Synthesis, Characterization andDNA binding affinities of mixed ligand Cu(II)complexes of 3-((E)-(2-hydroxyphenylimino)methyl)-4H-chromen-4-one

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Abstract

Three mixed ligand copper(II) complexes of 3-formylchromone and 2-aminophenol based Schiff base ligand (L) and N,N-donor ligands *viz.*, [Cu(L)(bpy)](L1),[Cu(L)(phen)](L2) and [Cu(L)(dmp)](L3),{Where bpy= 2,2'-bipyridine, phen = 1,10-phenanthroline, dmp = 2,9-dimethylphenanthroline} have been synthesized and characterized by ^{1}H-NMR, UV-Vis , FT-IR, ESI-mass and EPR spectral studies. These complexes were found to bind with CT DNA which was explored using absorption and emission spectral titrations and has been revealed that they could bind to DNA *via*, a mode of intercalation. The difference in the relative emission intensities of DNA-bound ethidium bromide observed upon interaction with the complexes found to parallel the trend in DNA binding affinities.

Keywords: 3-formylchromone, Schiff base, Copper(II) complex, CT-DNA, Intercalation.

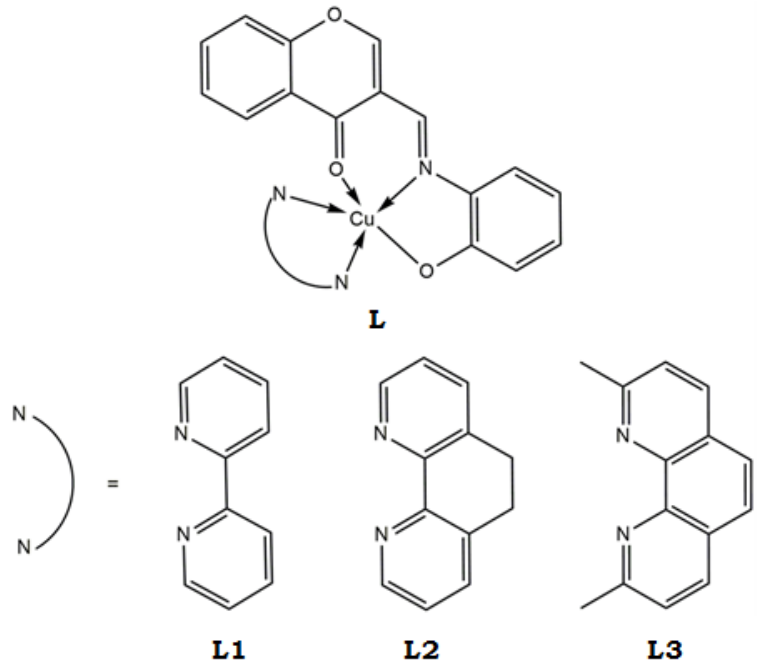
1. Introduction

Over the years, medicinal bioinorganic chemistry has played an important role towards designing innovative metal-based drugs. It is well known that the most prominent drug containing a metal centre is cis-platin, the most widely used anticancer drug. The mechanism by which cis-platin interacts with cancer cells was elucidated by *Lippard*, which involves its covalent binding to DNA. The metal complexes play an excellent role in the field of molecular biology and acts as excellent potential therapeutic agents[1-3]. There is still a major challenge for researchers to design and synthesis of such molecules to bind and cleave DNA. On account of diverse chemical reactivity and strange electronic properties, transition metal-polypyridyl complexes are used in these investigations since they could interact with DNA non-covalently[4,5]. Schiff bases derived from condensation ofa carbonyl compound and an amine are capable of coordinating to metal

ions via the imino nitrogen atoms. Schiff base ligands containing O, N, S as donor atoms have gained the attention of the researchers since it can bind with various metal ions and hence show wide biological activities[6]. Metal complexes derived from Schiff bases are studied most widely dueto their importance in biochemical and analytical reagents.Recently mixed ligand copper(II) complexes has gained interest due to its strong binding and cleaving abilities of DNA and thereby exhibit anticancer activities and regulate apoptosis [7,8]. Also enormous work have been reported in the DNA binding affinities of metal complexes with 2,2'-bipyridyl, 1,10-phenanthroline ligands and their derivatives.Hence we decided in designing suitable copper complexes and study their ability to interact with DNA.

2. Experimental

All the chemicals utilized in this research work are of AR grade and also 99% puresolvents are used. The chemicals 3-formylchromone, 2-aminophenol, 2,2'-bipyridine, 1,10-Phenanthroline and 2,9-dimethylphenanthroline were obtained from Merck, India. CT-DNA (Genei, Bangalore, India), tris-Buffer (HIMEDIA) and copper(II) chloride dihydrate (LobaChemie) were purchased in pure form and used as such.The elemental contents (C, H, N) of the ligand and its Cu(II) complexes were analysed using Carlo Erba 1108 elemental analyzer. Bruker Advance DRX 300 FT-NMR spectrometer was involved in recording the ^{1}H NMR spectrum using $CDCl_3$ as solvent and TMS as standard. Bruker-Daltonics micro TOF-Q II mass spectrometer was used to record ESI mass spectraof the complexes.FT-IR spectra of the complexes between the range400-4000 cm^{-1} were obtained on JASCO FT-IR/4100 Spectrometer employing KBr disc method. The complexes (10^{-3} M) were dissolved in DMSO and their absorbance was measured in the range of 200-800 nm on a Perkin Elmer Lambda 35 spectrophotometer along with their molar conductivities using Elico model SX 80 conductivity meter.The EPR measurements of all the complexes were recorded at a temperature of liquid nitrogen and Varian E-4 X-band spectrometer was employed for the analysis taking the g-marker to be DPPH.



