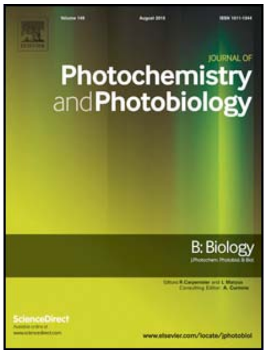

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Synthesis, characterization and biological approach of metal chelates of some first row transition metal ions with halogenated bidentate coumarin Schiff bases containing N and O donor atoms

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Abstract

The impregnation of halogen atoms in a molecule is an emerging trend in pharmaceutical chemistry. The presence of halogens (Cl, Br, I and F) increases the lipophilic nature of molecule and improves the penetration of lipid membrane. The presence of electronegative halogen atoms increases the bio- activity of core moiety. In the present study, Co(II), Ni(II) and Cu(II) complexes are synthesised using Schiff bases (**HL^{I}** and **HL^{II}**), derived from 8-formyl-7-hydroxy-4-methylcoumarin/3-chloro-8-formyl-7-hydroxy-4-methylcoumarin with 2,4-difluoroaniline/o-toluidine respectively. The synthesized compounds were characterized by spectral (IR, NMR, UV-visible, Mass, ESI-MS, ESR), thermal, fluorescence and molar conductivity studies. All the synthesized metal complexes are completely soluble in DMF and DMSO. The non-electrolytic nature of the metal complexes was confirmed by molar conductance studies. Elemental analysis study suggest $[ML_2(H_2O)_2]$ stoichiometry, here M = Co(II), Ni(II) and Cu(II), L = deprotonated ligand. The obtained IR data supports the binding of metal ion to Schiff base. Thermal study suggests the presence of coordinated water molecules. Electronic spectral results reveal six coordinated geometry for the synthesized metal complexes. The Schiff bases and their metal complexes were evaluated for antibacterial (*Pseudomonas aureginosa* and *Proteus mirabilis*), antifungal (*Aspergillus niger* and *Rhizopus oryzae*), anthelmintic (*Pheretima posthuma*) and DNA cleavage (Calf Thymus DNA) activities.

Keywords: Halogenated Coumarin Schiff bases; Metal complexes; Fluorescence; DNA cleavage; Anthelmintic

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1. Introduction

Coumarin is a white crystalline compound having typical benzopyrone framework, occurs naturally in plants and it can be synthesized chemically. Plant species of Compositae, Rutaceae and Umbelliferae families contain higher level of coumarin and it can be isolated using various extraction procedures [1]. Chemically coumarin can be synthesized by different methods [2-7]. The simple and common method used for synthesis of coumarin is Pechmann condensation, in which, different phenols are used. 7-Hydroxycoumarin (*Umbelliferone*), on its structural and biogenetic aspects is regarded as the parent coumarin among the structurally more complex coumarins [8]. Coumarin derivatives have the ability to express numerous pharmacological properties such as antimicrobial [9], antioxidant [10,11], antiplasmodial [12], anticancer [13], cytotoxic [14], anthelmintic [15], inhibition of platelet aggregation [16], antineurodegenerative [17], anti HIV [18,19] and anti-inflammatory [20] activities.

In recent years, many structural modifications have been made at various positions of the coumarin ring system to get more pharmaceutically active coumarin derivatives. In an aromatic ring, the presence and position of the electron withdrawing group may enhance its reactivity. The best pharmaceutical results can be expected by replacing a hydrogen atom of a molecule by halogen. Many of such derivatives are used as antibiotics, antineoplastic agents, CNS depressant, CNS stimulant and antipsychotic etc. Recently, K.N. Venugopala et al., showed that, halogenated coumarin derivatives express anti-malarial activity [21]. These compounds act as inhibitors against various protein kinases [22]. Recent studies on halogen-bonding or X-bonding show the increased affinity of ligand as potential anticancer drugs [23]. The presence of halogen atoms in a molecule enhances the lipophilic nature and is less soluble in water, which also improves the penetration of lipid membrane. Fluorine atom being less bulky among all the halogens (Cl, Br, I and F) has the ability to block metabolically active positions.

In the light of all these observations, we have planned to synthesize new transition metal complexes of halogen substituted coumarin Schiff bases and study their pharmacological activity. In this part of research work, we have synthesized Co(II), Ni(II) and Cu(II) complexes of coumarin Schiff bases derived from 8-formyl-7-hydroxy-4-methylcoumarin and 3-chloro-8-formyl-7-hydroxy-4-methylcoumarin with 2,4-difluoroaniline and o-toluidine respectively. The synthesized compounds were characterized by spectral (IR, NMR, UV-visible, Mass, ESI-MS, ESR), thermal, fluorescence and molar conductivity studies. The bioassay of these synthesized compounds was also carried out.

2. Experimental

2.1 Reagents

All chemicals are of reagent grade, used without further purification.

2.2 Physical measurements

Carbon, hydrogen and nitrogen were estimated using TRUSPEC CHN analyzer. The IR spectra of the Schiff base and their Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the region 4000-400 cm^{-1} using KBr disc. The electronic spectra of the metal complexes were recorded in HPLC grade DMF solvent on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1100 nm. The ^{1}H and ^{13}C NMR spectra of Schiff bases were recorded in D_6-DMSO on a BRUKER 400 MHz spectrometer at room temperature using TMS as an internal reference. The fluorescence studies of Schiff base and their metal complexes were carried out on HITACHI F-7000 Fluorescence Spectrophotometer. Mass spectrum of the Schiff bases and electro spray mass spectra of metal complexes were recorded using QP 2010S and THERMO Finnegan LCQ Advantage max ion trap mass spectrometer respectively. The ESR spectrum of Cu(II) complex (**5**) was recorded on Varian-E-4X-band EPR spectrometer and the field set was 3000 G at modulation frequency of 100 KHz under liquid nitrogen temperature using TCNE as ‘g’ marker. Thermo gravimetric analyses data measured from a range of room temperature to 1000 $^{\circ}C$ at a heating rate of 10 $^{\circ}C/min$. The data obtained by using a PERKIN-ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements recorded on ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51 and magnetic moment was carried out using faraday balance for all the synthesized metal complexes.

2.3 Synthesis

2.3.1 Synthesis of 7-hydroxy-4-methylcoumarin

A mixture of dry resorcinol (0.2 mol) and ethylacetoacetate (0.2 mol) was cooled to 0-5 $^{\circ}C$ and Conc. sulphuric acid (25 mL) added gradually with constant shaking. The reaction mixture was then kept in a refrigerator for 24 h. and poured into crushed ice with stirring. The separated solid was filtered washed with water and recrystallized from ethanol as cream colored needles. Yield: 82%; Melting Point: 180-182 ºC.

2.3.2 Synthesis of 3-chloro-7-hydroxy-4-methylcoumarin

A mixture of dry resorcinol (0.2 mol) and ethyl-2-chloroacetoacetate (0.2 mol) was cooled to 0-5 ^{o}C and Conc. sulphuric acid (25 mL) added gradually with constant shaking. The reaction mixture was then kept in a refrigerator for 24 h. and poured into crushed ice with stirring. The separated solid was filtered and washed with water. Finally, recrystallized using ethanol to get a pure compound. Yield: 79%; Melting Point: 240-243 º C.

2.3.3 Synthesis of 8-formyl-7-hydroxy-4-methylcoumarin

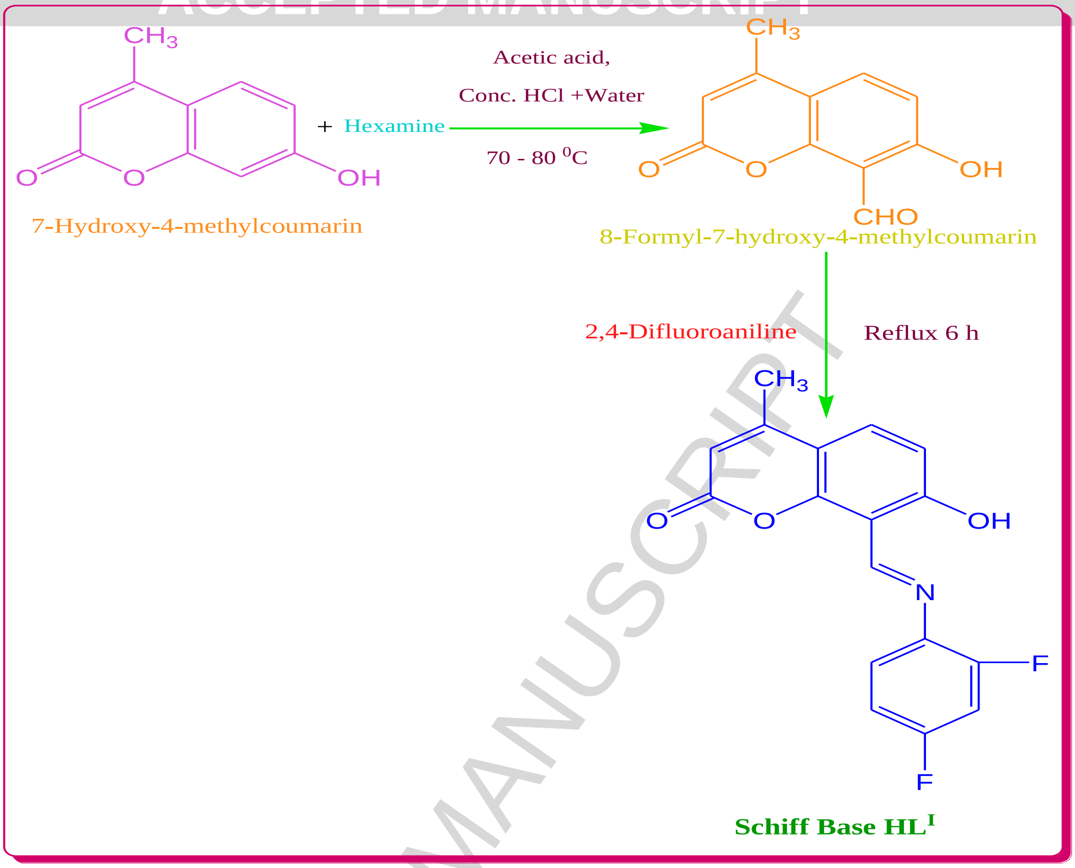
A mixture of 0.03 mol of 7-hydroxy-4-methylcoumarin and 0.07 mol of hexamine in 50 ml of glacial acetic acid were heated for 4-5 h and then 75 mL of 20% hydrochloric acid was added, heating was continued for 20 min. Resulting mixture was cooled and extracted with ether. Pale yellow solid of 8-formyl-7-hydroxy-4-methylcoumarin was obtained after evaporating the ether. Yield: 20.0 % ; Melting Point: 176-178 ºC.

