

## DNA BINDING INTERACTION STUDIES OF BINUCLEAR SCHIFF BASE Cu(II) COMPLEXES DERIVED FROM SUBSTITUTED SALICYLALDEHYDE AND TEREPHTHALDEHYDE BASED ON ABSORPTION AND VISCOMETRIC MEASUREMENTS

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### Abstract

The 1:2:2 condensation of benzene1,4-dicarbaldehyde, pyridine-2,6-diamine and 2-hydroxy-benzaldehyde/5-bromo-2-hydroxybenzaldehyde/1-(2-hydroxyphenyl) ethanone gives two sites for accommodating metal ion. The binucleating Schiff bases are then allowed to react with the ethanol solution of copper(II) acetate. The complexes have been characterized in the light of elemental analyses, spectral (IR, Uv-Vis., and ESR) and magnetic studies. The elemental analyses of the complexes confine to octahedral geometry for binuclear Cu(II) complexes. The interaction of copper complexes containing Schiff bases with calf thymus (CT) DNA was investigated by spectroscopic methods. Viscosity measurement and UV-vis spectroscopy were conducted to assess their binding ability with CT DNA. The results showed that the copper(II) complexes could bind to DNA with an intercalative mode.

**Keywords:** DNA interactions, Intercalative mode, hypochromism, Electronic absorption spectra

### Introduction

Astonishing properties of Schiff base and its complexes in various field have gained much attention in the group of budding scientists. DNA binding activity is the significant step for DNA activity and hence the effective chemotherapeutic agents and better anticancer drugs are essential to explore the interactions of metal complexes with DNA [1]. Transition metal complexes with their unique electronic and spectroscopic properties offer a multitude of coordination geometries and thus structure-activity relationship pave way for various pharmacological properties [2]. Moreover, several Cu(II) Schiff base complexes have been subject of intense investigation for DNA binding and cleavage studies[3].

In this report we continued our work [4,5] with DNA binding studies of binuclear Schiff base Cu(II) complexes.

### Materials and physical measurements

Tris(hydroxymethyl)aminomethane or Tris buffer (Sigma) were used as received. Disodium salt of calf thymus DNA (CT-DNA) and DMF (Sigma-Aldrich) were utilized as received. All other solvent and compounds are reagent grade compounds which were used without further purification.

### Synthesis of Schiff base ligand

The Schiff base ligand was synthesized by adding benzene 1,4-dicarbaldehyde (1 mmole) in 20 ml of ethanol and pyridine-2,6-diamine (2 mmole) in 20 ml ethanol slowly with constant stirring followed by the addition of 2-hydroxy-benzaldehyde (2 mmole). The mixture was then refluxed for 3 hrs. The solution was kept for slow evaporation and coloured precipitate was collected. The precipitate was washed with ethanol and then dried in air.

The same procedure was followed for synthesis of L<sub>2</sub> and L<sub>3</sub> (instead of 2-hydroxy-benzaldehyde, 5-bromo-2-hydroxybenzaldehyde used for L<sub>2</sub> and 1-(2-hydroxyphenyl) ethanone for L<sub>3</sub>).

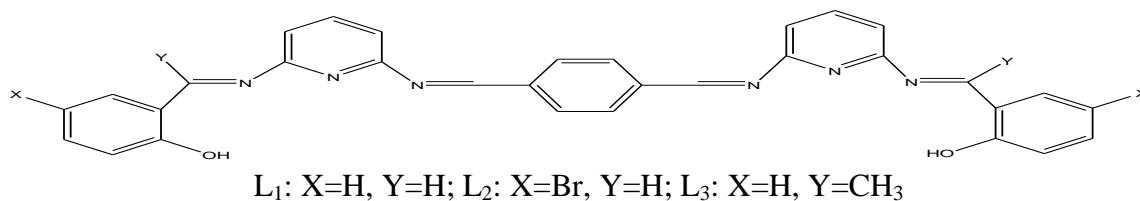


Fig.1 Structure of Schiff base ligands

### Synthesis of binuclear Schiff base Metal complexes

The ethanolic solution of synthesized Ligand (H<sub>2</sub>L) (1 mM) was added dropwise stirring to an ethanolic solution of the Copper(II)acetate (2 mM) with constant stirring, followed by the addition of 2,2'-bipyridyl and the mixture was boiled under reflux for 5 hrs. Then, the volume of the reaction mixture was reduced to 10 ml by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried in vacuo.