Scheme 1. Schematic representation for the synthesis of Cu(II) complexes.

2.1 Synthesis of Schiff base ligand (L)

Equimolar quantities of 3-formylchromone (3.30 g) and 2-aminophenol (3.30 g) were dissolved in 50 mL of methanol and the contents weresubjected to stirring for 1 hour. The product obtained was washed repeatedly with diethyl ether and dried *in vacuum* over anhydrous calcium chloride.

2.2 Synthesis of mixed ligand copper(II) complexes

To a methanolic solution of the Schiff base ligand **(**L) (1mmol),copper(II) perchlorate (1 mmol) was added at constant stirring for 30 min. Then about 1 mmol of the ancillary ligands 1,10-phenanthroline, 2,9-dimethyl phenanthroline and 2,2'-bipyridine dissolved in methanol was added drop wise to the reaction mixture. The resulting solution was then refluxed at 40 $^{\circ}$C with constant stirring for 2 hours. Finally, the precipitate obtained was then filtered, washed with ethanol and dried *in vacuo.*

2.3 DNA binding experiments

2.3.1 Absorption spectral method

All the binding experiments with calf thymus DNA (CT-DNA) were performed in tris-HCl buffer solution (pH = 7.5), prepared using deionized water.The concentration ofCT-DNA was obtained from its absorbance value at a wavelength of 260 nm using ε_{260}= 6600 $Lmol^{-1}cm^{-1}$[9]. The stock solutions of the copper(II) complexes were prepared by dissolving them in certain percent of DMSO and diluting to a final concentration of $1x10^{-5}$. The UV spectral titrations were performed by maintaining the concentration of the copper(II) complexes to be constant and there by varying the CT-DNA concentration.

2.3.2 Fluorescence spectral method

In order to further explain the interaction between the complexes and DNA, competitive binding experiments involving ethidium bromide (EB) were carried out, that emits intense fluorescence at about 612 nm in the presence of DNA on account of its strong intercalation between the adjacent base pairs of DNA [10]. The binding efficiency of the complexes was determined from the classical Stern-volmer equation;

$$\frac{I_0}{I} = 1 + K_{sv}[Q]$$

In this equation, I_0 and I represent the intensities of emission maxima in the absence and presence of the quenchers respectively and [Q] the corresponding complex concentration; K_{sv} the Stern-Volmer quenching constant which isobtained from the slope in the linear plot of I_0/I *vs*[Q]. The following equation paves the route to calculate the apparent binding constant (K_{app}) values which are provided in Table 4.

$$K_{app} = K_{EB}[EB]/K_{app}[complex]$$

The concentration of EB was 3.3 μM and the value of K_{EB} was found to be 1.0×10^7 M^{-1} [11].

3. Results and Discussions

The ligand L is synthesized by the condensation of 3-formylchromone and 2-aminophenol, and it is coordinated with Cu(II) metal ion using the ancillary ligands 2,2'-bipyridine (L1), 1,10-phenanthroline (L2)and 2,9-dimethylphenthroline (L3).The ligand is found to be stable in air and soluble in common

organic solvents like chloroform,ethanol and methanol whereas the synthesized complexes are stable in air and soluble in solvents like DMF, DMSO etc., but insoluble in common organic solvents.

The physical and analytical data of the ligand and the complexes are provided in Table 1.

Table 1. Physical and analytical data of ligand and its complexes.

Compound	Anal. Found (calculated) %			
	C	H	N	Cu
L	72.89(72.45)	3.90(3.94)	4.91(4.98)	-
L1	65.14(65.12)	4.02(4.05)	8.37(8.44)	12.47 (12.51)
L2	66.78(66.72)	3.87(3.86)	8.06(8.05)	12.77 (12.79)
L3	67.64(67.69)	4.32(4.40)	7.67(7.69)	12.47 (12.51)

3.1 ^{1}H-NMR spectral studies

The ^{1}H-NMR spectrum of the ligand (Fig. 1) shows a signal at 9.37 ppm which is assigned to azomethine proton (CH=N). The –OH proton is observed as a broad singlet at 9.06 ppm. The signals in the range of 7.03-7.47 ppm are due to aromatic protons the ligand [12]. This confirms the formation of Schiff base ligand by the condensation of 3-formylchromone and 2-aminophenol.