2.3.4 Synthesis of 3-chloro-8-formyl-7-hydroxy-4-methylcoumarin

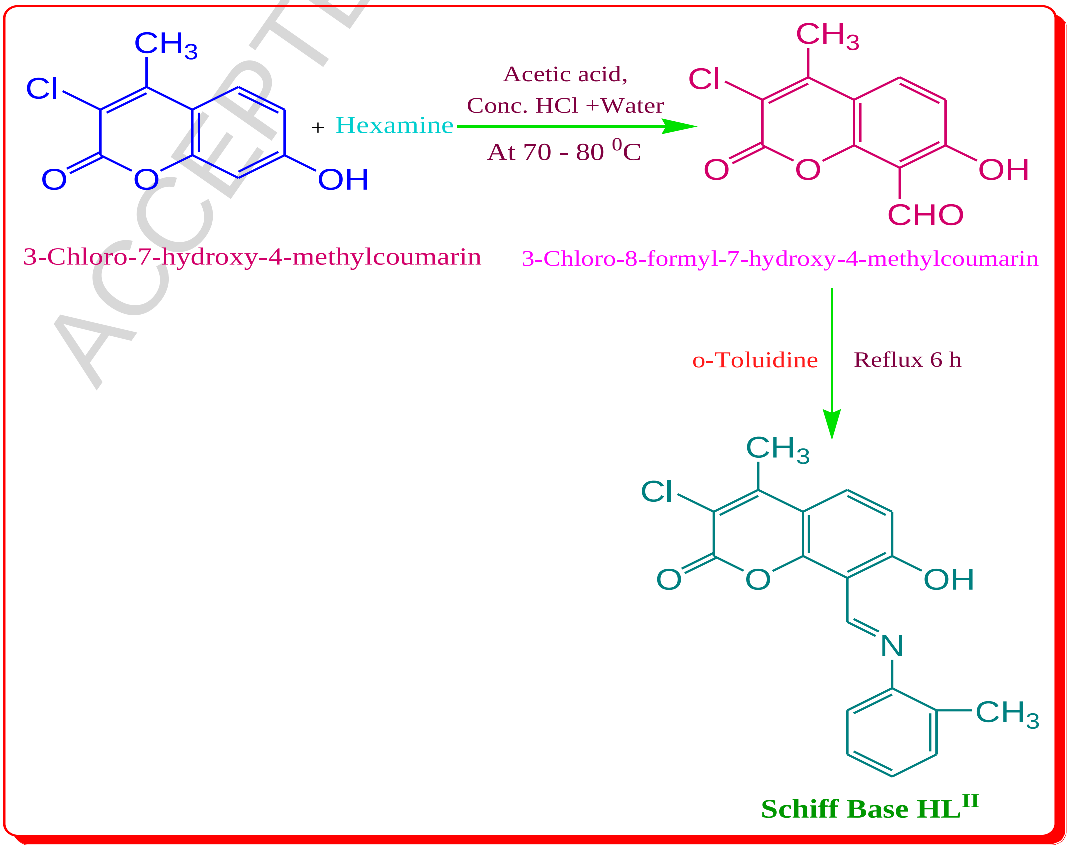
A mixture of 0.03 mol of 3-Chloro-7-hydroxy-4-methylcoumarin and 0.07 mol of hexamine in 50 ml of glacial acetic acid were heated for 4-5 hr and then dilute hydrochloric acid was added, heating was continued for 20 min. Cooled the resulting mixture and extracted with ether. Pale yellow solid of 3-Chloro-8-formyl-7-hydroxy-4-methylcoumarin was obtained after evaporating the ether. Yield: 25.0 %; Melting Point: 163-166 ^{o}C.

2.3.5 Synthesis of Schiff bases **HL^{I}** and **HL^{II}**

The steps involved in the synthesis of Schiff bases **HL^{I}** and **HL^{II}** are depicted in the **Scheme 1** (**a** and **b**). By refluxing the reaction mixture of hot ethanolic solutions of 2,4-difluoroaniline/o-toluidine with hot ethanolic solutions of 8-formyl-7-hydroxy-4-methylcoumarin/3-chloro-8-formyl-7-hydroxy-4-methylcoumarin respectively for about 6 h with addition of 1-2 drops of glacial acetic acid. The precipitate formed during refluxion was filtered, washed with cold ethanol and recrystallized with acetic acid.



Scheme 1(a). Synthesis of Schiff base **HL^{I}**.



Scheme 1(b). Synthesis of Schiff base **HL^{II}**.

2.3.5.1 NMR spectral study

The Schiff bases have been characterized by ^{1}H and ^{13}C NMR spectra to elucidate the differently positioned proton and carbon respectively. The ^{1}H NMR spectra of both the Schiff bases **HL^{I}** and **HL^{II}** are shown in **Figs. 1** and **2** respectively. The representative ^{13}C NMR spectrum of Schiff base **HL^{I}** is reproduced in **Fig. 3**.

In ^{1}H NMR spectrum of the **HL^{I}**, a singlet signal at 14.68 ppm is ascribed to phenolic -OH. A characteristic singlet proton signals at 9.35 ppm and 2.43 ppm are assigned to -CH=N and $-CH_3$ respectively. In addition to these, signals in the 6.29-7.90 ppm region are due to the aromatic protons. In case of **HL^{II}**, the singlet at 15.32, 9.21, 2.56 and 2.36 ppm are attributed to phenolic -OH, -CH=N, $-CH_3$ of o-toluidine, $-CH_3$ of coumarin respectively. The signals in the region 6.98-7.91 ppm are of aromatic protons.

The ^{13}C NMR spectrum of Schiff base **HL^{I}** shows a signal observed at 164.51 ppm is ascribed to lactonyl carbon (C=O) and a signal at 162.30 ppm is assigned to (C=N) group. Signal observed at 18.28 ppm is due to $-CH_3$ group. The characteristic signals in the region 104.63-162.19 ppm are assigned to aromatic carbons.

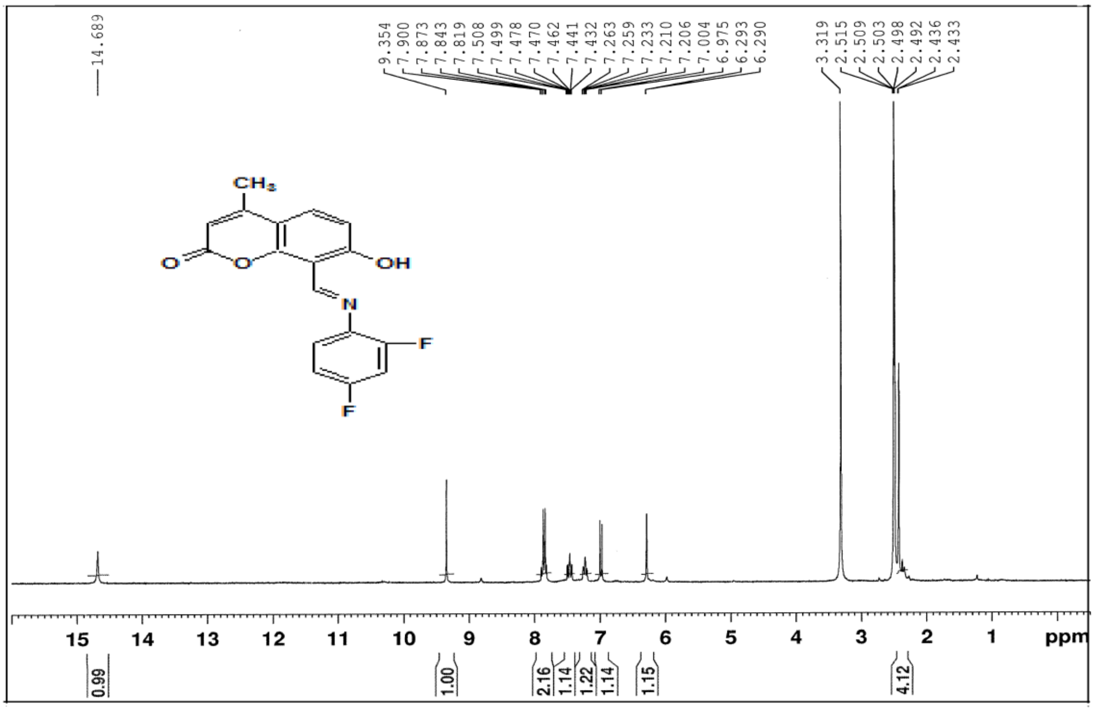


Fig. 1. ^{1}H NMR Spectrum of Schiff base **HL^{I}**.

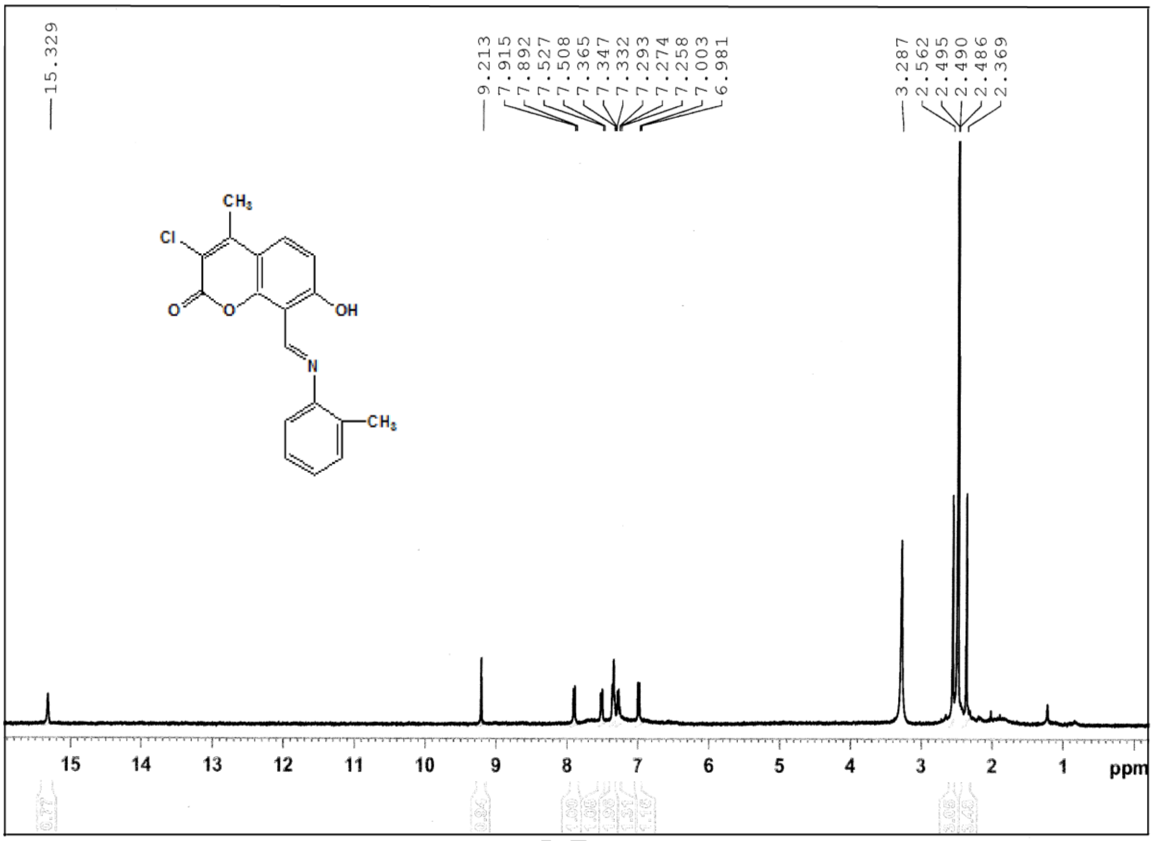
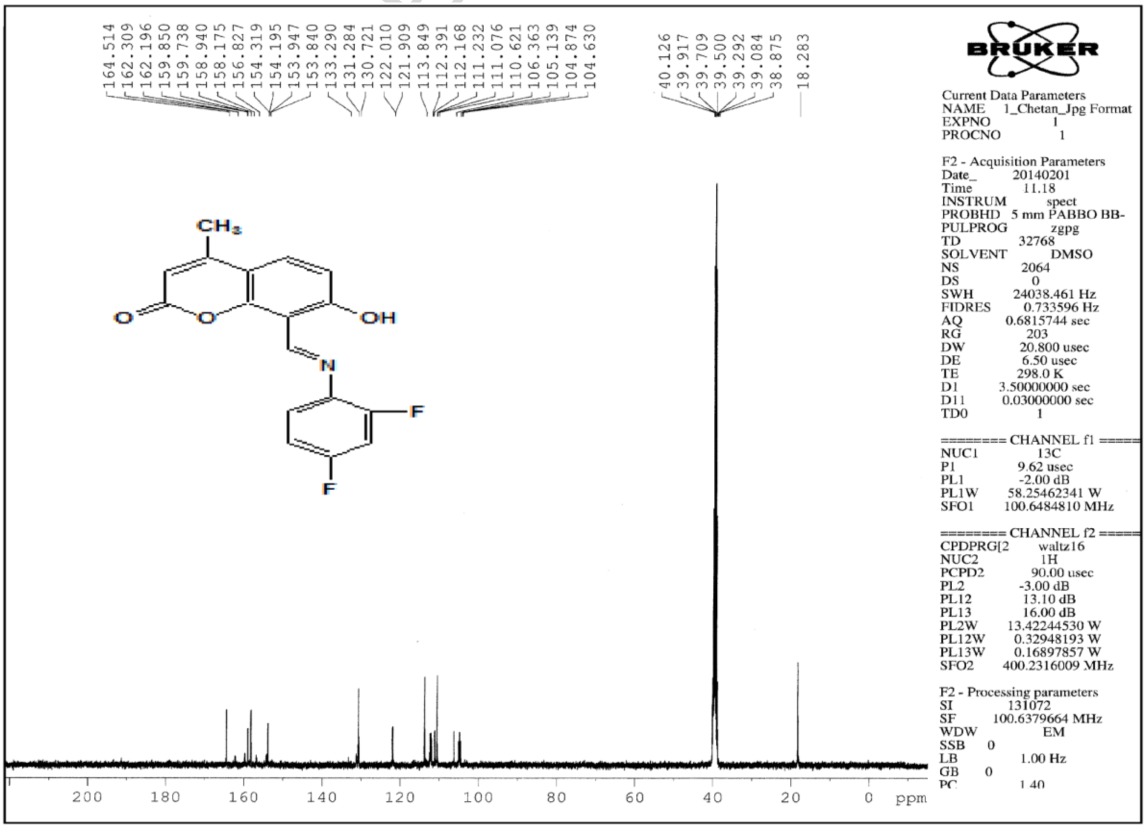


