

## SYNTHESIZED AND HYPOTHESIZED SCHIFF BASE LIGAND AND ITS METAL(II) COMPLEXES DNA BINDING MODE

G. Puthilibai<sup>1</sup>, V. Devatarika<sup>2</sup>, Byeon Haewon<sup>3</sup>, Shyam Sundar Behura<sup>4</sup>, Hitendra Kumar  
Lautre<sup>5</sup>, V. Subha<sup>6</sup>, Pardeep Sangwan<sup>7</sup> and J. Sunil<sup>8\*</sup>

<sup>1</sup>Department of Chemistry, Sri Sairam Engineering College, Chennai, Tamil Nadu, India

<sup>2</sup>Sri Muthukumaran Medical College Hospital & Research Institute, Chennai, Tamil Nadu, India

<sup>3</sup>Department of Digital Anti-aging Healthcare (Bk21), Inje University, Republic of Korea

<sup>4</sup>Department of Oral Pathology & Microbiology, Kalinga Institute of Dental Sciences, Kalinga  
Institute of Industrial Technology (KIIT), Deemed to be University, Bhubaneswar - 751024,  
Odisha, India

<sup>5</sup>BYOS Scientific Lab, Mowa, Raipur, Chhattisgarh - 492007, India

<sup>6</sup>Department of Chemistry, R M D Engineering College, Chennai, Tamil Nadu, India

<sup>7</sup>Maharaja Surajmal Institute of Technology, New Delhi, India

<sup>8</sup>Department of Mechanical Engineering, Annai Vailankanni College of Engineering,  
Kanyakumari, Tamil Nadu, India

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**ABSTRACT.** Metal complexes play an important role in agriculture, pharmacology, medicine, and industrial chemistry. Schiff base metal compounds are the fusion products of propene and aldehydes, require a metal surrounded by an ion or cluster of molecules to form. Metal complexes of Co(II), Ni(II), Cu(II), and Zn(II) have also been created. Elemental analysis is used to analyses the structure and related compounds of the produced Schiff base ligand. According to deoxyribose nucleic acid (DNA) binding tests, Schiff base metal(II) complexes attach to DNA in an intercalative form with weak covalent connections. The predicted DNA binding mode supports an increase in the complexes' binding activity in the presence of newly generated ligand. The cleavage activities of the DNA were recorded using gel-electrophoresis in the presence and absence of the complexes. The findings of the cleavage experiment show that all of the produced chelates can successfully cut DNA.

**KEY WORDS:** Schiff base ligand, DNA, Metal(II) complex, Hetero-ligand cobalt(II), Aldehydes

## INTRODUCTION

The interaction of transition metal(II) ions with bioactive ligands important in many physiological processes has been studied in bioinorganic chemistry. Anticancer medicines such as cis-platin and trans-platin are now widely used. Cis-platin has been demonstrated to be a successful chemotherapeutic drug for a range of cancers. Diffusion and active transport allow the medication to pass past the cell membrane [1, 2]. This substance platinates the DNA inside the cell membrane by forming adducts and crosslinking, generally through the amino acid guanine, which is the most electron rich location in the cell and hence easily oxidized. The distortion caused by the adduct formation causes DNA replication to be inhibited. Protein binding to the platinized DNA adduct has been suggested as a way to boost the drug's anticancer effects. Apart from its capacity to kill cancer cells, cis-platin has a number of unpleasant side effects.

The vast range of uses for Schiff base compounds, such as chemo sensors, light emitting diodes (LEDs), photochromic materials, catalysts, and anticorrosion agents, has piqued attention. Antimicrobial, anticancer, antibacterial, antifungal, and antioxidative actions are all demonstrated by base ligand of Schiff and their complexes [3-5]. Schiff base complexes have previously been

\*Corresponding author. E-mail: sunil0520@gmail.com

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shown to cleave plasmid DNA. Docking experiments have also been utilized to explore these chemicals' interactions with biomacromolecules. Schiff bases are imine or azomethine-containing compounds ( $-RC=N-$ ) produced by carbonyl compound fusion with propene bioactive carbonyl:



The reaction to prepare Schiff bases is reversible in order to achieve high yields, passing via a group of specialized intermediate and necessitating the removal of water, which is generally accomplished by azeotropic distillation with benzene. Although the reaction is acid-catalyzed, when aliphatic amines are involved, catalysts are usually not required. Schiff developed an experimental procedure for producing metal-imine complexes by reacting a metal salicylaldehyde molecule with a primary amine. This method is a forerunner of the "metal-template" approach, which has emerged as a fast way to produce macrocyclic ligands and complexes. Schiff developed a novel approach for producing transition metals by combining a completed metal salicylaldehyde molecule with a primary amine [5-7].