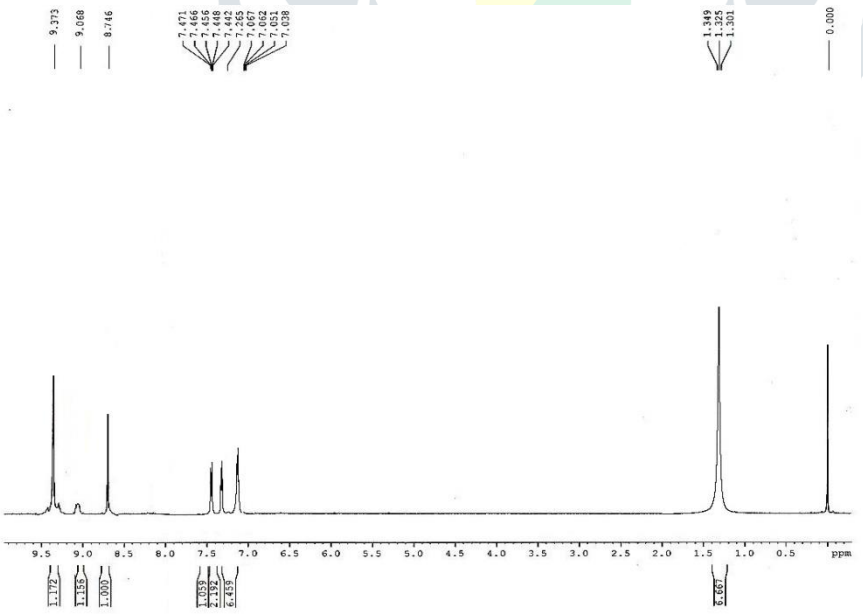


Figure 1. ^{1}H NMR spectrum of the Schiff base ligand.

3.2 Mass spectral studies

The ESI-mass spectra of the ligand (Fig. 2a) exhibits a molecular ion peak at m/z = 282.25 which corresponds to the molecular weight of the ligand. Molecular ion peaks for the complexes (Fig. 2b) L1, L2 and L3 were observed at m/z = 497.17, 521.10 and 549.42 respectively. These were in agreement with the molecular weight of the synthesized complexes.The ESI-mass thus confirms the authenticity of the complexes.

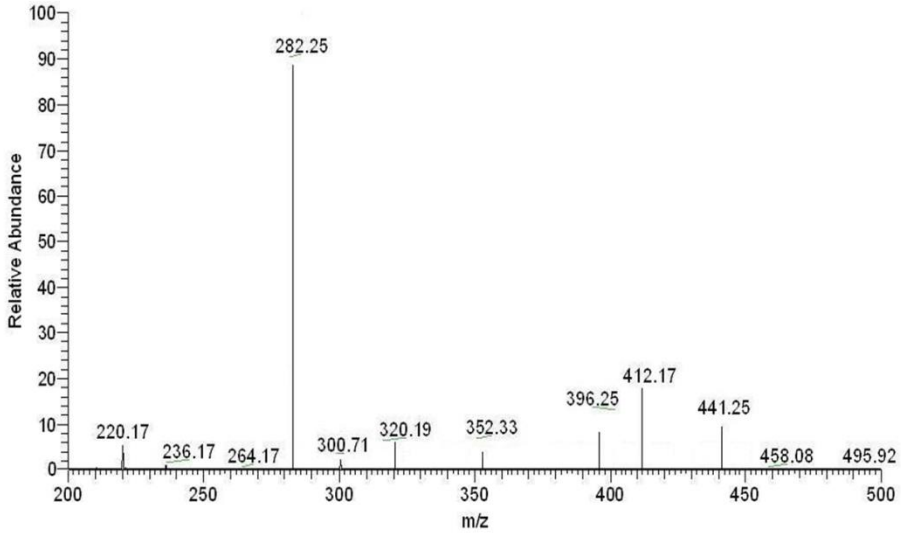


Figure 2a. ESI mass spectrum of the Schiff base ligand.

