microorganisms and Aspergillus Niger fungi and moderate activity against Candida albicans. C₄₂H₃₆N₂O₈Cu.2H₂O complex show maximum activity against bacteria as well as fungi. The cobalt complex (C₄₂H₃₆N₂O₈Co.2H₂O) is weakly activity against the microorganism but is moderately active against fungi. C₄₂H₃₆N₂O₈Ni.2H₂O complex is moderately active against Staphylococcus aureus and fungi and weakly active against Pseudomonas aeruginosa. The results were compared with standard antibiotics like Gentamycin and Streptomycin.

The hemolytic activity of the synthesized ligand and its metal complexes was determined using Wister rat erythrocytes. The RBCs were prepared as follows.2-3 ml of rat blood was drawn into 12 ml of heparinized 5 mM HEPES buffer pH 7.4 containing 150 mM NaCl and centrifuged at 4000 rpm for 5 min.

The cell pellet was washed three times with buffer and the Buffy coat was removed. A working stock of RBCs was made by diluting the RBCs pellet (0.5-0.8 ml) to about 15 ml with buffer. For standardization of the volume for lysis assay, different volumes (5-40 μ l) of diluted blood samples were drawn into 1 ml of water containing 0.1 % of Triton X-100 and buffer with RBCs was used as blank. The solution was centrifuged at 4000 rpm. Hemoglobin released in the supernatant was measured spectrophotometrically at 540 nm. The volume of RBCs was standardized so as the absorbance was about \sim 0.2 OD (about 2×10^6 cells / ml) range. This suitable volume was used for the hemolysis assay with synthesized compounds.

Different volumes of compounds were taken in 1.5ml eppendorf tubes containing 5mM HEPES buffer pH 7.4 containing 150 mM NaCl. To this, diluted blood suspension (volume as determined above) was added and total volume in the reaction tube was made upto 1 ml with buffer. The tubes were incubated at 37°C for 30 min in a gentle shaking water bath. After incubation, the samples were centrifuged in a Kubota centrifuge at 4000 rpm for 5 min. to remove the unhemolysed cells. The absorbance of the supernatant was measured at 540 nm. Buffer containing RBCs suspension was taken as buffer blank (A_0). The lysis obtained with 0.1% of Triton X-100 with RBCs was taken for 100% lysis control (A_{100}). The assay was carried out in duplicates and was repeated thrice. The average values were taken for plotting. The percentage hemolysis was calculated by using following equation

$$H\% = (A_{\text{sample}} - A_0) \times 100 / (A_{100} - A_0)$$

Where A_{100} and A_0 absorbance of 100 % and 0% hemolysed cells respectively

The same procedure was repeated for human erythrocytes also and the results are recorded in **Table 4**. The cobalt complex shows less hemolytic activity. The results are very interesting and much helpful for drug designing.

Table 4: Hemolytic activity data

S. No	Compounds	% RBC Lysis	
		Rat	Human
1	HL	18	19
2	HL-Co	17	17
3	HL-Ni	22	20
4	HL-Cu	33	23

4. CONCLUSION

All the complexes have octahedral geometry and paramagnetic nature. The synthesized ligand shows moderate activity against *Candida albicans*. The cobalt ($C_{42}H_{36}N_2O_8Co.2H_2O$) and the nickel complexes ($C_{42}H_{36}N_2O_8Ni.2H_2O$) are moderately active against the *Staphylococcus aureus* fungi *Candida albicans* and *Aspergillus Niger*. The highest activity is observed for copper complex.

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