

Synthesis, Characterisation and Biological Activity of Schiff Base Metal Complexes Derived from N'-(3,5-Dichlorohydroxybenzylidene) Benzohydrazide

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Abstract

A new series of transition metal complexes of Co(II), Ni(II), and Cu(II) Chlorides have been synthesized from Schiff base ligand derived from 3,5-dichlorosalicylaldehyde and benzohydrazide. The structural features of Schiff base and its metal complexes were determined from their elemental analysis, magnetic susceptibility, molar conductance, mass spectrometry, IR, UV-Vis, ¹H-NMR and ESR spectral studies. This data analysis predicts the complexes are in 1:2 ratio of ML₂ type. The UV-Vis, magnetic susceptibility and ESR spectral data values suggest an octahedral geometry around the metal ion. Biological screening of the metal complexes with Schiff base ligand reveals that the Schiff base transition metal complexes show more antibacterial and antifungal activity.

Key words: Schiff base ligand, Metal complexes, Spectral studies, Antifungal activity, Antibacterial activity

Schiff bases were important class of ligands and played an important role in the development of coordination chemistry as they readily form stable complexes with most transition metals [1–5]. There have been several reports on metal complexes of the Schiff base ligands having a variety of applications including biological, clinical, analytical and industrial in addition to their important roles in catalysis and organic synthesis [6-10]. It is well known that some organic ligands exhibit increased activity when administered as metal complexes [11–13] and several metal chelates have been shown to inhibit tumor growth [14]. The biomedical properties of free organic molecules depend upon chelation with suitable metal ions leading to the implementation of metal complexes for several biomedical applications. The direct use of transition metal salts, as antimicrobial agents cannot be recommended as they are toxic to both the microbes and the host animals. Metal ions on complexed with Schiff base ligand exhibit better antimicrobial, anti-cancer and anti-tumor activities. Schiff bases derived from salicylaldehyde represent an important class of ligands due to their ability to be used in various fields [15–19].

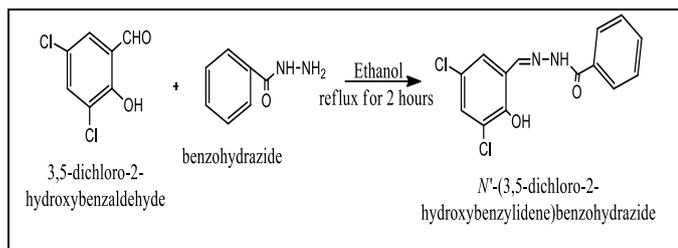
The coordinating property of benzohydrazide ligand has been modified to give a flexible ligand system, formed by condensation with a variety of reagents like aldehydes and ketones [20-22]. Several model systems, including those with bidentate, tridentate, tetradentate, multidentate Schiff base ligands, and their coordination chemistry of cobalt (II), Nickel (II) and copper (II) attracts much attention because of its biological relevance and its own interesting coordination chemistry such as geometry, flexible redox property. In this paper to discuss the synthesis, spectroscopic and antimicrobial

studies of Schiff's base and its cobalt (II), nickel (II) and copper (II) complexes. The structures of the compounds are characterized by using IR, UV, NMR, mass and EPR spectroscopic techniques.

MATERIALS AND METHODS

All the chemicals used were of analytic grade, and were purchased from Sigma-Aldrich. Metal salt were purchased from E. Merck and were used as received. All solvents used were of standard/spectroscopic grade. The elemental analyses (Carbon, Hydrogen and Nitrogen) were performed using a FLASH EA 1112. The infrared spectra were recorded on the Perkin Elmer RX1 FTIR spectrometer using KBr disc in the region 4000–400cm⁻¹. The conductance measurements were carried out on Direct Reading Conductivity meter 304 with dip type conductivity cell. The cell constant K was determined by measuring the conductance of the aqueous KCl solution of known specific conductance. The value of cell constant was found to be 1.06 cm⁻¹. The conductance C values of the complexes were determined by using 10-3 (DMF) solution. Electronic absorption spectra in the ultraviolet (UV) – Visible range were recorded on Perkin Elmer Lambda - 35 spectrophotometer between 300 and 800nm by using DMSO as the solvent. The mass spectral study of the ligands carried out using JEOL GCMS GC-Mate II GC-Mass Spectrometer. ESR spectra were recorded on a Varian JEOL-JES-TE100 ESR spectrophotometer at X-band microwave frequencies for powdered samples at room temperature and liquid nitrogen

temperature. The antibacterial and antifungal studies were conducted by Disc-diffusion method.