In this study, metal complexes of Co(II), Ni(II), Cu(II), and Zn(II) were synthesized and their related compounds of the produced Schiff base ligand which has intercalative form with weak covalent connections. The experiments involving the interaction of the complexes with DNA were carried out in Tris buffer and the absorption titration experiment was performed by maintaining the metal complex concentration constant (2  $\mu$ M) and varying the concentration of nucleic acid from (0 to 20  $\mu$ M). The cleavage activities of the DNA were recorded using gel-electrophoresis in the presence and absence of the complexes to demonstrate that all synthesised chelates through cleavage experiment results.

## EXPERIMENTAL

### *Ligand synthesis*

Combined 0.1 mol 4-nitro-*o*-phenylenediamine and 0.2 mol alkyl in 50 mL of concentrated sodium borohydride and mix gently with reflux for 2 hours, until the liquid turns yellow. The precipitate was removed by washing several times and rinsed with deionized water and it was ready for use. Methanol was used to re-crystallize the precipitate, which was then dried overnight at 50 °C.

### *Metal complex synthesis*

Schiff base metal(II) complexes were constructed utilizing three saturated metal salts (CoCl<sub>2</sub>·6H<sub>2</sub>O, NiCl<sub>2</sub>·2H<sub>2</sub>O and CuCl<sub>2</sub>·2H<sub>2</sub>O). 0.18 g CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.10 g NiCl<sub>2</sub>·2H<sub>2</sub>O, and 0.26 g CuCl<sub>2</sub>·2H<sub>2</sub>O were combined with Schiff base ligand in a 20 mL ethanol solution. When the combination is suitable at 60 °C, the colored precipitate was filtered off, washed with ethanol, and dried, colored complexes were created [5-7].

### *Elemental analysis*

The sophisticated analytical instrument facility obtained the materials' fast atomic bombardment mass spectra (FAB–Mass). The complex's FAB-mass signal is acquired on a JEOL SX 102/DA-6000 mass spectrometer/communication utilizing Argon/Xenon (6 kV, 10 mA) as the FAB-gas. The wavelengths are measured with a 10 kV accelerating voltage and m-nitro benzyl alcohol (NBA) as a lattice.

At room temperature, magnetic susceptibility measurements for solid metal(II) complexes were performed using a Gouy balance. The electromagnet had a field strength of 8000 G and a pole gap of 2 cm. The Gouy tube was suspended with a torsion-free string using a semi-micro

analytical balance. A pair of magnet poles ringed an empty tube that was half-way between the poles and parallel to the interpoles axis. An empty tube's weight was determined in the existence and lack of a magnetic field. To reduce the possibility of error, the tube was next filled with finely ground complex and the procedure was repeated.

The Gouy tube was standardized with mercuric tetra cobaltate(II),  $\text{Hg}[\text{Co}(\text{NCS})_4]$ , and the diamagnetic correction from Pascal's constant was made by summing the diamagnetic contributions of various atoms and structural units. From the adjusted molar magnetic susceptibilities, the moments of effective magnetic were determined. ( $x_m$ ), using the relation,  $\mu_{eff} = 2.83 (x_m T)^{1/2}$ , where,  $x_m$  = molar of magnetic susceptibilities and T = Kelvin temperature. The potential state of a metal(II) ion can be determined by counting the number of unpaired electrons in a complex. These numbers can be used to determine whether a complex has a high or low spin.

The electron absorption method was used to determine the ability to attach hetero-ligand cobalt(II) complexes to DNA. The spectra showed that the hetero-ligand system remained stable during the course of the tests, which lasted 24 hours. The absorbance of subsequent Co(II)-PicHA-AlaSal (PicHA-AlaSal is (hydroxybenzyl)alanine-Salicylaldehyde) samples containing especially associated of DNA hydrochloride from calf thymus (CT-DNA) declined between 304 and 440 nm. Because of the high stacking contact between the ligands' planar aliphatic fluorescent dyes and the DNA pairs. PicHA binary complexes, as well as other Co(II) compounds with pyridyl groups and reduced Schiff base ligands, have shown this type of interaction. The stacking contact between the intercalated ligand's\*-orbital DNA of lowers the energy levels of the complex, causing the -\* transitions to diminish, resulting in hypochromic absorption spectra. The ligand rings' electron-withdrawing function can boost the affinity of complexes for DNA. In addition, the development of the intercalative binding mode is favored by a substantially flattened PicHA conformation.

The time dependent DNA cleavage reaction in the presence and absence of the complexes was also studied to calculate rate of hydrolysis. The extent of increase of conversion of SC to NC forms are observed. Upon the addition of increasing amounts of the complexes, we observed the conversion of SC form to NC form with continuous increase with respective concentration. A complete conversion is observed at a concentration of 500  $\mu\text{M}$  for all the complexes [8].