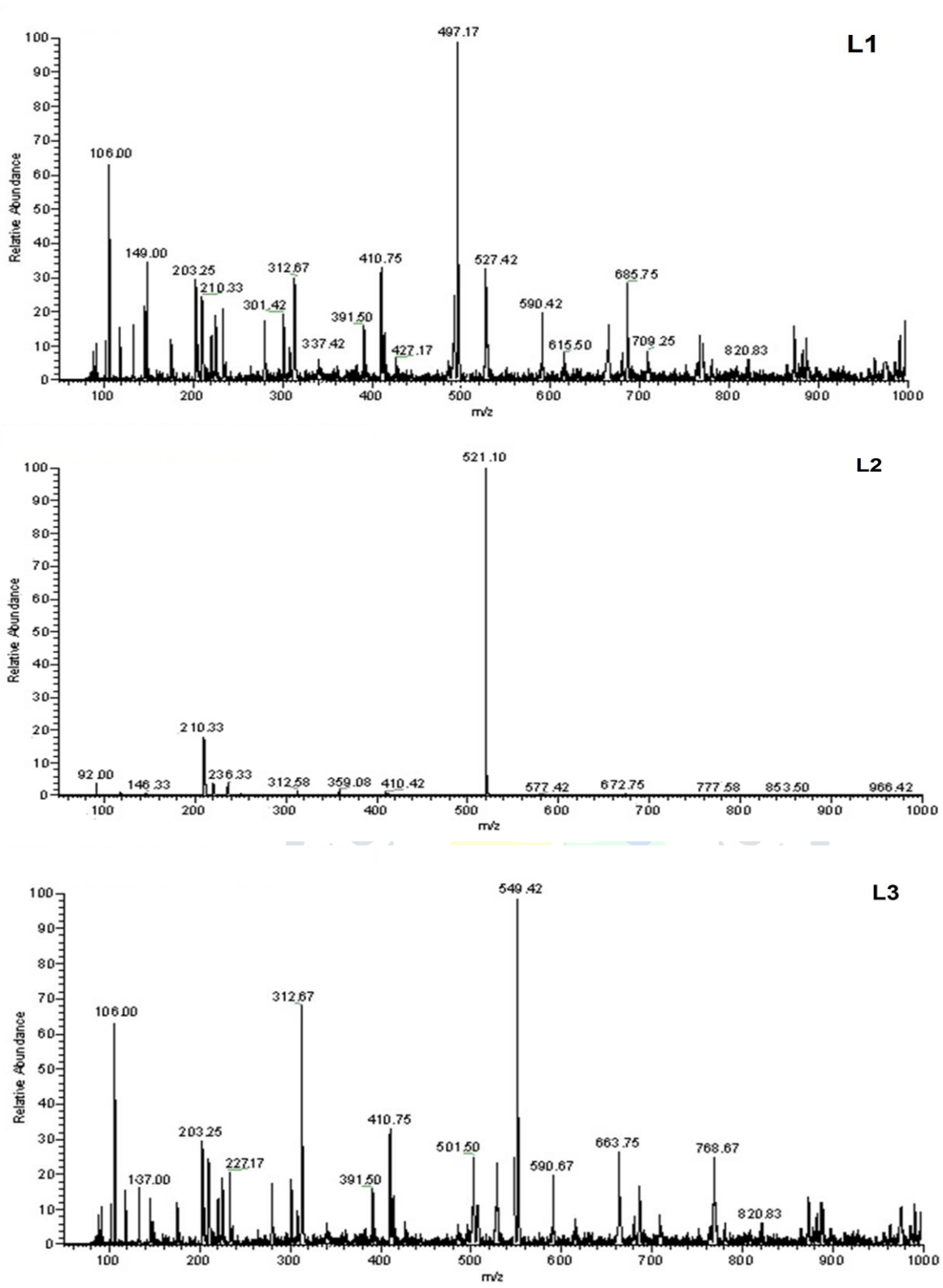


Figure 2b. ESI mass spectra of Cu(II) complexes.

3.3 FT-IR spectral studies

In the vibrational spectrum of ligand L (Fig. 3), the band for azomethine(C=N) group appeared at 1597cm^{-1} [13]. In the FT-IR spectra of complexes (Fig. 3), this band has been shifted to lower frequency indicating the involvement of azomethine nitrogen in coordination to the Cu(II) ion. The band at 3241cm^{-1} in the ligand is due to OH stretching vibration which disappeared in metal complexes thereby confirming the participation of oxygen in coordination to metal ion. The carbonyl stretching of pyrone ring (C=O) exhibits a band at 1650 cm^{-1} forthe ligand [14] which was shifted to lower frequency region for complexes suggesting the participation (C=O) group in co-ordination to the metal ion. All the complexes show broad anti-symmetric stretching bands and a sharp anti-symmetric bending band around 1100 - 1085 and 626 - 621 cm^{-1}, indicating the presence of ionic perchlorate anions [15]. Additional bands were observed for complexes around 450 cm^{-1}and 550 cm^{-1} corresponding to M-N and M-O stretching respectively [16].

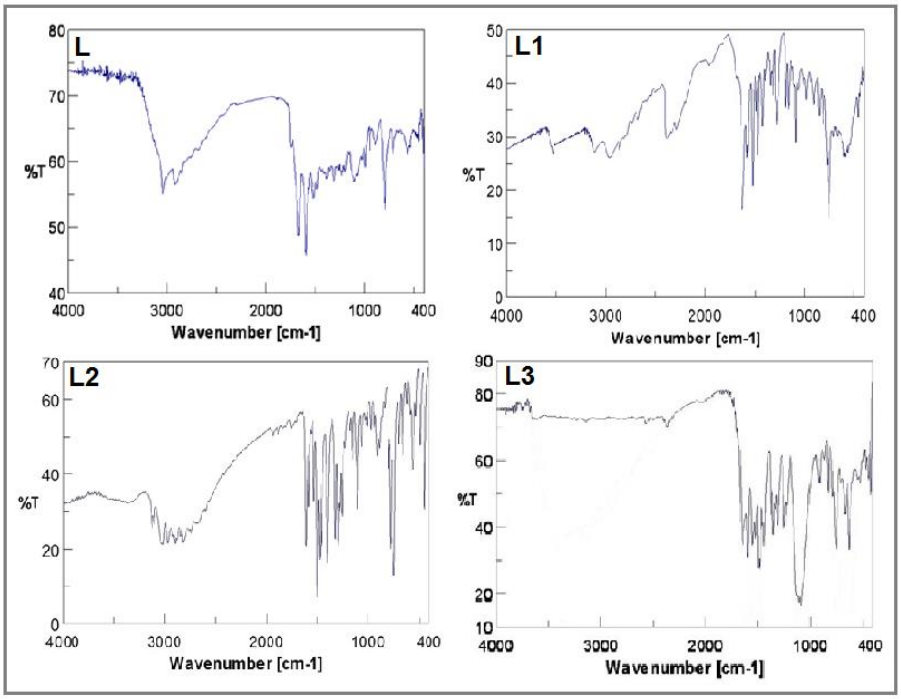


Figure 3. FT-IR spectra of the Schiff Base ligand and its Cu(II) complexes.