Synthesis of the Schiff base ligand $C_{14}H_{10}Cl_2N_2O_2$ and metal complexes

Benzohydrazide (0.01mmol, 1.36g) dissolved in about 20 mL absolute ethanol was added slowly to a magnetically stirred solution of 3,5-dichloro-2-hydroxybenzaldehyde (0.01mmol, 1.91g). The mixture was refluxed for two hours. White crystals were obtained on cooling at room temperature. The product was filtered off and recrystallized from ethanol and

dried in anhydrous calcium chloride. The different complexes were prepared by addition of 0.01mmol of $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ and $CuCl_2 \cdot 2H_2O$ dissolved in about 20 mL ethanol, into a solution of the ligand (0.02mmol in 20mL ethanol). The mixture was refluxed for three hours. The obtained solid products were filtered, washed twice with diethylether and dried. The synthesis of ligand shown in (Fig 1).

RESULTS AND DISCUSSION

Elemental analysis

All the metal complexes were colored, non-hygroscopic in nature, and stable at room temp. They were insoluble in common organic solvents but soluble in DMF and DMSO. The results of the elemental analysis are in good agreement with the calculated values. The molar conductance value indicates their non-electrolytic nature. Physical and analytical data of complexes are summarized in (Table 1). On the basis of analytical and spectral data, octahedral geometry has been assigned to the complexes.

Table 1 Analytical data and physical data of ligand and its metal complexes

Empirical formula	Molecular weight	Melting point ($^{\circ}C$)	Yield (%)	Elemental analysis found/(calculated)				Λ_M
				C	H	N	M	
$C_{14}H_{10}Cl_2N_2O_2$	309	190	80	54.45 (54.39)	3.34 (3.26)	8.99 (9.06)	-	-
$C_{28}H_{18}CoN_2O_4$	675	>300	72	49.99 (49.81)	2.75 (2.69)	8.47 (8.30)	8.80 (8.73)	42.86
$C_{28}H_{18}NiN_2O_4$	674	>300	68	50.08 (49.82)	2.76 (2.69)	8.48 (8.30)	8.81 (8.70)	39.04
$C_{28}H_{18}CuN_2O_4$	679	>300	75	49.54 (49.47)	2.72 (2.67)	8.36 (8.24)	9.39 (9.35)	44.54

Table 2 IR spectral data of ligand (DAB) and its metal complexes

Compound	γ_{OH}	$\gamma_{C=N}$	$\gamma_{C=O}$	M-O	M-N
Ligand (1)	3231	1573	1612	-	-
Co(II) complex	-	1568	1605	537	417
Ni(II) complex	-	1547	1602	517	451
Cu(II) complex	-	1564	1603	538	489

IR spectral study

The IR data of the spectra of Schiff base ligand and its complexes are listed in (Table 2). On comparing the IR values of Schiff base ligand and metal complexes, the coordinating sites were azomethine, phenolic oxygen and carbonyl oxygen groups respectively. The $C=N$ stretching frequency is found in the free Schiff base ligand at $1573cm^{-1}$, the band is shift to lower number in the complexes indicating the participation of the azomethine nitrogen atom in coordination. The broad band at $3231cm^{-1}$ attributed to phenolic $-OH$ group, this band is disappeared in all complexes, which can be attributed to the involvement of phenolic ion in coordination. The peak at $\gamma_{C=O}$ has frequency at $1397cm^{-1}$, shift to the lower numbers in all the metal complexes. The band at $1612cm^{-1}$ attributed to $\gamma_{C=O}$ group and shift to the lower frequency numbers in metal complexes indicates that the $\gamma_{C=O}$ is involved in coordination. The appearance of two new band observed in all the spectra of the metal complexes were assigned to γ_{M-O} , γ_{M-N} respectively.