Fig. 2. ^{1}H NMR Spectrum of Schiff base **HL^{II}**.



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======== CHANNEL f2 =====
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LB              1.00 Hz
GB      0
PC              1.40
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Fig. 3. ^{13}C NMR Spectrum of Schiff base **HL^{I}**.

2.3.5.2 Mass spectral study

The mass spectrum **HL^{I}** of showed a molecular ion peak at *m/z* 315 which is equivalent to its molecular weight. In case of the molecular ion peak at *m/z* 327 is ascribed to $C_{18}H_{14}ClNO_3$. The fragmented peak at *m/z* 310 is assigned to the cleavage of -OH group. The representative mass spectrum of **HL^{II}** has been depicted in **Fig. 4**.

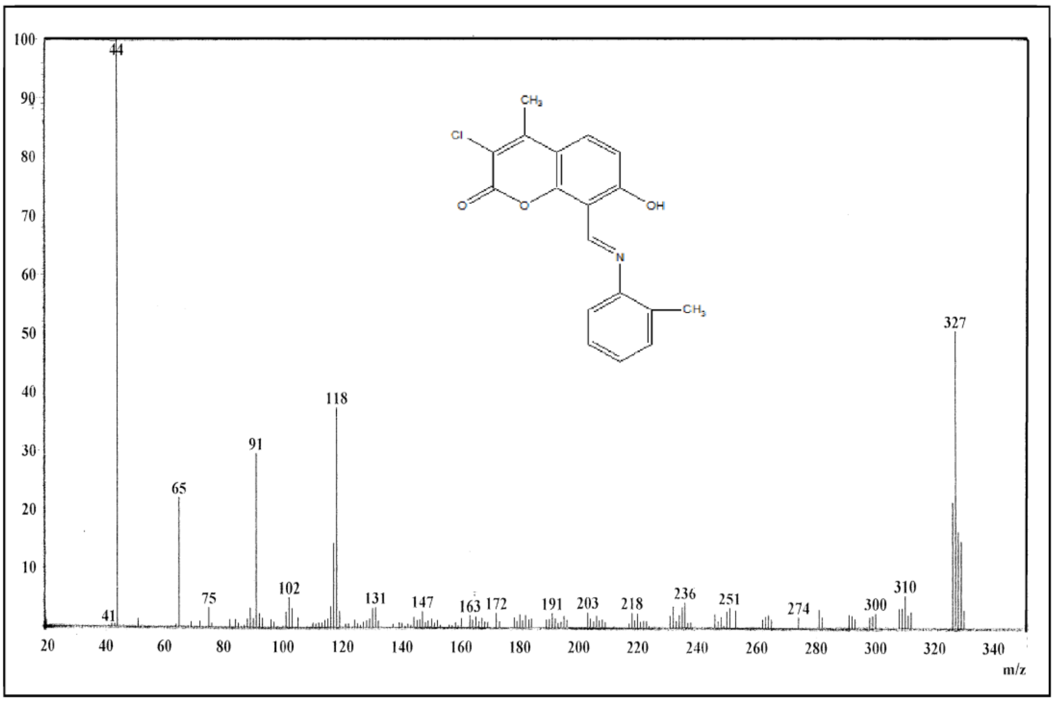

Fig. 4. Mass spectrum of Schiff base **HL^{II}**.

2.3.6 Synthesis of Co(II), Ni(II) and Cu(II) complexes (1-6)

An alcoholic solution of Schiff bases **HL^{I}** and **HL^{II}** (2 mmol) was refluxed with (1 mmol) of $CoCl_2.6H_2O/NiCl_2.6H_2O/CuCl_2.2H_2O$ on water bath for 1h. Then, to the reaction mixture 1mmol of sodium acetate was added and refluxion was continued for 3 h. The separated metal complex was filtered, washed thoroughly with water, ethanol and ether and then dried in vacuum over fused $CaCl_{2.}$

2.4 Pharmacology

2.4.1 *In vitro* antibacterial and antifungal activities

The biological activities of the newly synthesized Schiff base and their metal complexes were studied for their antibacterial and antifungal activities by disc diffusion method [24].

The antibacterial and antifungal activities were carried out at 200 μg/mL concentrations of metal complexes dissolved in DMF solvent, against four bacterial species (*Pseudomonas auregenosa* and *Proteus* *mirabilis*) and two fungal species *(Aspergillus niger* and *Rhizopus oryzae)*. Bacteria and fungi cultures were sub cultured on nutrient agar and potato dextrose agar medium respectively. Bacteria and fungi culture petridishes treated with synthesised Schiff base and their metal complexes were incubated for 24 h and 48 h at 30 ºC respectively. The standard Antibacterial drug (*Gentamicin*) and antifungal drug (*Fluconazole*) were also screened as explained above. The zone of inhibition for each fungi against respective organism was recorded in triplicate sets.

2.4.2 Anthelmintic activity

The assay was executed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological similarity with the intestinal roundworm parasite of human beings [25]. The anthelmintic assay was studied as per the method given in the literature [26] with minor modifications. The earthworms, *Pheretima posthuma* collected from moist soil, washed with normal saline (0.5%) for about 30 s to remove all faecal matter, were used for the anthelmintic study. The earthworms of 2-4 cm in length were used for all the experimental protocol. All the synthesized compounds were subjected to study anthelmintic activity against the earthworms at 2 and 10 µg/mL concentrations. DMSO in distilled water was used as control and Albendazole as a reference drug. The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was recorded by placing the earthworms in warm water (50 ºC) and observed for stimulated movement, if the worm was alive.

2.4.3 DNA Cleavage activity

The DNA cleavage activity was studied for the synthesised metal complexes of coumarin Schiff bases using Calf-thymus DNA (Purchased from Bangalore Genei, Bengaluru, Cat. No. 105850). The synthetic compounds were added separately to the Calf- thymus DNA, sample mixtures were incubated at 37 ºC for 2h. The cleaved product were run on agarose gel electrophoresis according to the procedure [27] and observed under UV transilluminator for DNA cleavage.

2.4.3.1 Agarose gel electrophoresis

In brief, 250mg of agarose was dissolved in 25 ml of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 L) by boiling. The warmed agarose was poured into the gel cassette fitted with comb. Later comb was removed carefully after the gel solidified and gel placed in electrophoresis chamber flooded with TAE buffer. 20μL of sample mixture, Calf-thymus DNA (mixed with bromophenol blue dye @ 1:1 ratio), were loaded carefully into individual wells, along with standard DNA marker and gel was run in electrophoresis chamber by passing constant 50 V of electricity for 45 min. Gel was then immersed in ETBR solution (10 μg/mL) for 10-15 min to stain and then observed for bands under UV transilluminator and gel band pictures were recorded. The cleavage ability of samples were compared with the calf-thymus DNA and standard DNA marker (λ DNA/ HindIII digest, purchased from Merck, Bengaluru).

3. Results and discussion

The Co(II), Ni(II) and Cu(II) complexes are non-hygroscopic coloured solids, stable at room temperature and soluble in DMF and DMSO. Carbon, hydrogen and nitrogen were estimated by using a C, H, N analyzer. The low conductance values confirm the non-electrolytic nature of all the metal complexes. Elemental analysis and molar conductance values are listed in **Table 1**.

3.1 Infrared studies

Infrared spectra of both the Schiff bases were studied. Characteristic high intense bands at 1623 and 1617 cm^{-1} for $\mathbf{HL^{I}}$ and $\mathbf{HL^{II}}$ respectively are attributed to the ν(C=N) vibrations [28]. The broad band at 3020 and 3026 cm^{-1} and a strong band at 1324 and 1320 cm^{-1} in the infrared spectra of $\mathbf{HL^{I}}$ and $\mathbf{HL^{II}}$ Schiff bases are assigned to -OH stretching and phenolic *ν*(C-O) vibrations respectively. Another strong band at 1720 and 1724 cm^{-1} is assigned to *ν*(C=O), lactonyl carbon of the coumarin moiety of $\mathbf{HL^{I}}$ and $\mathbf{HL^{II}}$ respectively [29]. Typical infrared spectrum of Schiff base $\mathbf{HL^{II}}$ is reproduced in **Fig. 5**.

In order to study the binding mode of Schiff base to metal ion in complexes, IR spectra of free ligands were compared with the spectra of metal complexes. Characteristic IR spectra assignments of all the synthesized metal complexes are compared with the corresponding free ligands. On comparison, the band positions of various vibrations are ascertained.