#### *DNA binding experiment*

The experiments involving the interaction of the complexes with DNA were carried out in Tris buffer (5 mM Tris and 50 mM NaCl, pH 7.2). Absorption titration experiment was performed by maintaining the metal complex concentration constant (2  $\mu\text{M}$ ) and varying the concentration of nucleic acid from (0 to 20  $\mu\text{M}$ ). The intrinsic DNA binding constant  $K_b$  was determined using the following equation through a plot of  $[\text{DNA}]/(\epsilon_A - \epsilon_f)$  vs  $[\text{DNA}]$ , of the straight line.

$$[\text{DNA}] / (\epsilon_A - \epsilon_f) = [\text{DNA}] / (\epsilon_b - \epsilon_f) + 1 / K_b (\epsilon_b - \epsilon_f) \quad (2)$$

where  $\epsilon_A$ ,  $\epsilon_f$  and  $\epsilon_b$  correspond to  $A_{\text{obsd}} / [\text{Ru}]$ , the extinction coefficient for the free ruthenium complexes and the extinction co-efficient for the complex in the fully bound form respectively.

The DNA binding constant of compounds have been estimated to be  $5.2 \times 10^4 \text{ M}^{-1}$  to  $6.4 \times 10^4 \text{ M}^{-1}$  through absorption spectral titration. The experiments involving the interaction of the complexes with DNA were carried out in Tris buffer (5 mM Tris and 50 mM NaCl, pH 7.2). Absorption titration experiment was performed by maintaining the metal complex concentration constant (2  $\mu\text{M}$ ) and varying the concentration of nucleic acid from (0 to 20  $\mu\text{M}$ ) [9-18].

*Antibacterial investigation*

The ligands and their complexes were screened *in vitro* for their microbial activity against two pathogenic bacterial species *S. aureus* and *P. mirabilis* using the well diffusion method. The test solutions were prepared in dimethyl formamide (DMF). The effectiveness of an antimicrobial agent in sensitivity is based on the zones of inhibition. The diameter of the zone is measured to the nearest millimeter (mm). These compounds were found to exhibit moderate activity against both the organisms. The complexes are more active than their parent ligands, which is consistent with earlier reports [15]. The increased activity for the metal chelates as compared to the free ligands can be explained on the basis of Tweedy's chelation theory. The activity increases with increase in concentration of test solution containing the new complexes. The different compounds exhibit microbial activity with small variations against the bacterial species and this difference in activity could be attributed to the impermeability of the cell of the microbes or differences in the ribosomes of the microbial cells [16]. Although the complexes are active, they did not reach the effectiveness of the conventional bacteriocide ampicillin.

**RESULTS AND DISCUSSION***Biochemistry*

Ligands and associated compounds are put through a battery of biological assays, including DNA binding, cleavage, and antimicrobial activity investigations, to see how they interact with DNA and bacteria.

*Schiff base ligand*

The chemical structure of the base ligand of Schiff was identified using the programmed SHELEXS97. The molecular compositions of a Schiff base ligand is calculated using the atomic numbering scheme for ligand unit cell packing [6-8]. The ligand's geometric properties are compared to previously known ligand structures. An angle of 8.1 (2) degrees was produced by the heterocyclic planes (C5–C10) and (C12–C17). The ethanol group in the compound's terminal region (N1) are disordered at two places with field occupancy values of 0.775 (9) and 0.225 (9). The ligand's crystal structure is sustained by strong intra - molecular interactions [17].

Table 1. Electronic spectrum and magnetic moment values of Schiff base ligand and complexes.

Compound	$\pi - \mu^*(nm)$	$n - \pi^*(nm)$	LMCT (nm)	d-d (nm)	$\mu_{ef}(BM)$
[Co(L)]	275	335.426	468	593	2.15
[Ni(L)]	273	338.377	478	648	-
[Cu(L)]	273	327.387	479	661	1.79
[Zn(L)]	271	333.417	471	-	-

\*LMCT – Ligand to metal charge transfer.

*Electronic absorption spectra*

The structure of metal complexes can be determined using electronic spectrum transitions and flux density values. The electronic spectra and magnetic moments of base ligand of Schiff and metal complexes are shown in Table 1. The electronic absorption spectral bands of the complexes were recorded over the range of 200–1100 nm in MeCN together with tentative assignments (Table 1) are discussed in detail. All complexes exhibit three bands in the range of 203–630 nm. The spectral profiles below 400 nm are very similar and are ligand centered transitions. The

intense bands appeared below 450 nm which can be assigned to  $\pi\text{-}\pi^*$  and  $\pi\text{-}\mu^*$  transition was due to transitions involving molecular orbitals located on the phenolic chromophore. Another band appeared in the region 468–479 nm can be assigned to LMCT transition was due to transitions involving molecular orbitals of the C N chromophore and the benzene ring [18]. The other broad appearing above 500 nm, which can be attributed to d–d transitions in the metal orbitals.