3.4 UV Spectra and Magnetic studies

The electronic spectra of the complexes were recorded in 450-900 nm range in DMSO solvent and are depicted in Fig. 4. The electronic spectra of all the complexes exhibit broad bands around 536-550 nm and

660-680 nm which can be assigned to $^2B_1 \rightarrow {}^2B_2$ and $^2B_1 \rightarrow {}^2E$ transitions suggesting square pyramidal geometry [17].

The magnetic moment values of the complexes L1, L2 and L3 were found to be 1.88, 1.89 and 1.89 B.M indicates that copper ion is in d^9 configuration with one un-paired electron. Further the molar conductance values explain the non-electrolytic nature of the complexes. The electronic spectral data, magnetic susceptibility values and molar conductance values of the complexes are provided in Table 2.

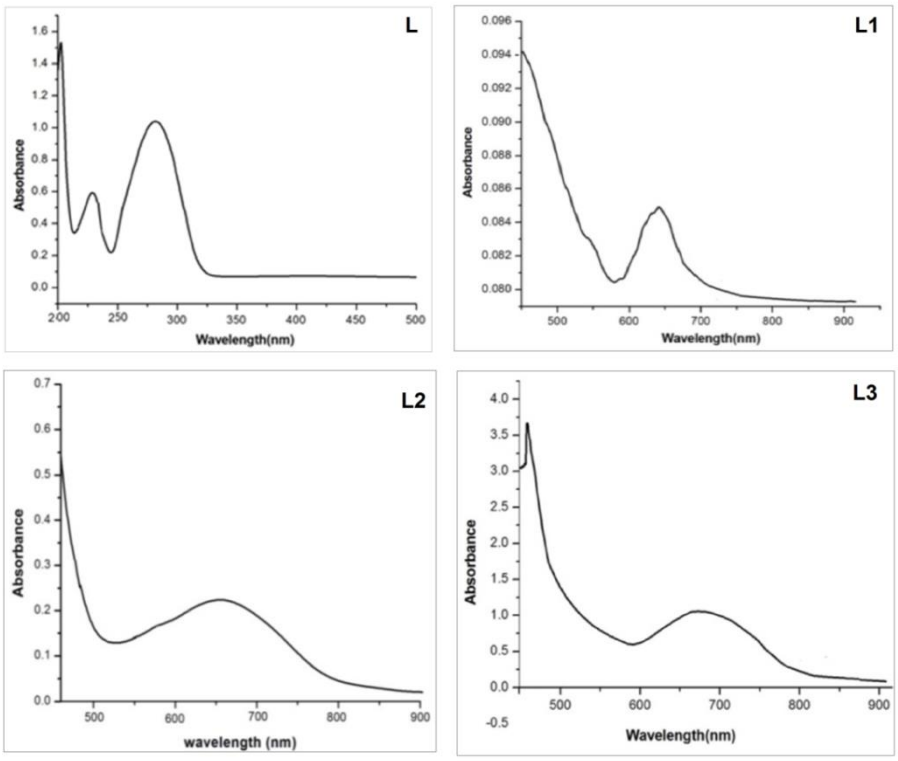


Figure 4. Electronic spectra of the Schiff Base ligand and its Cu(II) complexes.

Table 2. Electronic spectra data of ligand and its Cu(II) complexes.

Compound	Frequency (nm)	Transition	Geometry	μ_{eff}	Molar conductance $ohm^{-1}cm^2mol^{-1}$
L	226, 264	ILCT	-	-	
L1	546, 674	$^2B_1 \rightarrow {}^2B_2$ & $^2B_1 \rightarrow {}^2E$	Square pyramidal	1.88	36.74
L2	535, 663	$^2B_1 \rightarrow {}^2B_2$ & $^2B_1 \rightarrow {}^2E$	Square pyramidal	1.89	39.48
L3	539, 677	$^2B_1 \rightarrow {}^2B_2$ & $^2B_1 \rightarrow {}^2E$	Square pyramidal	1.89	37.83

3.5 EPR spectral studies

EPR spectra of the complexes recorded in DMSO at 77K are given in Fig. 5. The spin Hamiltonian parameters are provided in Table 3. The Zeeman splitting or the g factors are found to be shifted from the free-electron value of 2.0023 by spin-orbit coupling of the ground state to the excited states. The spectra of the copper complexes exhibits four well resolved peaks in the region of low field.

From the spectra, it is found that for all the complexes, $g_{11} > g_{\perp}$ suggesting that the ground state of Cu(II) is predominantly d_{x2-y2} with d^9 configuration [18]. The g_{11} and A_{11} values are in consistent with the square pyramidal geometry[19]. The quotient of g_{11} /A_{11} explains that all the complexes exhibited a slight distortion from planarity. The covalency factor α^2 is found to be less than unity which shows that all the complexes have some covalent character.

Table 3. EPR spectral data of Cu(II) complexes.