Table 1

Elemental analyses of Schiff bases [**HL^{I}** and **HL^{II}**] and their Co(II), Ni(II) and Cu(II) complexes along with their molar conductance and magnetic moment data.

Metal Complex	**Empirical Formula**	**Yield (%)**	**M%**		**C%**		**H%**		**N%**		**Molar Conductance (Ohm^{-1} cm^{2} mole^{-1})**	**μ_{eff} (BM)**
			Obsd.	**Calcd.**	**Obsd.**	**Calcd.**	**Obsd.**	**Calcd.**	**Obsd.**	**Calcd.**		
HL^{I}	$C_{17}H_{11}F_2NO_3$	82	--	--	64.73	64.76	3.50	3.52	4.43	4.44	--	--
HL^{II}	$C_{18}H_{14}ClNO_3$	78	--	--	65.95	65.96	4.33	4.31	4.28	4.27	--	--
1	$C_{34}H_{24}CoF_4N_2O_8$	56	8.13	8.15	54.39	54.44	3.40	3.34	3.81	3.87	18.10	4.32
2	$C_{36}H_{30}Cl_2CoN_2O_8$	50	7.90	7.87	57.73	57.77	4.02	4.04	3.72	3.74	22.32	4.54
3	$C_{34}H_{24}NiF_4N_2O_8$	65	8.14	8.12	54.45	54.46	3.35	3.34	3.85	3.87	18.36	3.19
4	$C_{36}H_{30}Cl_2NiN_2O_8$	49	7.88	7.84	57.80	57.79	4.06	4.04	3.70	3.74	12.57	3.15
5	$C_{34}H_{24}CuF_4N_2O_8$	47	8.69	8.73	56.13	56.09	3.33	3.32	3.83	3.85	28.45	1.79
6	$C_{36}H_{30}Cl_2CuN_2O_8$	69	7.40	7.38	57.47	57.42	4.05	4.02	3.74	3.72	27.34	1.77

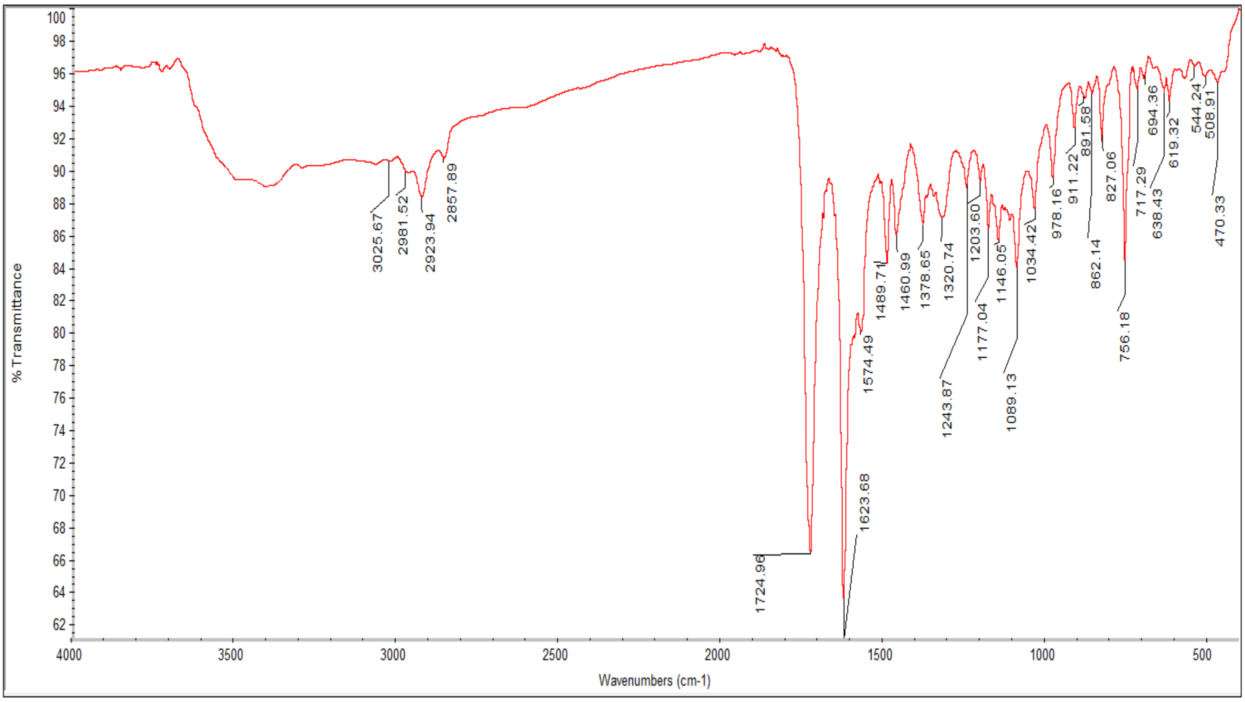

Fig. 5. Infrared spectrum of Schiff base **HL^{II}**.

The important infrared spectral data are presented in the **Table 2** and the representative IR spectrum of Cu(II) complex (**6**) is reproduced in **Fig. 6.**

The main features in infrared spectra of the complexes are as follows. Shift in the stretching frequencies of azomethine (-CH=N) groups to lower values is observed after the formation of transition metal complexes in the range 1602-1590 cm^{-1} compared with free ligand due to the coordination of azomethine nitrogen atom. Further, evidence of bonding was given by the new bands observed in the spectra of the metal complexes as a medium or weak intense in the region 467-415 cm^{-1} due to (M-N) stretching vibrations supporting the involvement of nitrogen atom of the azomethine group via coordination [30,31]. The new weak intensity bands of (M-O) stretching vibrations occur at 591-575 cm^{-1} [32]. The presence of coordinated water molecules in metal complexes are confirmed by a broad band in the region 3433-3449 cm^{-1} and two weak bands in the region 800-763 and 721-710 cm^{-1} due to *ν*(OH) rocking and wagging modes of vibrations, respectively [33,34]. High intensity bands observed around 1323 cm^{-1} in the Schiff bases are due to phenolic C-O, appeared as a medium to high intensity band in the region 1343-1326 cm^{-1} of the metal complexes. These observations suggest that, -OH groups have been replaced by the metal ion forming M-O bonds via deprotonation. Slightly altered position of ν(C=O) (lactonyl carbon) in all the metal complexes confirms its non involvement in coordination.

3.2 Fluorescence study of Schiff bases

The emission spectra of Schiff bases are studied in DMSO. Schiff base HL^I was characterized by an emission band around 480 nm is due to formation of phenoxide anion. Upon addition of 2-3 drops of aqueous alkali (2% NaOH) to Schiff base solution, we observed λ_{max} value at 476 nm in DMSO. The emission spectrum of HL^{II} in DMSO shows an emission band around 513 nm. On addition of 2-3 drops of aqueous alkali (2% NaOH) to the Schiff base solutions, we observed the λ_{max} value at 503 nm. The shift in λ_{max} values after addition of 2-3 drops of aqueous alkali (2% NaOH) to both the Schiff bases clearly indicates that, the proton transferred (H-bonded ion pair) species exist in equilibrium [35,36]. **Fig.7**, showing emission spectra of both the Schiff bases individually and overlapping after adding alkali.

3.3 Solution electronic spectra study

In order to obtain the information regarding stereochemistry of metal ions, UV-Visible spectra of the ligands and their metal complexes were recorded in DMF. The electronic absorption spectra are often very helpful in the evaluation of results furnished by other methods of structural investigation. The electronic spectral measurements were used to assign the stereochemistry of metal ions in complexes based on the positions and number of d–d transition peaks.

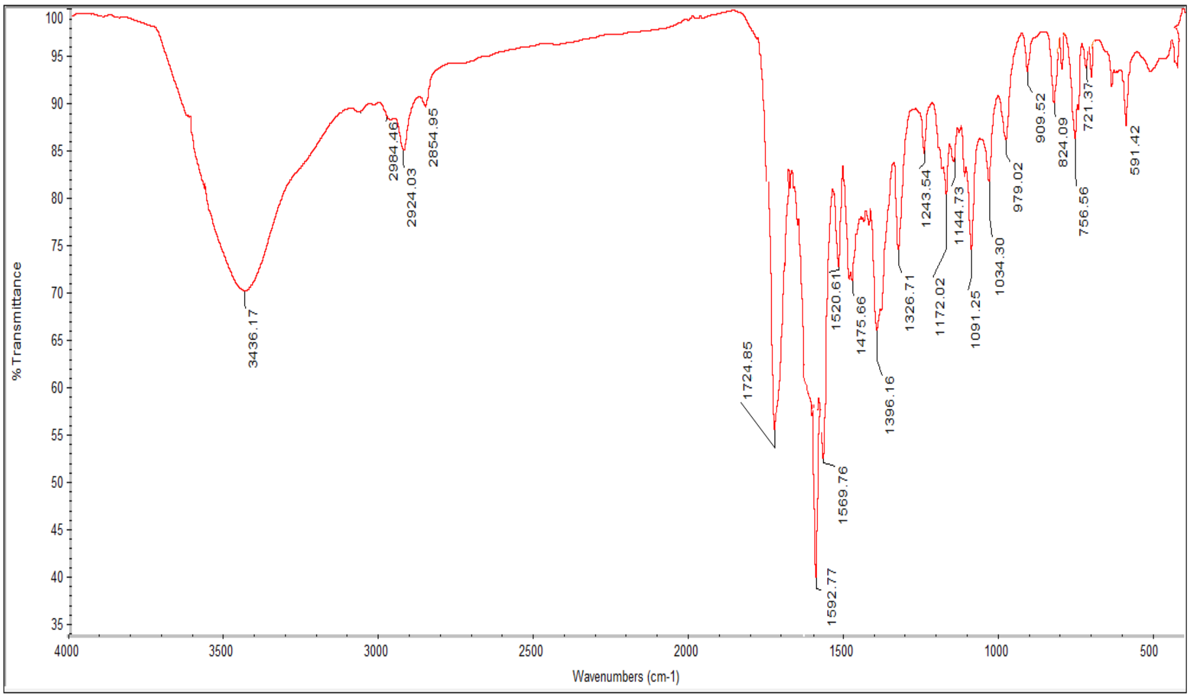

Fig.6. Infrared spectrum of Cu (II) complex (**6**).

Table 2

Prominent infrared frequencies (in cm^{-1}) of Schiff bases and their metal complexes.