^1H-NMR spectral study

The ^1H-NMR spectra of the Schiff base ligand is recorded in d_6 -DMSO using TMS as internal standard. The peak at $7.96\delta ppm$ is due to the presence of azomethine proton. The phenyl proton appeared as multiplet in the range $7.2-7.9\delta ppm$. While the signal due to $-OH$ and $-NH$ was observed at 12.5 and $8.5\delta ppm$.

Molar conductivity measurements

The molar conductivities of 10^{-3} M of the complexes (dissolved in DMF) at room temperature were measured (Table 1). The results were in the range $40-50 \Omega^{-1} mol^{-1} cm^2$ for Co(II), Ni(II) and Cu(II) complexes. Conductivity measurements reveal that all the metal complexes have conductivity values in the range characteristic for non-electrolytic nature.

Electronic Spectral Analysis and Magnetic moment

The electronic spectral data of the Schiff base ligand and its metal (II) complexes are given in the experimental section. The Schiff base ligand showed three bands at 226 nm ($44247cm^{-1}$), 267 nm ($37453 cm^{-1}$), and $345nm$ ($28977cm^{-1}$). The band at 226 nm is due to the $\pi-\pi^*$ transition in benzene. The band appearing at 267 nm is assignable to $n-\pi^*$ transition of nonbonding electrons present on the nitrogen of the azomethine group ($-HC=N$). The band at 345 nm is due to $n-\pi^*$ transition of the phenolic group [23-25].

In the Co (II) complex, two bands were observed at 249 nm ($40144 cm^{-1}$) and 401 nm ($24881 cm^{-1}$). This was a shift towards longer wavelength with respect to the spectrum of the Schiff base ligand. The band at 249 nm was due to intraligand transition, and the band at 401 nm was as a result of d-d transition for $3T_{1g}(F) \rightarrow 3T_{2g}$ which is within the range for octahedral configuration as reported in many octahedral cobalt (II) complexes [26-28].

The electronic spectrum of Ni (II) complex showed three bands at 245 nm ($40783 cm^{-1}$), 325 nm ($30759 cm^{-1}$) and 421 nm ($23702 cm^{-1}$). The band at 245 nm was probably due to

intraligand transition, while the band at 325 nm was due to charge transfer. The observed band at 421 nm was as a result of

d-d transition for $3A_{2g} \rightarrow 3T_{2g}$ which favours an octahedral geometry for the Ni (II) complex [29].

Table 3 Electronic spectra of ligand and metal complexes

Compound	UV-Visible (nm)	UV-Visible (cm^{-1})	Transitions	μ_{eff} (BM)
Schiff base	226;267;345	44247;37453; 28977	$\pi-\pi^*$; $n-\pi^*$	-
Co(II) complex	249;401	40144; 24881	$^3T_{1g}(F) \rightarrow ^3T_{2g}$	4.85
Ni(II) complex	245;325;421	40783; 30759;23702	$^3A_{2g} \rightarrow ^3T_{1g}(P)$	3.25
Cu(II) complex	229;266;451	43649; 37579;22,172	$^2E_g, ^2T_{2g} \rightarrow$	1.75

The copper (II) complex spectrum showed three bands at 229 nm (43649 cm^{-1}), 266 nm (37579 cm^{-1}) and 451 nm ($22,172\text{ cm}^{-1}$), which were all shifted to longer wavelength. The band at 229 nm and 266 nm was due to intraligand transition, and the one at 451nm was as a result of d-d spin allowed transition for. This transition is suggestive of octahedral geometry around Cu (II) ion [30].