### DNA-binding experiments

Electronic absorption spectroscopy has been widely employed to determine the binding characteristics of metal complexes with DNA. The DNA binding experiments were performed in Tris-HCl/ NaCl buffer (50 mM Tris-HCl/ 1 mM NaCl buffer, pH 7.5) using DMF (dimethylformamide) solution (10%) of the metal complexes. The concentration of calf-thymus (CT) DNA was determined from the absorption intensity at 265 nm. Absorption titration experiments were made using different concentrations of CT-DNA [0-100  $\mu$ M], keeping the concentration of the complexes constant, with due correction for the absorbance of the CT-DNA itself. The absorbance (A) was recorded after successive additions of CT-DNA. While measuring the absorption spectra an equal amount of CT-DNA was added to both the compound solution and the reference solution to eliminate the absorbance of the CT-DNA itself. Samples were equilibrated before recording each spectrum.

Viscosity measurements were conducted on Ostwald's viscometer at  $30 \pm 0.01$  °C using fixed concentration of DNA solution (100  $\mu$ M) with increasing concentration of chiral Schiff base metal complexes (0-50  $\mu$ M) in phosphate buffer (10 mM, pH 7.0) for flow time measurements. Each sample was measured in triplicate and the average flow time was calculated with a digital stopwatch. Data were presented as  $(\eta/\eta^0)^{1/3}$  versus the ratio of the concentration of the compound and DNA, where  $\eta$  is the viscosity of DNA in the presence of the complex, and  $\eta^0$  is the viscosity of DNA alone.

## Results and discussion

The analytical data and physical properties of the ligand and its complexes are listed in Table 1. The resultant Schiff base complexes are soluble in DMF and DMSO and insoluble in other common organic solvents. The molar conductance of all the complexes was measured in DMF using  $10^{-3}$  M solutions at room temperature. The molar conductivity values of the metal complexes (Table 1) suggest the electrolytic nature [6].

**Table 1: Analytical data of the Schiff base ligands and its binuclear metal complexes**

Compound	Molecular Formula	colour	M. Pt (°)	% of Nitrogen		% of Metal		scm <sup>2</sup> mol <sup>-1</sup>
				Cal	Exp	Cal	Exp	
L	C <sub>32</sub> H <sub>24</sub> N <sub>6</sub> O <sub>2</sub>	Yellow	136	16.02	16.01	-	-	-
L <sub>2</sub>	C <sub>32</sub> H <sub>22</sub> Br <sub>2</sub> N <sub>6</sub> O <sub>2</sub>	orange	142	12.31	12.25	-	-	-
L <sub>3</sub>	C <sub>34</sub> H <sub>28</sub> N <sub>6</sub> O <sub>2</sub>	Yellow	165	15.20	15.15	-	-	-
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>56</sub> H <sub>44</sub> Cu <sub>2</sub> N <sub>10</sub> O <sub>6</sub>	Dark green	>200	12.97	12.95	11.76	11.73	135
[Cu <sub>2</sub> L <sub>2</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>56</sub> H <sub>42</sub> Br <sub>2</sub> Cu <sub>2</sub> N <sub>10</sub> O <sub>6</sub>	Dark green	>200	11.31	11.30	10.27	10.24	140
[Cu <sub>2</sub> L <sub>3</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>58</sub> H <sub>48</sub> Cu <sub>2</sub> N <sub>10</sub> O <sub>6</sub>	Dark green	>200	12.63	12.62	11.49	11.47	143