Schiff Base / Metal Complex	**Coordinated Water *v*(OH)**	**Lactonyl *v*(C=O)**	***v*(C=N)**	***v*(OH) Stretching**	**Phenolic *v*(C-O)**	***v*(M-N)**	***v*(M-O)**
HL^{I}	--	1720	1617	3020	1326	-	-
HL^{II}	--	1724	1623	3026	1320	-	-
1	3443	1723	1599	-	1343	419	583
2	3445	1722	1590	-	1335	423	589
3	3439	1719	1592	-	1333	467	587
4	3449	1723	1597	-	1331	445	575
5	3433	1721	1591	-	1336	459	582
6	3436	1724	1602	-	1326	415	591

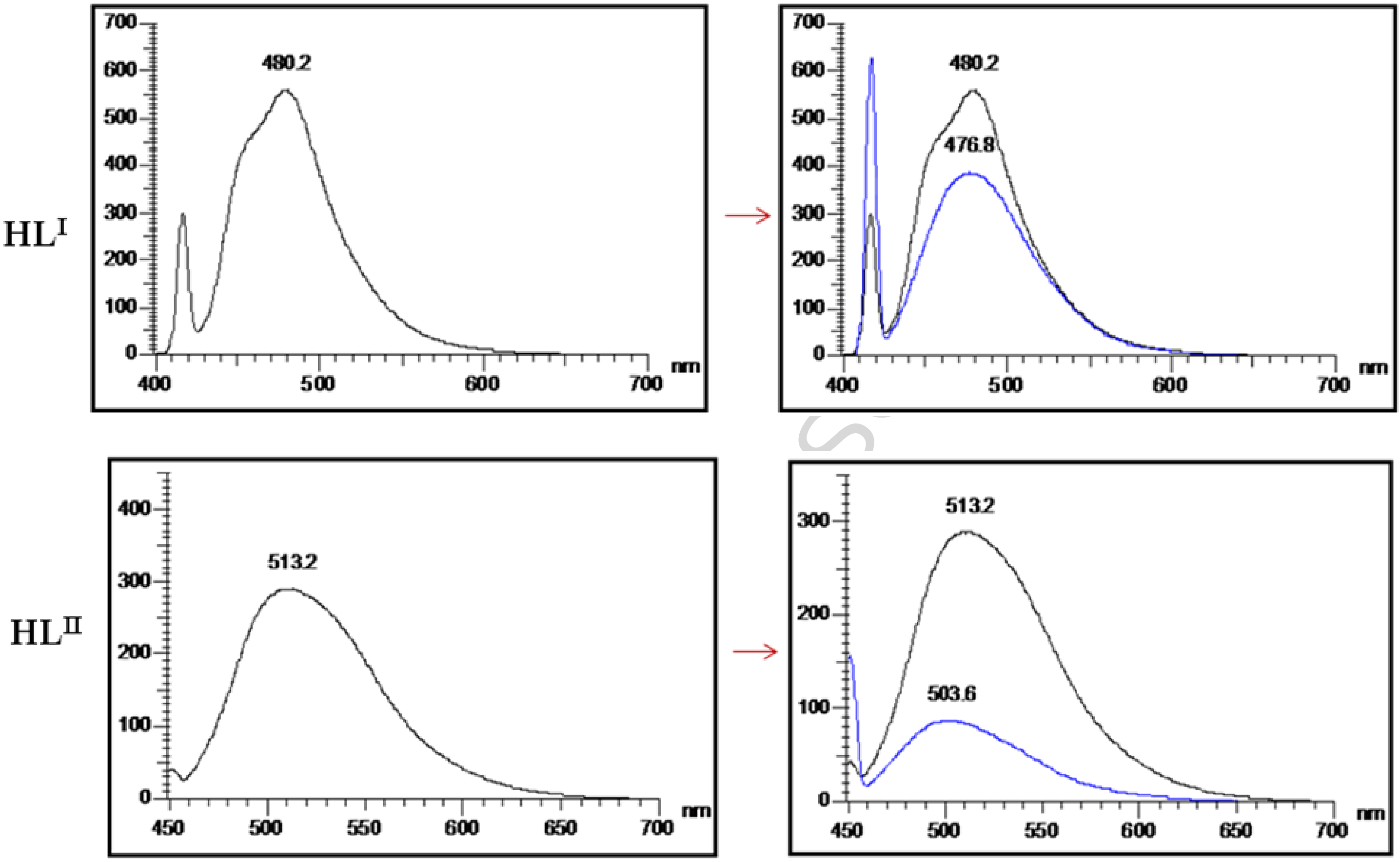

Fig. 7. Emission spectra of the Schiff bases **HL^{I}** and **HL^{II}** in DMSO.

The Schiff bases exhibited two absorption bands around 275 and 330 nm. Spectral data below 350 nm are ligand centred transitions (intraligand π-π* and n-π*) of aromatic ring and non-bonding electrons present on nitrogen of the azomethine group in the Schiff bases [37,38]. In the metal complexes imino π-π* transitions were shifted to longer wavelengths as a consequence of coordination to metal, conforming the formation of Schiff base metal complexes [39].

Solution electronic absorption spectra of all the synthesized metal complexes were recorded in DMF (**Table 3**). The electronic spectra of Co(II) complexes exhibited absorption bands around 1005 and 517 nm are corresponding to $^{4}T_{1g}$ (F) → $^{4}T_{2g}$ (F) (ν_1) and $^{4}T_{1g}$ (F) → $^{4}T_{1g}$ (P) (ν_3) transitions respectively. These bands are characteristic of high spin octahedral Co(II) complexes [40]. Observed magnetic moment values of Co(II) complexes were 4.32 and 4.54 BM and these results supports octahedral geometry for Co(II) complexes [41, 42].

The electronic spectra of present light green Ni(II) complexes exhibited three bands around 920, 601 and 372 nm, presumably due to the three spin-allowed transitions $^3A_{2g}$ (F) → $^3T_{2g}$ (F) (ν_1), $^3A_{2g}$ (F) → $^3T_{1g}$ (F) (ν_2) and $^3A_{2g}$ (F) → $^3T_{1g}$ (P) (ν_3) respectively, which indicate octahedral geometry around Ni(II) ion [43]. Magnetic moment values obtained were 3.15 and 3.19 BM, which are agreeable with the octahedral environment around Ni(II) ion [44,45].

The electronic spectra of Cu(II) complexes showed two absorption bands. A low intensity broad band around 627 nm is assignable to ^2E_g → $^2T_{2g}$ transition in support of distorted octahedral configuration of metal complex [46]. Another high intensity band around 370 nm are due to symmetry forbidden ligand → metal charge transfer. Obtained magnetic moment value for Cu(II) complexes were 1.77 and 1.79 BM, further these values support the electronic spectral results [47,48]. **Fig. 8** shows the representative solution electronic spectrum of Cu(II) complex (**6**).

Table 3

Solution electronic spectral results of the synthesized metal complexes

Metal Complex	**λ_{max} in cm^{-1} (nm)**	**Band Assignments**	**Geometry**
1	9984 (1001) 18970 (527)	$^4T_{1g}$(F)→ $^4T_{2g}$(F) (ν_1) $^4T_{1g}$ (F)→ $^4T_{1g}$ (P) (ν_3)	octahedral
2	9907 (1009) 19709 (507)	$^4T_{1g}$(F)→ $^4T_{2g}$(F) (ν_1) $^4T_{1g}$ (F)→ $^4T_{1g}$ (P) (ν_3)	octahedral
3	10910 (916) 16740 (597) 26860 (372)	$^3A_{2g}$(F)→$^3T_{2g}$(F) (ν_1) $^3A_{2g}$(F)→$^3T_{1g}$(F) (ν_2) $^3A_{2g}$(F)→$^3T_{1g}$(P) (ν_3)	octahedral
4	10810 (925) 16500 (606) 26750 (373)	$^3A_{2g}$(F)→$^3T_{2g}$(F) (ν_1) $^3A_{2g}$(F)→$^3T_{1g}$(F) (ν_2) $^3A_{2g}$(F)→$^3T_{1g}$(P) (ν_3)	octahedral
5	16021 (624) 26960 (370)	$^2T_{2g}$← ^2E_g L→M	distorted octahedral
6	16105 (631) 26901 (371)	$^2T_{2g}$← ^2E_g L→M	distorted octahedral

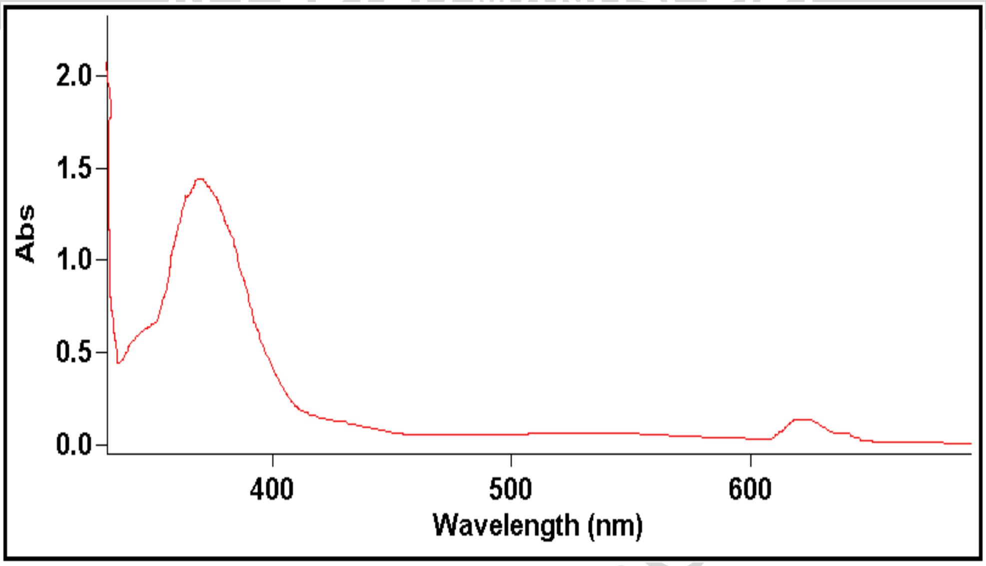

Fig. 8. Solution electronic absorption spectrum of Cu(II) complex (**6**).

3.4 ESI-MS studies of metal complexes

ESI mass spectra of all the synthesized complexes showed M+1 peaks, which are equivalent to their molecular weight, these facts support the formation of respective metal complexes of the type $ML_2(H_2O)_2$. The representative $Co(C_{17}H_{10}F_2NO_3)_2(H_2O)_2$ is shown in **Fig. 9**. Here, a spectrum showed M+1 peak at *m/z* 724 equivalent of its molecular weight, it is an evidence that supports the formation of respective metal complex of the type $ML_2(H_2O)_2$. All fragments of the species undergo demetallation to form ‘L’ gave a base peak at *m/z* 314**.**

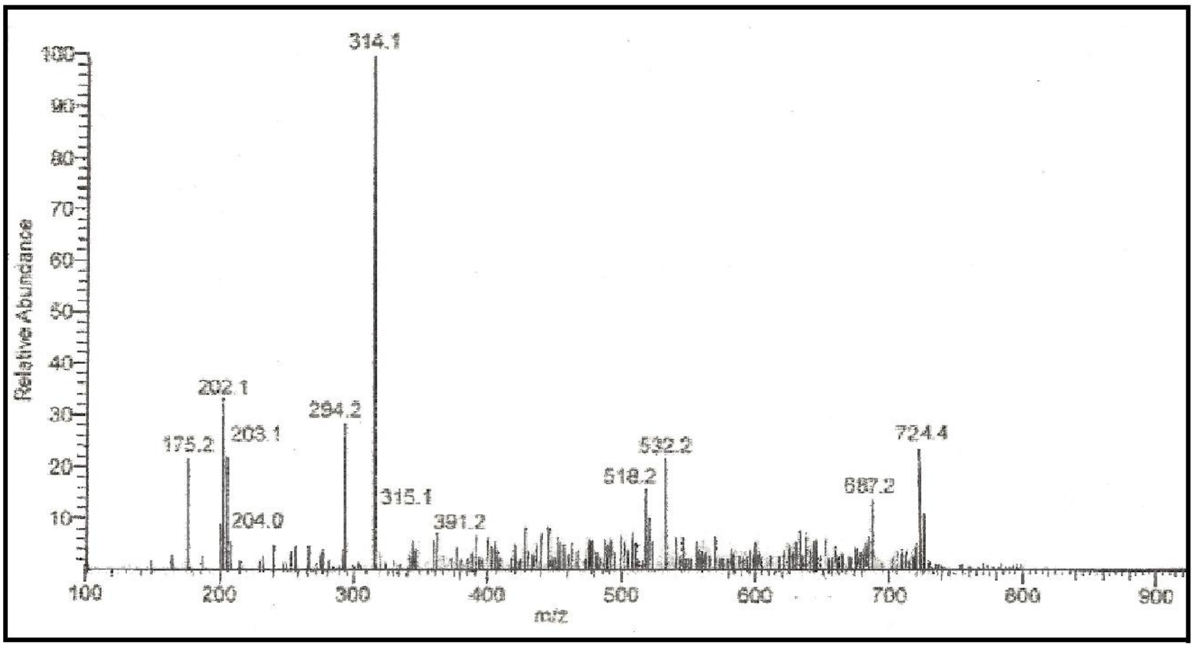

Fig. 9. ESI-MS of Co(II) complex (**1**).