#### FTIR spectra

The IR spectra of the free Schiff bases were compared with their respective ruthenium complexes in order to determine the coordination mode of the ligands. The free ligands show a broad band of medium intensity observed at ca.  $3430\text{--}3460\text{ cm}^{-1}$  is assigned to (O–H) of the phenolic group. In complexes the two intense bands centered at ca.  $1611$  and  $1360\text{--}1455\text{ cm}^{-1}$  assigned to (C–N) and (C–O), respectively. The bands observed in the region of  $400\text{--}425\text{ cm}^{-1}$  has been assigned to (M–O) and (M–N). The disappearance of (O–H), the downward shift of (C–O) and the lower frequency of (C–N) on complexation proves the bonding of ligands through imine nitrogen and deprotonated phenolic oxygen [15].

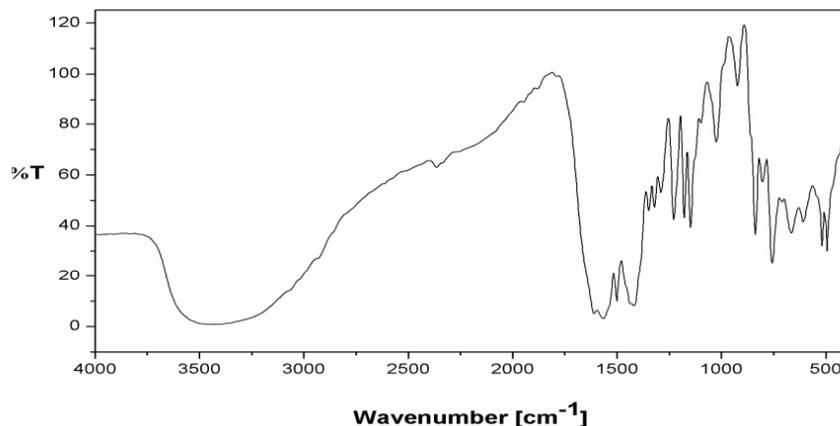


Figure 1. Representative IR spectra of [Cu(L)].

#### Magnetic moment

Magnetic moment is the measure of magnetic strength and orientation of a magnet or other object that produces a magnetic field and it is a vector quantity. The objects have a tendency to place themselves in such a way that the magnetic moment vector becomes parallel to the magnetic field lines. The direction of the magnetic moment points from the south to the north pole of a magnet. The magnetic field created by a magnet is directly proportional to the magnetic moment. The magnetic moment value signifies that whether the complex is paramagnetic or diamagnetic.

Table 2. ESR spectral data of copper and cobalt complexes.

Complex	$g_{\parallel}$	$g_{\perp}$	$g_{\text{iso}}$	$A_{\parallel} \times 10^{-4} (\text{cm}^{-1})$	$A_{\perp} \times 10^{-4} (\text{cm}^{-1})$
[Cu(L)]	2.20	2.07	2.05	145	69
[Co(L)]	2.35	2.02	2.12	127	54

The spin Hamiltonian parameters have been calculated (Table 2). From the observed 'g' values,  $g_{\parallel} > g_{\perp} > 2$ , it is apparent and shows that both the copper and cobalt complexes are paramagnetic, also the unpaired electron lies predominantly in  $d_{x^2-y^2}$  orbital giving  ${}^2B_{1g}$  as the ground state. As per the data it is understood that cobalt and copper complexes are paramagnetic whereas nickel and zinc complexes are diamagnetic.

### CONCLUSION

Base ligand of Schiff and metal(II) complexes were produced using spectroscopic techniques. The molar conductivity, magnetic susceptibility, and spectrum data all point to a tetrahedral/octahedral environment surrounding Cu(II) ions. With the exception of Cu(II) and Zn(II) groups, thermal analysis confirmed that coordinated water molecules were present in all compounds. The FAB–Mass confirmed the complex formed. In the presence of newly produced ligand, the projected DNA binding mechanism suggests that the complexes' binding activity will rise. The results of the cleavage test demonstrate that all of the chelates created are capable of cutting DNA. Furthermore, the Cu(II) and Zn(II) complex outperform AlaSal samples forms other complexes in antibacterial activity against a wide spectrum of microorganisms [5, 6]. The Cu(II) and Zn(II) interactions be situated less active than the others in terms of DNA binding and oxidative cleavage.

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