Compound	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$	$A_{\perp}$	A_{iso}	$g_{\parallel}/A_{\parallel}$ (cm)	α^2	g_{iso}
			10^{-4} cm^{-1}					
L1	2.2310	2.0623	184	115	124	121	0.74	2.12
L2	2.2562	2.0781	180	103	119	125	0.81	2.17
L3	2.2715	2.0744	182	113	131	124	0.86	2.14

3.6 DNA binding studies

3.6.1 Absorption spectral studies

Addition of incremental amounts of CT-DNA to the complexes L1, L2 and L3 (Fig. 6), found to decrease the absorption band intensity along with a small red-shift. This arises as a result of the binding of DNA with metal complexes. Also it is well known that hypochromism along with batho-chromism occurs only if the complexes could bind to DNA through intercalation [20]. The binding constant(K_b) values, were calculated from the equation provided as follows[21].

$$\frac{[DNA]}{(\varepsilon_a - \varepsilon_f)} = \frac{[DNA]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

Where ε_a is the molar extinction coefficient for the charge transfer absorption of DNA at a given concentration, ε_f and ε_b is the extinction coefficients of the complex in free as well as bound states respectively and K_b, the equilibrium binding constant. The K_b value is the ratio of the slope to the intercept in the linear fitting plot of [DNA]/(ε_a-ε_f) versus [DNA].

The calculated intrinsic binding constant values K_b for the complexes are found to be in the order of L2> L3> L1. This explains that the coordinated phen and bpy planar rings are engaged in partial interaction between the base pairs of DNA. Generally planar intercalative ligand would increase the strength of interaction of the metal complexes with DNA [22].

3.6.2 Fluorescence spectral studies

The intense emission of EB in presence of DNA is due to its strong intercalation between the base pairs of DNA [23]. On adding various concentrations of the complexes L1, L2 and L3 to DNA-EB system, the emission intensity of EB is quenched around 612 nm.This shows that the complexes can displace EB from DNA-EB system via intercalative mode [24]. The relative binding of the complexes L1, L2 and L3 with EB bound CT-DNA solution as shown in Fig. 7. The decrease in the emission intensity gives a measure of the binding tendency of the complexes to DNA. The apparent binding constants (K_{app}) of the complexes are given in Table 4. From the results observed, it could be concluded that the complexes interact with CT-DNA *via.*, intercalative mode.

Table 4: Binding constant (K_b) and Apparent binding constant (K_{app}) for the complexes with interaction of CT-DNA

Complexes	**Binding constant (K_b) M^{-1}**	**Apparent binding constant (K_{app}) M^{-1}**
L1	3.60×10^4	4.91×10^5
L2	2.30×10^5	1.14×10^6
L3	1.07×10^5	7.64×10^5