3.5 Electron spin resonance

ESR spectra of metal chelates provide the important information, regarding the environment around metal ions. **Fig. 10,** shows the ESR spectrum of Cu(II) complex (**5**) at liquid nitrogen temperature using TCNE as g-marker (2.00277). From the figure, $g_{||}$ and $g_{\perp}$ values have been found to be 2.1230 and 2.0370 respectively. Here, $g_{||} > g_{\perp}$ indicate that, the unpaired electron predominantly lies in $d_{x^2-y^2}$ orbital. The g_{av} and G value calculated to be 2.0656 and 3.4783 respectively. G < 4 indicates there is an interaction in the metal complex. Observed $g_{||}$ value of the metal complex is less than 2.3 which is in agreement with the covalent character of metal-ligand bond.

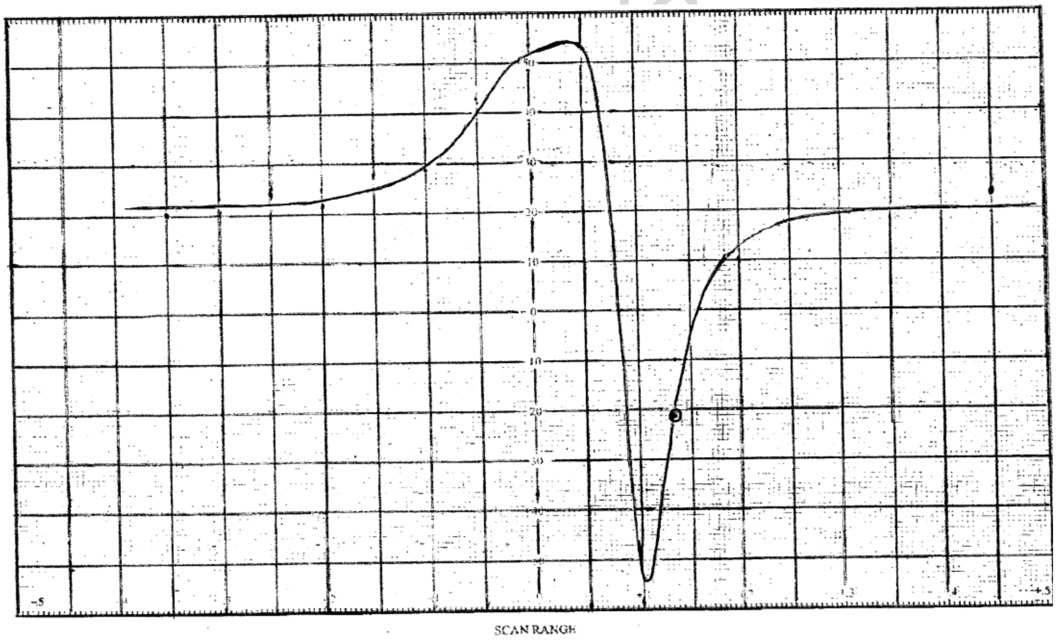

Fig. 10. ESR spectrum of Cu(II) complex (**5**).

3.6 Thermal studies of the metal complexes.

As a function of temperature, thermal behaviour of the synthesized Co(II), Ni(II) and Cu(II) complexes of Schiff base **HL^{II}** were studied. The nature of proposed chemical change, the percentage weight loss and the percentage of metal oxide obtained are tabulated in **Table 4**. It shows that, thermal features of all the metal complexes were almost same. Here, the representative TG/DTA spectrum (**Fig. 11**) of $Cu(C_{18}H_{13}ClNO_3)_2.(H_2O)_2$ is explained. First decomposition occured in the region 225-247 ºC centred at 236 ºC is due to loss of coordinated water molecules. Decomposition in the regions 310-440 ºC centred at 385 ºC and 440-800 ºC centred at 472 ºC corresponding to loss of organic moieties. Finally metal oxide was formed above 880 ºC.

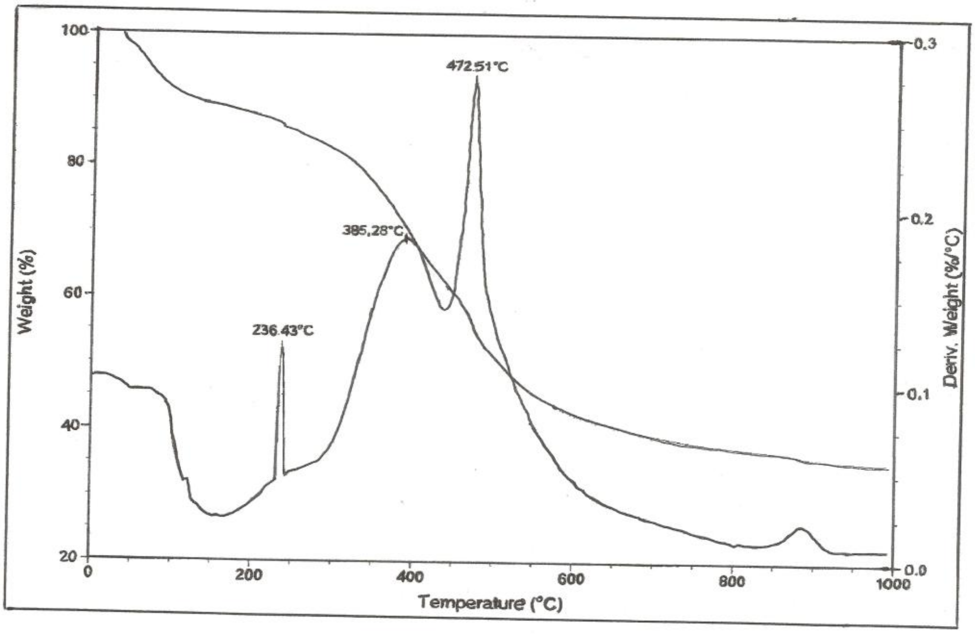

Fig. 11. TG/DTA spectrum of Cu(II) complex (**6**).

3.7 Electrochemical study

The electrochemical property of synthesized Cu(II) complex (**5**) was studied in order to monitor spectral and structural changes accompanying electron transfer. Here, cyclic voltammogram (**Fig. 12**) of the representative $Cu(C_{17}H_{10}F_2NO_3)_2.(H_2O)_2$ is discussed. It displays an oxidation peak at E_{pa}= 0.2101V with a corresponding reduction peak at E_{pc}= -0.0401V. The peak separation of this couple (ΔEp) is 0.17V at 100 mVs^{-1} suggested that Cu(II) / Cu(I) couple which is a one electron transfer. So, copper complex had a large separation between the cathodic to anodic peak, it increases with the scan rates, indicating a quasi-reversible character [49,50].

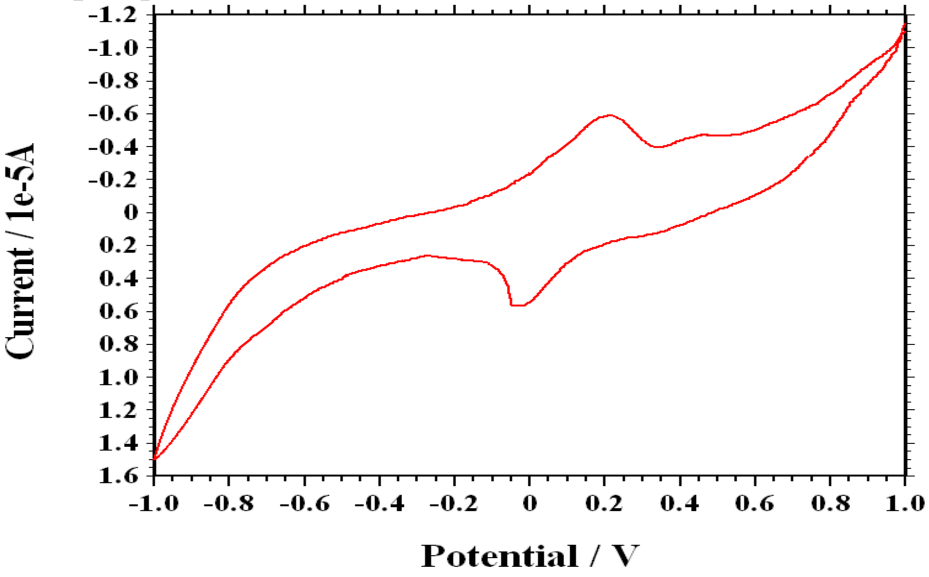

Fig. 12. Cyclic voltamogram of Cu(II) complex (**5**).

Table 4

Thermo gravimetric data of the metal complexes (**2**, **4** and **6**) of Schiff base **HL^{II}**.

Metal Complex	**Decomposition Temperature (°C)**	**Weight loss (%)**		**Metal oxide (%)**		**Inference**
		Obsd.	**Calcd.**	**Obsd.**	**Calcd.**	
2	215-238	4.83	4.82	10.00	10.04	Loss of coordinated water molecules
	325-789	85.17	85.14			Loss of organic moieties
4	220-248	4.84	4.83	9.89	9.92	Loss of coordinated water molecules
	318-782	85.27	85.25			Loss of organic moieties
6	225-247	4.82	4.79	10.51	10.52	Loss of coordinated water molecules
	310-800	84.67	84.69			Loss of organic moieties

3.8 Fluorescence study of Schiff bases **HL^I^** and its metal complexes

Fluorescence is a spectrochemical method of analysis where molecules of an analyte get excited by irradiation at a certain wavelength and emit the radiation of a different wavelength.

In this investigation, we have studied the fluorescent nature of the Schiff base **HL^{I}** and its metal complexes with same concentration of 10^{-3} M in DMF solution at room temperature. The overlapping spectra of Schiff base **HL^{I}** and its Co(II), Ni(II) and Cu(II) complexes in DMF is shown in **Fig. 13**. The Co(II), Ni(II) and Cu(II) complexes showed strong emission bands at 486, 481 and 484 nm respectively. Schiff base exhibits a strong fluorescence intensity compared to other three metal complexes, it shows an emission band at 480 nm. The weak intensity bands of metal complexes are due to decrease in electron density on ligand [51, 52]. The decreased fluorescence intensities of synthesized metal complexes were compared with **HL^{I}** is in the order Co(II) > Ni(II) > Cu(II). Here, Co(II) complex (**1**) exhibits the weakest quenching whereas, strongest quenching has been obtained for Cu(II) complex (**5**).