## Infrared spectra

The comparative IR spectra of the ligands and Cu(II) complexes gave elaborate details about the binding behavior of the ligand with metal ions. The IR spectra of the Schiff bases display strong absorptions between 1621-1618 cm<sup>-1</sup>, respectively, which are assigned to the C=N stretching mode and this band shift to lower frequency by 20-15 cm<sup>-1</sup> in metal complexes [7]. The band corresponding to  $\nu(\text{C-O})$  was observed around 1280-1274 cm<sup>-1</sup> and appeared higher wave frequency in the region 1299-1280 cm<sup>-1</sup> in the metal complexes, suggesting the phenolic oxygen coordinates in its deprotonated form to the metal ion [8]. In the lower frequency region the weak bands observed at 568-537 and 456-446 cm<sup>-1</sup> have been assigned respectively to the  $\nu(\text{M-O})$  and  $\nu(\text{M-N})$  vibrations[9-11].

**Table 2: Infrared spectra (cm<sup>-1</sup>) of the Schiff base ligands and its binuclear metal complexes**

Compound	Free-OH	-C=N	In-plane & Out-of-plane Pyridine ring Deformations	C-O	M-O	M-N
L <sub>1</sub>	3362 b	1620 s	567, 402 m	1274 m	-	-
L <sub>2</sub>	3362 b	1618 s	570, 410 m	1274 m	-	-
L <sub>3</sub>	3436 b	1621 s	565, 406 m	1280m	-	-
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1606 s	633, 413 m	1288 m	537 w	446 w
[Cu <sub>2</sub> L <sub>2</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1600 s	621, 420 m	1299 m	568 w	456 w
[Cu <sub>2</sub> L <sub>3</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1605 s	618, 426 m	1297 m	557w	452 w

b-broad, s-strong, w-weak, m-medium

### Electronic spectra and magnetic studies

The electronic spectrum of free Schiff base ligands show a broad band around 255-294 nm, which is assigned to  $\pi-\pi^*$  transition of the C=N chromophore. On complexation this band was shifted to lower wavelength region suggesting the coordination of azomethine nitrogen to the central metal ion. The synthesized Cu(II) complexes displayed four bands at 35,087–34,013  $\text{cm}^{-1}$ , 27,777–26,737  $\text{cm}^{-1}$ , 20,833–19,607  $\text{cm}^{-1}$ , 14492–13,888  $\text{cm}^{-1}$  due to  $\pi-\pi^*$  within organic molecule, (L  $\rightarrow$  Cu<sup>2+</sup>) charge transfer after complex formation; while the last band can be assigned to  $^2B_{1g} \rightarrow ^2E_g$  transition of the Cu(II) ion in of octahedral geometry [12].

**Table 3: Electronic Spectral data of Schiff base ligand and its binuclear metal complexes.**

Compound	Electronic spectra (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L $\rightarrow$ M	d-d	
L <sub>1</sub>	294	386	-	-	-
L <sub>2</sub>	287	395	-	-	-
L <sub>3</sub>	255	345	-	-	-
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	285	374	480	690	Octahedral
[Cu <sub>2</sub> L <sub>2</sub> X <sub>2</sub> ]Y <sub>2</sub>	290	365	510	720	
[Cu <sub>2</sub> L <sub>3</sub> X <sub>2</sub> ]Y <sub>2</sub>	294	360	495	695	

### Electron Spin Resonance

The ESR spectrum of the binuclear Schiff base Cu(II) complexes exhibits two signals  $g_{\perp}$ ,  $g_{\parallel}$  and  $g_{av} = 1/3(g_{\parallel} + 2g_{\perp})$ , these values follows the same trend  $g_{\parallel} > g_{\perp} > g_e$  which suggest that the presence of unpaired electron  $dx_{2-y_2}$  orbital giving octahedral geometry[13].

In the axial spectra, the g-values are related with exchange interaction coupling constant (G) by the expression,  $G = g_{\parallel} - 2 / g_{\perp} - 2$  reflects the spin-interaction between Cu(II) centers of the binuclear Cu(II) complex. In the present case G value comes out to be 4.8, which indicated negligible exchange interaction between the two copper ions and evidenced the monomeric nature of the complex [14]. These data are in agreement with those obtained from the electronic spectra and confirm the octahedral geometry for Cu(II) Schiff base complexes.