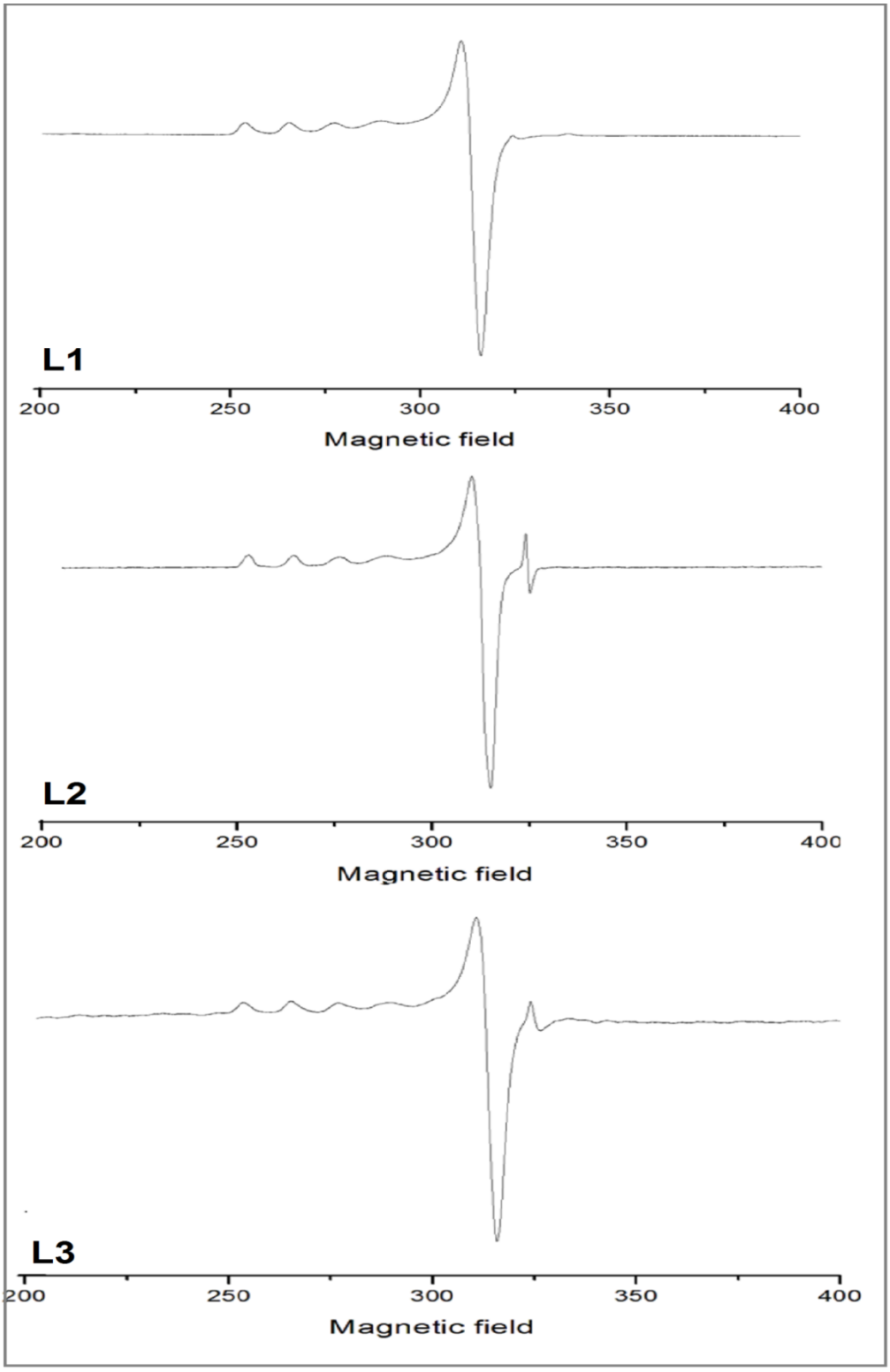


Figure 5. EPR spectra of Cu(II) complexes

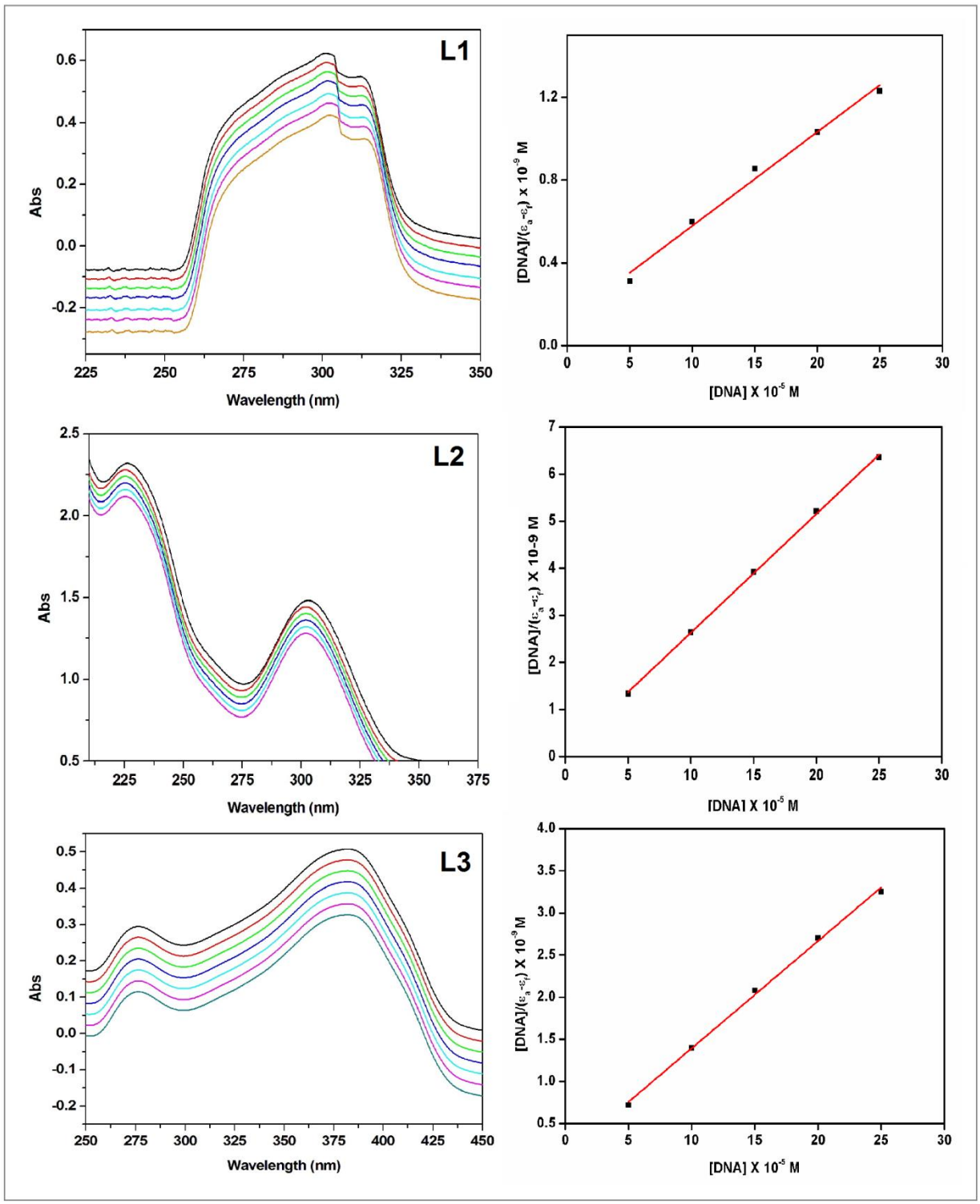


Figure 6. Absorption spectra of Cu(II) complexes (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10^{-5} M) at room temperature in 50 mM tris-HCl/NaCl buffer (pH = 7.5)