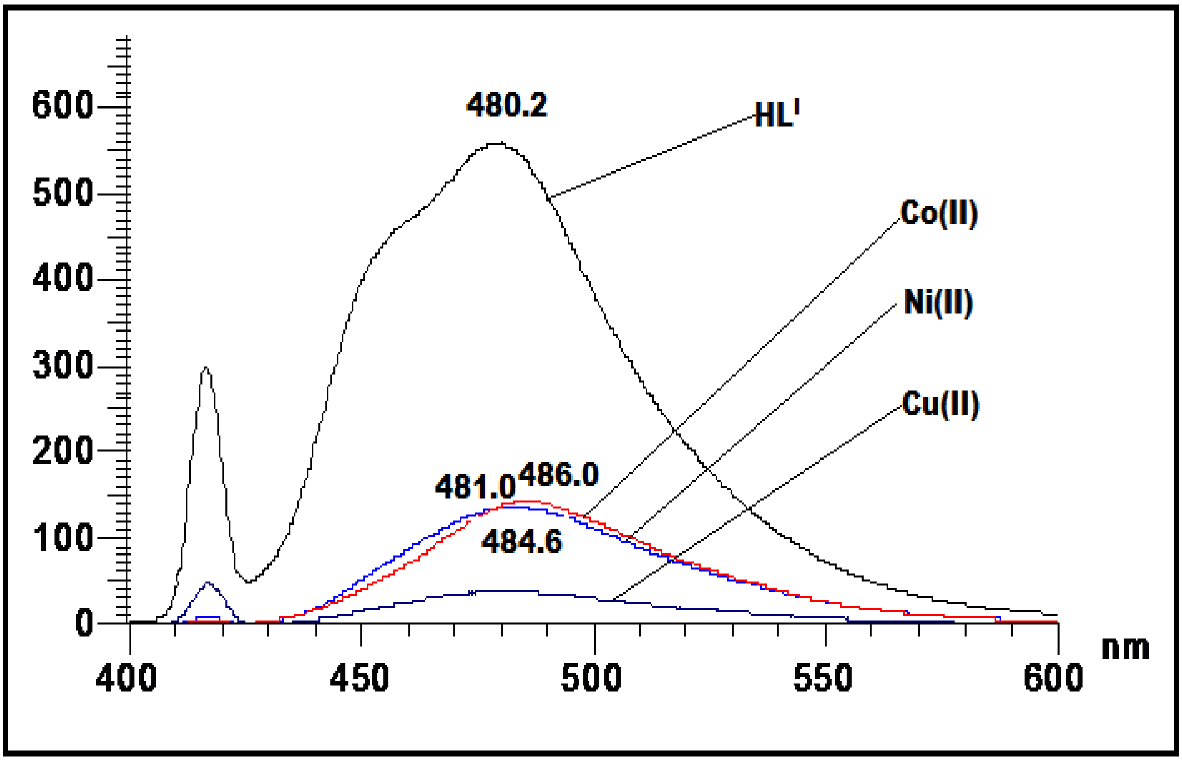

Fig. 13. Fluorescence study of the **HL^{I}** and its Co(II) (**1**), Ni(II) (**3**) and Cu(II) (**5**) complexes.

4.0 Bio-assay investigations

4.1 *In-vitro* antibacterial and antifungal activity

All the synthesized Schiff bases (**HL^{I}** and **HL^{II}**) and their corresponding Co(II), Ni(II) and Cu(II) complexes were screened for their antibacterial and antifungal activity by using two species of bacteria, namely *Pseudomonas aeruginosa, Proteus mirabilis* and two fungi species namely *Aspergillus niger, Rhizopus oryzae* using Gentamicin and Fluconazole as standard drugs respectively at 200 µg/mL concentration by disc diffusion method.

M.A. Phaniband *et al.*, in the year 2011, synthesised similar compounds and studied antimicrobial activities against two bacteria (*S. aureus* and *E. coli*) and two fungi (*A. niger* and *C. albicans*) species at 100 µg/mL concentration using Norfloxacin and Griseofulvin as standard drugs respectively. The results shows that, halogen substituted Cu(II) more complex showed zone of inhibition against *A. niger* than non halogenated one [53].

Copper has antimicrobial property. The importance of copper in biological system is reported with the use of Cu(II) Schiff base complexes. Copper(II) forms stable complexes with nitrogen and oxygen donor ligands [54-56]. Many of the other authors have showed that, Cu(II) complexes of the coumarin Schiff bases express more antimicrobial activity against various pathogens [57,58].

The obtained results are given in **Figs. 14** and **15**. A comparative study of ligands and their Co(II), Ni(II) and Cu(II) complexes indicates that, the metal complexes exhibit higher bio-activity than their free ligands. Such an increased activity of metal complexes can be explained on the basis of Overtone's and Tweedy's concepts [59,60]. According to the chelate hypothesis higher activity of metal chelates is due to the increased lipophilicity from inclusion of metal ion with Schiff base.

By observing the results, all synthesised metal complexes are comparatively active against four microbial species. The Cu(II) complexes showed higher bioactivity compare to Co(II) and Ni(II) complexes. While comparing the antifungal results of the newly synthesised Cu(II) complexes of both halogenated coumarin Schiff bases with previous studies, the present results have showed a significant activity against *Aspergillus niger* at 200 µg/mL.

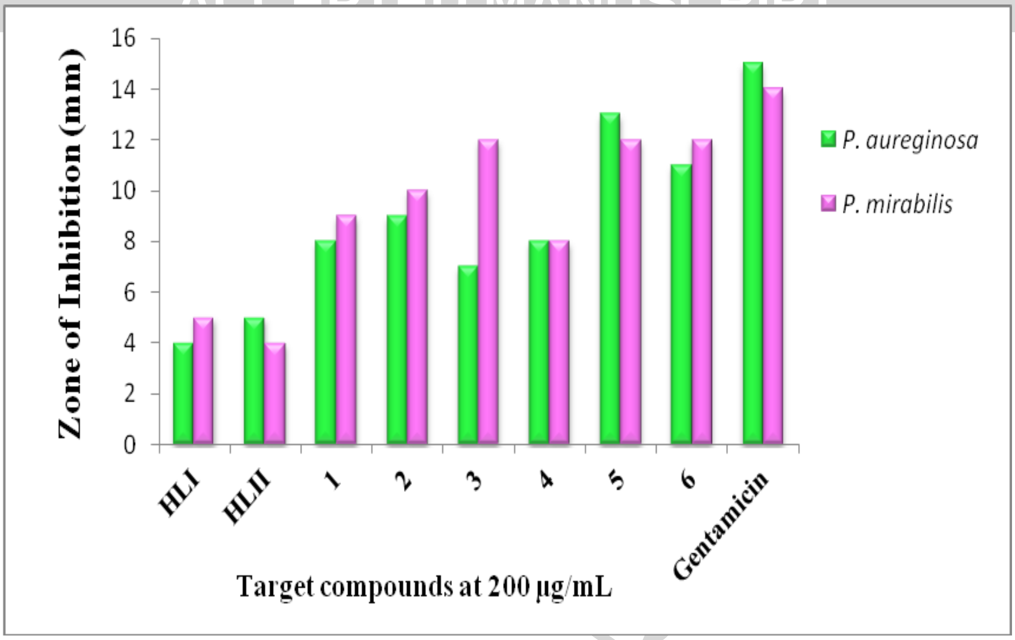

Fig. 14. Bacterial growth inhibition in millimetres (mm) for different compounds at 200 µg/mL concentration, Gentamicin used as reference compound.

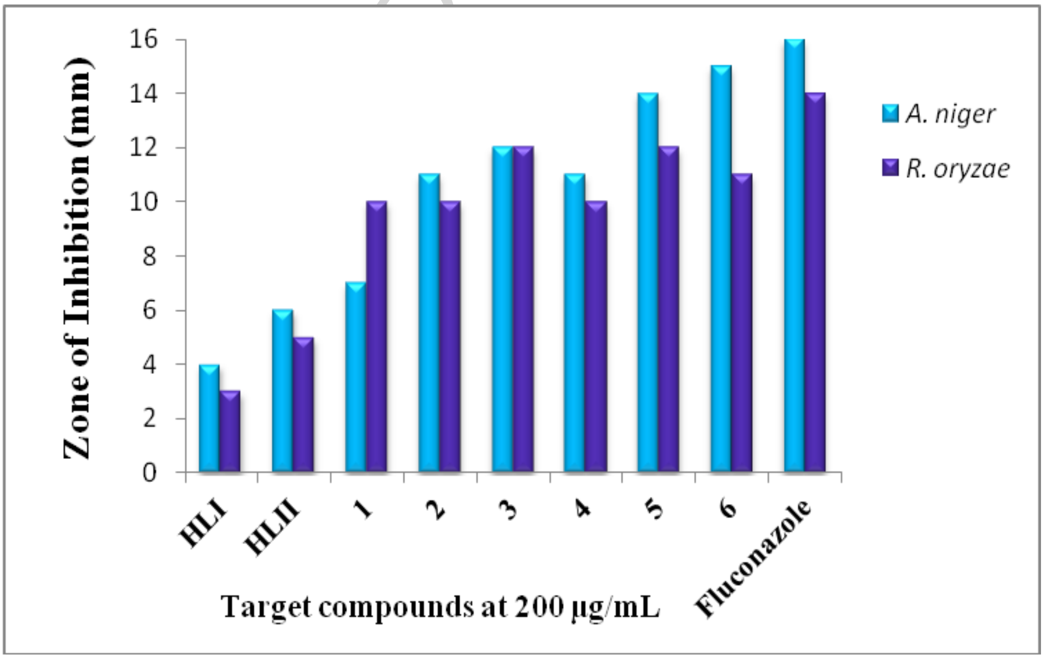

Fig. 15. Fungal growth inhibition in millimetres (mm) for different compounds at 200 µg/mL concentration, Fluconazole used as reference compound.

4.2 Anthelmintic activity

Anthelmintic activity data of the newly synthesized compounds are shown in **Table 5**. The obtained data shows that, the metal complexes have better anthelmintic activity compared to their respective Schiff bases **HL^{I}** and **HL^{II}** using Albendazole as standard drug at 10 µg/mL concentration.

In the previous study, M. Manjunatha et al.,[61] showed that, all the synthesized Schiff bases and their respective Co(II), Ni(II) and Cu(II) complexes have shown prominent anthelmintic activity at 10 µg/mL concentration using Albendazole as standard drug. Whereas, in our present study, both the copper (II) complexes have exhibited faster paralysis and death time of worms when compared to other synthesized compounds at 10 µg/mL concentration using the same standard drug.