**Table 4: Electron Spin Resonance Data of Binuclear Cu(II) Complex.**

Complex	$g_{\parallel}$	$g_{\perp}$	$g_{av}$	$\Delta g$	G
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	2.438	2.090	2.206	0.34	4.86
[Cu <sub>2</sub> L <sub>2</sub> X <sub>2</sub> ]Y <sub>2</sub>	2.347	2.064	2.158	0.28	5.42
[Cu <sub>2</sub> L <sub>3</sub> X <sub>2</sub> ]Y <sub>2</sub>	2.257	2.059	2.125	0.19	4.35

### DNA Binding properties

#### Absorption studies

The electronic absorption spectroscopy is one of the most common method to study the DNA-binding properties of Cu(II) complexes, since there are strong MLCT (metal-to-

ligand) and LMCT (ligand-to-metal) features were observed in the absorption spectra of these complexes. In general, Hypochromism is suggested to occur due to an intercalative mode of binding involving a tough stacking interaction between an aromatic chromophore and the base pairs of DNA [15]. Upon addition of CT-DNA, the observed absorption band of  $[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2$  complex at 285 nm exhibited hypochromism of about 47.2 % with a slight bathochromism of about 4 nm (Fig. 2). Similarly,  $[\text{Cu}_2\text{L}_2\text{X}_2]\text{Y}_2$  and  $[\text{Cu}_2\text{L}_3\text{X}_2]\text{Y}_2$  complexes exhibits an evident hypochromism of about 30.5 % and 33.7 % at 290 and 294 nm, respectively, with 3 nm of apparent bathochromic shift was observed (Figs. 3 and 4). The appearance of strong hypochromism in the absorption spectra of the complexes is attributable to the interaction between the electronic states of the binding chromophore and that of CT-DNA. This absorption suggests a mode of intercalative binding that involves a stacking interaction between the complexes and the base pairs of DNA.

**Table 5: Absorption properties of Binuclear Schiff Cu(II) complexes-DNA Binding Activity**

Complexes	$\lambda_{\text{max}}$ (nm)		$\Delta\lambda$ (nm)	Hypo (%)
	Free	Bound		
$[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2$	285	289	4	47.2
$[\text{Cu}_2\text{L}_2\text{X}_2]\text{Y}_2$	290	293	3	30.5
$[\text{Cu}_2\text{L}_3\text{X}_2]\text{Y}_2$	294	297	3	33.7

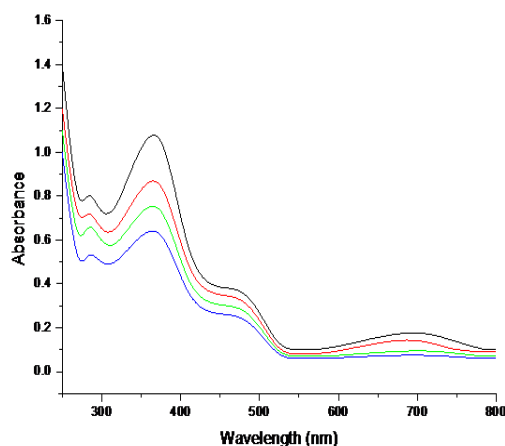


Fig.2 Absorption spectra of  $[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2$  in Tris-HCl buffer upon addition of DNA.  $[\text{Cu(II)}] = 0.5 \mu\text{M}$ ,  $[\text{DNA}] = 0-100 \mu\text{M}$ .