Magnetic moment susceptibility of the metal complexes was recorded at room temperature for Cu (II), Ni (II) and Co (II) complexes and they were found to be paramagnetic. The

observed magnetic moment for Cu (II) complex was 1.75 B. M. which was approximately equal to spin only value of one unpaired electron 1.73B.M. for octahedral geometry [31]. The magnetic moment value of Ni (II) complex was observed as 3.25 B.M., which was in the range of expected value for octahedral geometry of metal complex 2.83–3.50 B. M [32]. For Co (II) complex, the observed magnetic moment was 4.85 B.M. which well agreed with the expected value for octahedral complexes of Cobalt [33]. The magnetic moment values are given in (Table 3).

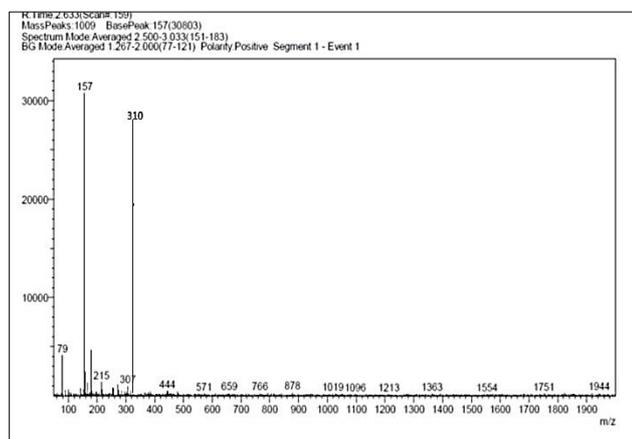


Fig 3 Mass spectrum of the ligand

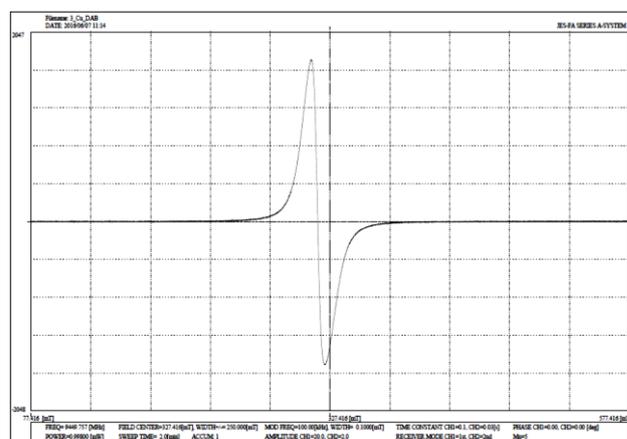


Fig 4 ESR Spectrum of Copper complex

Mass spectral study

The mass spectrum gives information about the structure of the ligand by using its molecular formula. The mass spectrum of the ligand gives the peak at $m/z = 310$ due to the molecular ion peak (m^{-1}). The value is in good agreement with the molecular formula of the ligand.

ESR study

ESR is an important tool in coordination chemistry since it gives valuable information about the distribution of unpaired electron in the metal complexes explaining the nature of metal-ligand bond. Among the complexes of transition metals, Cu(II) complexes are of more important, because it shows wide range geometries like octahedral, tetrahedral, square planar, and square pyramidal. Generally, the ground state of the copper is dx_2-y_2 based on spectral analysis as $g_{II} > g^{\perp}$ by the studies of paramagnetic transition metal ions yield a great deal of information about the symmetry and bonding of metal ion to ligand. The solid state of ESR spectra of the Cu(II) complex is recorded at liquid nitrogen temperature and is shown in figure. There are four resolved peaks corresponding to g_{II} (2.2752) and g^{\perp} (2.052). Kivelson and Neiman [35] showed that for an ionic environment g_{II} is normally 2.3 or larger, but for co-valent environment g_{II} are less than 2.3. The g_{II} value for Cu(II) complex is 2.2752 consequently the environment is covalent. The factor that the unpaired electron lies predominately in the

dx_2-y_2 orbital is also supported by the value of exchange interaction term G. According to Hathway [36].

$$G = g_{II} - 2.0023 / g^{\perp} - 2.0023.$$

If the value of G is greater than four, the exchange interaction between copper centers in the solid state is negligible, when it is less than four a considerable interaction is shown in solid complex. The G - value is found to be 5.49 which shows the negligible interaction in the Cu(II) complex. The tendency of AII to decrease with an increase of g_{II} is an index of an increase of the tetrahedral distortion the coordination sphere of Cu(II). The f - factor g_{II}/A_{II} decide the quantity of degree of distortion of the Cu(II) complexes. Its value ranges between 105 - 135 cm^{-1} for square planar complexes whereas value ranges from 135 - 200 cm^{-1} has distorted tetrahedron complexes. The predicted value for Cu(II) Schiff base ligand are greater than 139 suggesting the distorted octahedral geometry. The ESR parameters presented in (Table 4).