Table 5. Anthelmintic studies of Schiff bases and their metal complexes

Schiff Base/ Metal Complex	**Concn. (µg/mL)**	**Time of Paralysis (min)**	**Time of Death (min)**
HL^{I}	2 10	19.06±0.04 7.03±0.03	21.40±0.12 10.19±0.01
HL^{II}	2 10	18.19±0.08 8.15±0.04	19.20±0.10 9.10±0.10
1	2 10	12.14±0.04 6.00±0.03	15.40±0.04 9.22±0.12
2	2 10	14.20±0.05 7.23±0.11	15.10±0.13 9.41±0.20
3	2 10	13.03±0.03 6.29±0.01	15.10±0.02 9.11±0.04
4	2 10	11.20±0.01 5.14±0.03	14.21±0.02 8.45±0.12
5	2 10	9.10±0.01 4.45±0.01	10.05±0.03 8.23±0.10
6	2 10	10.04±0.03 4.20±0.04	12.20±0.09 7.25±0.12
Albendazole (Std.)	10	3.38±0.03	6.35±0.05
DMSO*	--	--	--

* Used as control

4.3 DNA Cleavage activity

DNA cleavage may be considered as an enzymatic reaction which comprises various biological processes as well as biotechnological manipulation of genetic material. It has wide applications in molecular biology, bioorganic chemistry and drug design [62,63]. Metal containing reagents that induce chemical DNA scission are often referred to as artificial metallonucleases [64] and the attention focused towards the development of new metallonucleases, which bind and cleave DNA at physiological conditions, have gained momentum.

The metal ions play a crucial role in DNA cleavage activity because nature of the metal complexes may be attributed to additional π-π interaction through the aromatic phenyl rings [65]. The presence of different substituent's in the intercalative ligand could affect changes in space configuration and electron density distribution around transition metal complexes.

All the synthesized Co(II), Ni(II) and Cu(II) complexes of bidentate ligands were subjected to their DNA cleavage activity by gel electrophoresis method. Lane **1** and **6** i.e. metal complexes **1** and **6** showed complete DNA cleavage whereas metal complexes **2**, **3**, **4** and **5** cleaved partially as shown in the **Fig. 16**. The cleavage efficiency of metal complexes compared with control is due to their efficient DNA-binding ability [66]. In the year 2012, M.B Halli et al., have studied DNA cleavage activity for their newly synthesized M(II) compounds by agarose gel electrophoresis method against Calf-thymus DNA and obtained the similar kind of results [67].

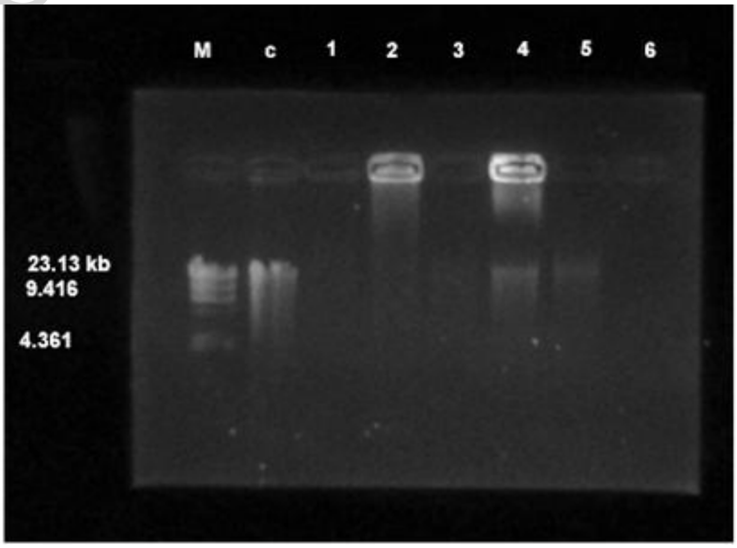

Fig. 16. DNA Cleavage activity of the synthesized metal complexes (**1-6**).

5.0 Conclusion

The newly synthesized Schiff bases act as bidentate ligands. The metal ion is coordinated through the azomethine nitrogen and oxygen atoms. The bonding of ligand to metal ion was confirmed by various spectral studies. Metal complexes exhibited promising antibacterial, antifungal and anthelmintic activities. The bioactivity significantly increased upon coordination. All these observations lead us to propose the following structures (**Figs. 17** and **18**) in which metal complexes having stoichiometry of the type $ML_2(H_2O)_2$ [M = Co(II), Ni(II) and Cu(II)].

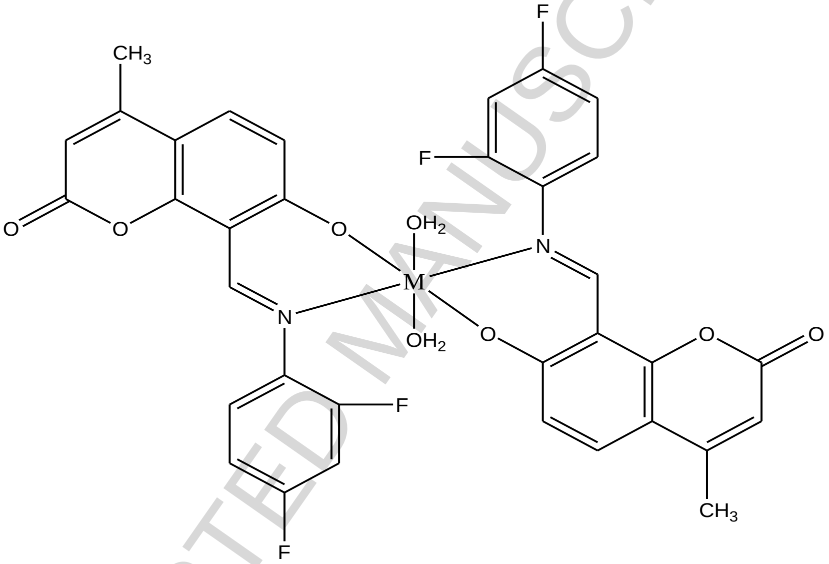

Fig. 17. Proposed structure of metal complexes (**1**, **3** and **5**) of Schiff base **HL^{I}**.

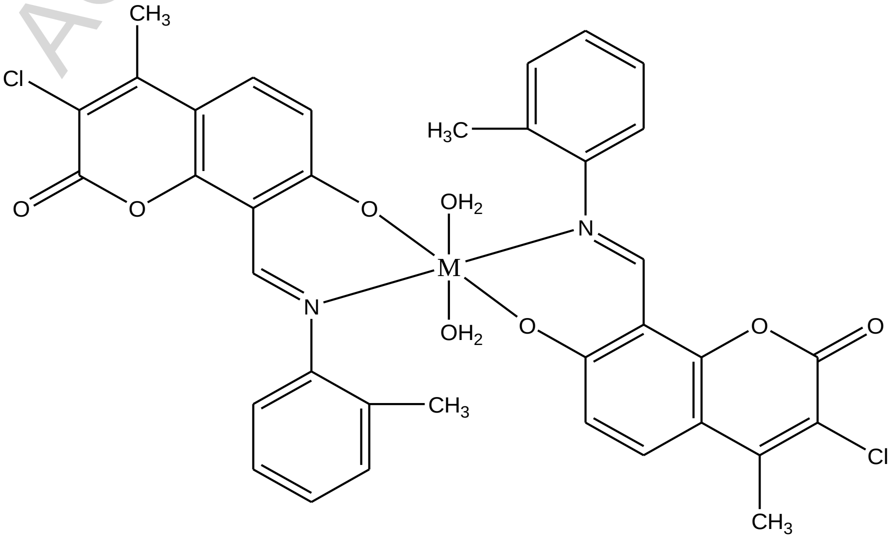

Fig.18. Proposed structure of metal complexes (**2**, **4** and **6**) of Schiff base **HL^{II}**.

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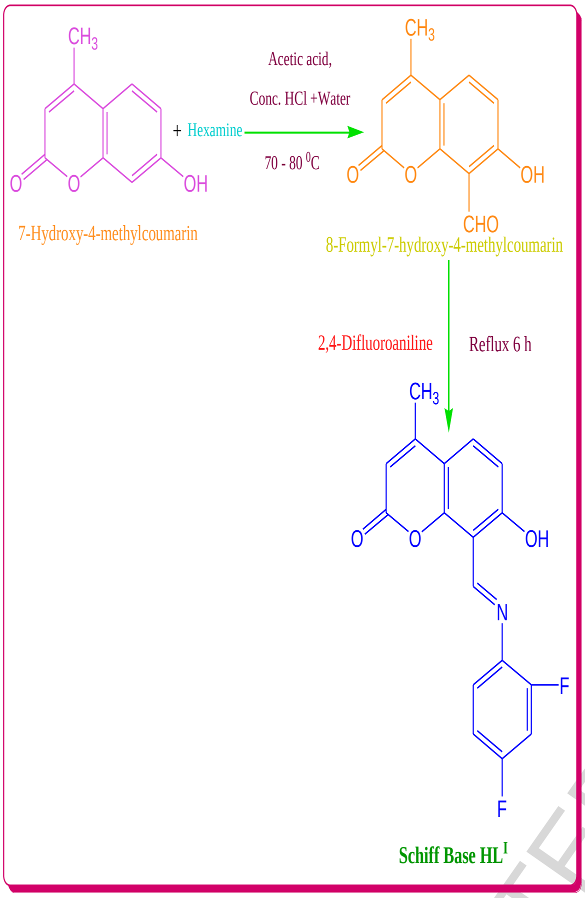
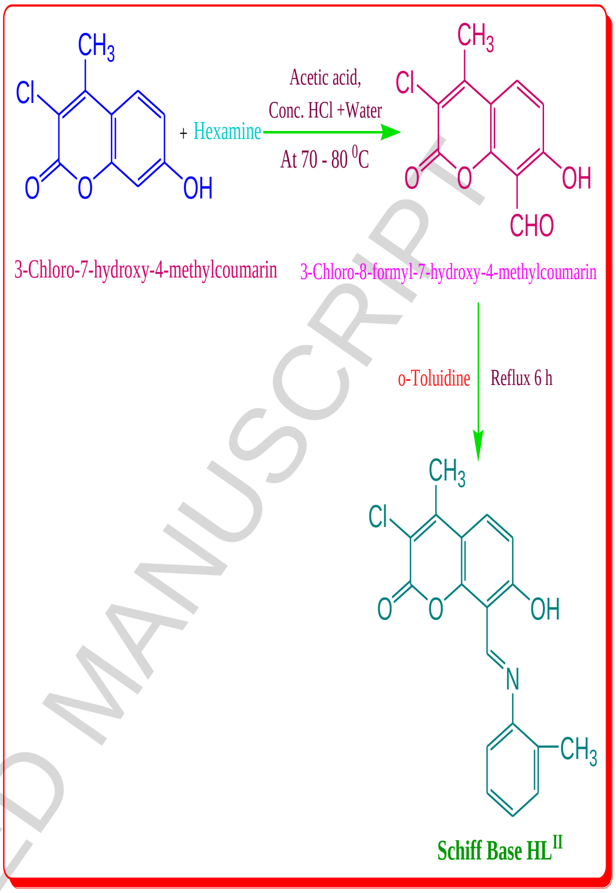
Graphical abstract : Synthesis of Schiff bases





Highlights

- Synthesis and depiction of halogenated coumarin Schiff base metal complexes.
- Characterization of synthesized compounds using various spectroscopic techniques.
- Confirmation of quenching property by fluorescence emission study.
- Cyclic voltammogram of Cu(II) complex suggests quasi-reversible one electron oxidation.
- The biological activity of metal complexes were significantly higher than the non-metal compounds.