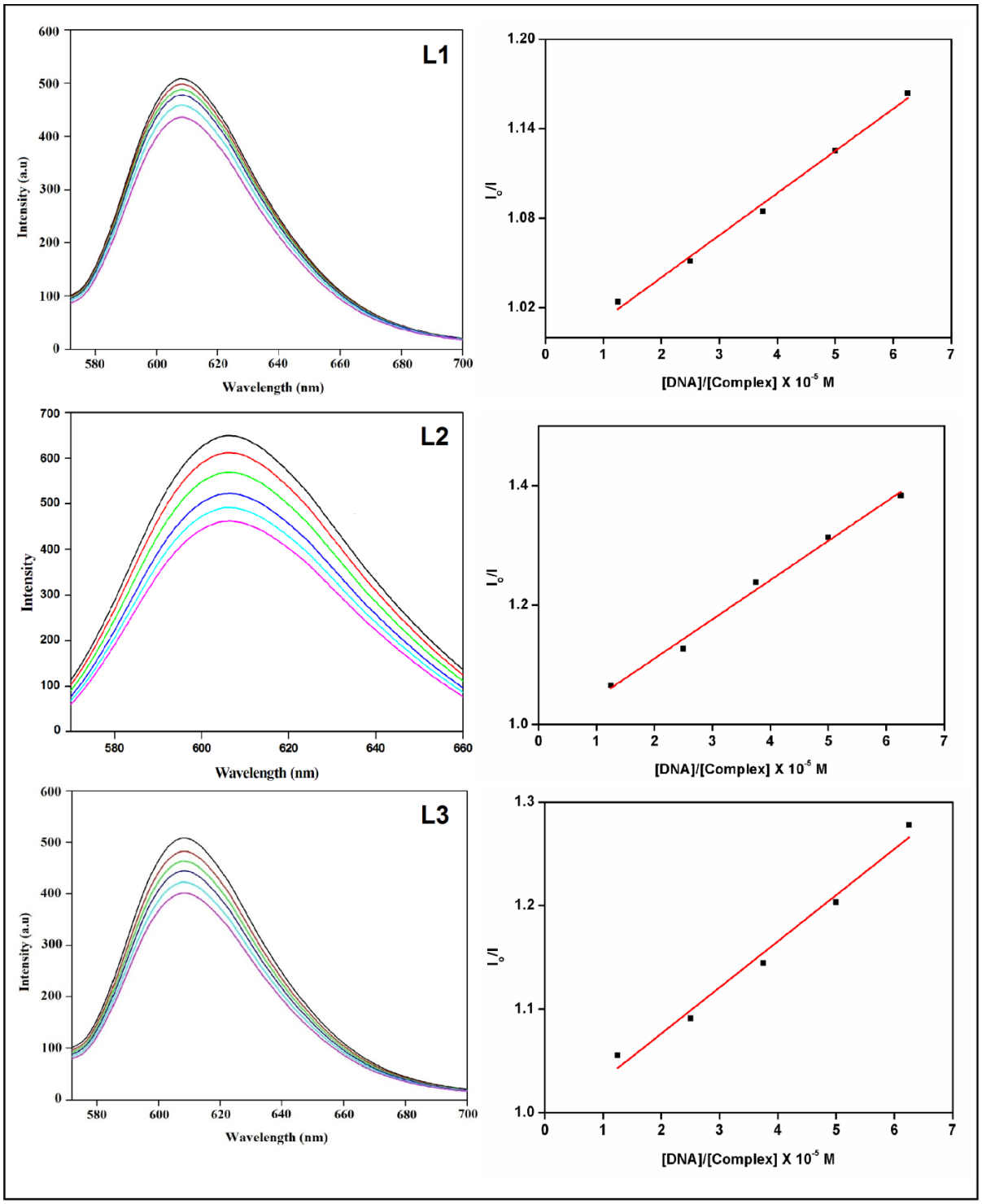


Figure 7. Emission spectrum of EB bound to DNA in the presence of Cu(II)complexes ([EB] = 3.3 μM, [DNA] = 40 μM, [complex] = 0-30 μM, λ_{ex}= 430 nm). In set shows the plots of emission intensity I_o/I *vs* [DNA]/[complex]

4. Conclusion

A Schiff base ligand (L) involving3-formylchromone and 2-aminophenol has been synthesized and coordinated with Cu(II) ion along with various ancillary ligands such as 2,2'-bipyridine, 1,10-phenanthroline and 2,9-dimethylphenanthroline. The ligand as well as the complexes was characterized using various spectral tools. The electronic and magnetic susceptibility values suggest that all the complexes possess square pyramidal geometry. The EPR spectra reveals that the ground state of Cu(II) is predominantly $d_{x^2-y^2}$ with d^9 configuration. The DNA binding studies of all the synthesized copper complexes explored using absorption and emission spectral techniques revealed that the complexes could bind to CT-DNA *via*., intercalation in an effective manner. Further the binding constant values substantiate that the complexes could effectively bind to CT-DNA.

5. References

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