Table 4 ESR spectral data of Cu(II) complex (EAP)

Complex	g_{II}	g^{\perp}	g_{avg}	g_{iso}	A_{II}	G
Cu(II) complex	2.2752	2.052	2.1264	2.2008	156	5.49

On the basis of the analytical and characterization studies, the structure of metal complexes is predicted.

The structure of metal complexes shown in (Fig 5).

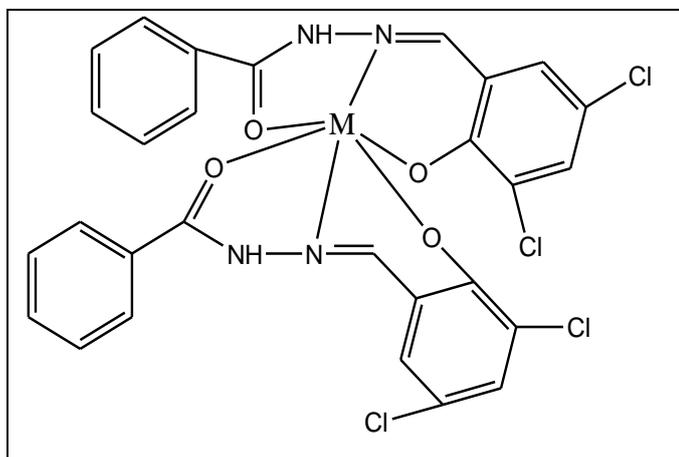


Fig 5 Structure of the metal complexes M = Co(II),Ni(II),Cu(II)

Biological activity

The Schiff base ligand and metal complexes were evaluated against two bacterial species *E. coli* and *S. aureus* and two fungal species *A. niger* and *C. albicans* using a disc diffusion method. The results show that all the metal complexes exhibit better activity than the ligand. The results of the antibacterial activity and antifungal activity towards the metal complexes and Schiff base ligand in (Table 5-6) and shown in figure 6-7. The metal complexes show greater activity than those of the free ligand, this indicates that the complexation

with metal enhances the activity of the ligand it was explained by Tweedy's Chelation theory [37-38].

According to the concept of cell permeability, the lipid membrane surrounds the cell favors the passage of only lipid-soluble materials due to the fact that liposolubility is considered to be an important factor that controls anti-microbial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the partial sharing of positive charge of metal ions with donor groups and the overlap of the ligand orbital. Furthermore, it increases the delocalization of the electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes into the lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms. These metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The variation in the activity of different complexes against different organisms depends either on the impermeability of the microbe's cells or difference in the microbe's ribosomes. In this study, it can be observed that all complexes have higher biological activities against most of the tested bacteria and fungi than the free ligand confirming that the complexation increases the activity of the compounds against microorganisms. The Cu(II) shows more activity than other metal complexes, therefore the order of the reactivity toward against microbial species is Cu(II)>Co(II)>Ni(II)>ligand (DAB).