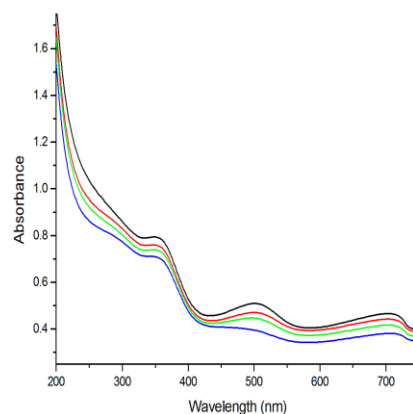


Fig.3 Absorption spectra of  $[\text{Cu}_2\text{L}_2\text{X}_2]\text{Y}_2$  in Tris-HCl buffer upon addition of DNA.  $[\text{Cu(II)}] = 0.5 \mu\text{M}$ ,  $[\text{DNA}] = 0-100 \mu\text{M}$ .

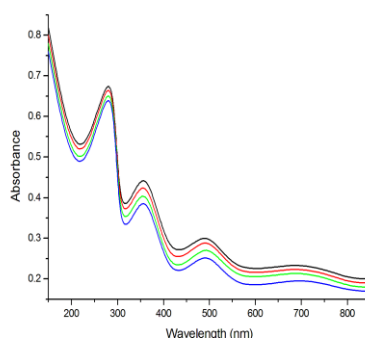


Fig.4 Absorption spectra of  $[\text{Cu}_2\text{L}_3\text{X}_2]\text{Y}_2$  in Tris-HCl buffer upon addition of DNA.  $[\text{Cu(II)}] = 0.5 \mu\text{M}$ ,  $[\text{DNA}] = 0\text{--}100 \mu\text{M}$ .

### Viscosity studies

In order to clarify the interactions between the complexes and DNA, viscosity measurements were carried out[16]. The effects of all the synthesized complexes on the viscosity of DNA at  $30 \pm 0.1^\circ\text{C}$  are shown in Fig. 5. Viscosity experimental results clearly show that all the complexes can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the viscosity of DNA. The complexes can intercalate strongly, leading to a greater increase in viscosity of the DNA with an increasing concentration of complexes. The increased degree of viscosity which follows the order of  $[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2 > [\text{Cu}_2\text{L}_3\text{X}_2]\text{Y}_2 > [\text{Cu}_2\text{L}_2\text{X}_2]\text{Y}_2$ , may depend on its affinity to DNA. The results obtained from viscosity measurements confirm intercalation between the complexes and DNA.

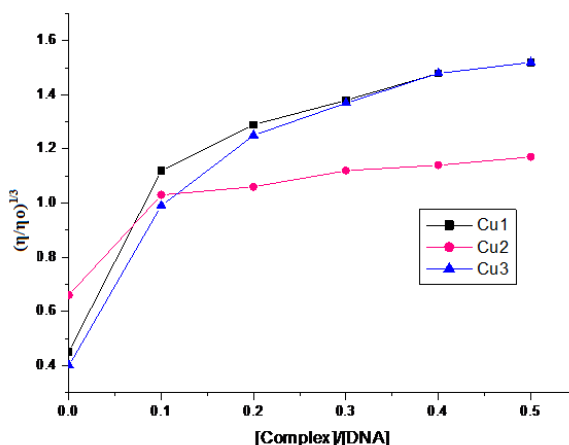


Fig.5 Plot of relative viscosity vs  $[\text{Complex}]/[\text{DNA}]$ .  $R = [\text{complex}]/[\text{DNA}]$ . Complex =  $0\text{--}50 \mu\text{M}$ ,  $[\text{DNA}] = 100 \mu\text{M}$ .

### Conclusion

Condensation reaction of benzene1,4-dicarbaldehyde, pyridine-2,6-diamine and 2-hydroxy-benzaldehyde/5-bromo-2-hydroxybenzaldehyde/1-(2-hydroxyphenyl) ethanone resulted in the production of Schiff base ligand in excellent yield. The ligand coordinates in 1:2 ligand to metal ratio as a dibasic two (NNO) donor in all the complexes. The analytical,

molar conductance, vibrational and electronic spectral study suggested the structures in octahedral. The DNA-binding properties of the synthesized complexes have been examined by absorption spectroscopy and viscosity measurements. Evidences are presented that the complexes could interact with DNA via intercalation mode.

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