Table 5 Antibacterial activity of ligand and its metal complexes

Ligand /complexes	Zone of inhibition(mm)			
	Antibacterial			
	<i>S. aureus</i>		<i>E. coli</i>	
Conc ($\mu\text{g/ml}$)	50	100	50	100
Ligand (DAB)	7	9	8	10
Co(II) complex	9.5	11.5	10.5	12
Ni(II) complex	8	10	9	11
Cu(II) complex	11	13.5	13	14

Table 6 Antifungal activity of ligand and its metal complexes

Ligand /complexes	Zone of inhibition(mm)			
	Antifungal			
	<i>A. niger</i>		<i>C. albicans</i>	
Conc ($\mu\text{g/ml}$)	50	100	50	100
Ligand (DAB)	8	10	8	9
Co(II) complex	11	12	11	12
Ni(II) complex	9	10.5	10	10.5
Cu(II) complex	14	15	12	14

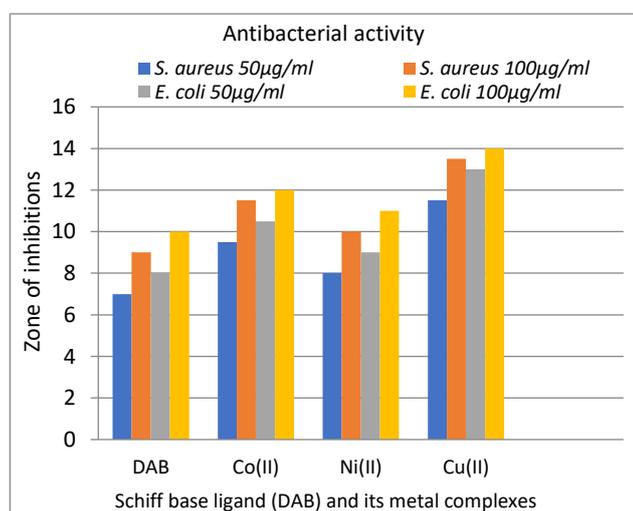


Fig 5 Correlation diagram of antibacterial activity

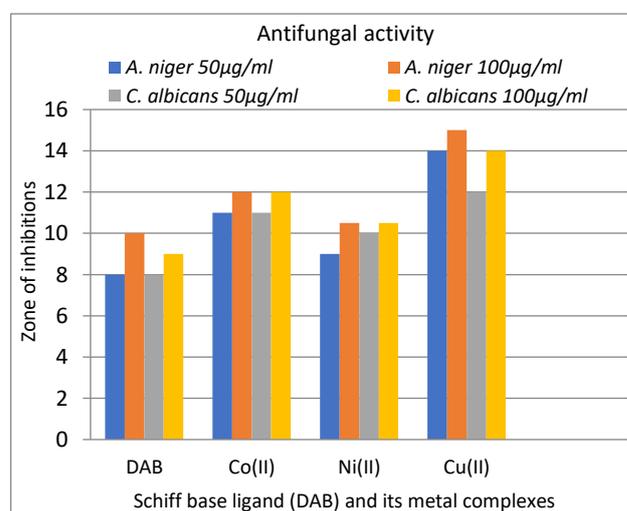


Fig 6 Correlation diagram of antifungal activity

CONCLUSION

Schiff based metal complexes have been synthesized from the Schiff base ligand which was synthesized by the condensation of 3,5-dichlorosalicylaldehyde and benzoylhydrazide. The bonding of ligand to the metal ions has been confirmed by elemental analysis, IR, electronic spectra 1H-NMR, mass, ESR, molar conductance measurements and magnetic study. The IR values of ligand and its metal complexes predicts the coordinating atom of the ligand. The electronic and magnetic study results the complexes are octahedral. The synthesized complexes exhibited enhanced antimicrobial activity than the corresponding free Schiff base ligand. All the synthesized compounds possess higher antibacterial and antifungal properties than the free ligand.

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Declarations

I, the undersigned Dr. K. Jansi Rani declare that the work embodied in this research entitled "Synthesis, Characterisation and Biological Activity of Schiff Base Metal Complexes Derived from N'-(3,5-Dichlorohydroxybenzylidene) Benzohydrazide" on my own contribution to the research work under the Guidance and contributions of Dr. S. R. Bheeter. This work has not been published and submitted in any other journals.

Ethical Approval

Human and animal studies are not done.

No Competing interests as defined by Springer

Authors' contributions

One of the author Dr. S. R. Bheeter has been contributed in the area of ESR study.

No Funding has been received

Availability of data and materials

ESR studies were conducted in SAIF IIT Mumbai.

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