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Thermal, physico-chemical, *in vitro* anti-microbial and DNA cleavage investigations of newly synthesized Co(II), Ni(II) and Cu(II) complexes of O, N donor Schiff base

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ABSTRACT

Cobalt(II), Nickel(II), Copper(II) complexes have been synthesized with newly synthesized biologically active ligand. The novel ligand has been synthesized by the condensation of 2-amino-4-methyl-5-carbethoxy-1,3-thiazole and 8-formyl-7-hydroxy-4-methylcoumarin. The probable structure of the complexes has been proposed on the basis of analytical and spectroscopic data (IR, UV-Vis, ESR, ESI-mass and TG-DTA). Electro chemical study of the complexes has also been recorded. The elemental analyses of the complexes confine to the stoichiometry of the type $ML_2.2H_2O$ *[M=Co(II), Ni(II) and Cu(II)]. The Schiff base and its metal(II) complexes have been screened for their antibacterial (Staphylococcus aureus (MRSA), Escherichia coli, Salmonella typhi*) and antifungal activity (*Aspergillus niger, Candida albicans, Aspergillus flavus*) by MIC method. The DNA cleavage is studied by agarose gel electrophoresis method.

Keywords: Synthesis; Thiazole; Coumarin; DNA Cleavage; Metal complex.

INTRODUCTION

The Schiff bases and their metal complexes are found to be of greater interest in coordination chemistry [1-3]. Various cobalt, nickel and copper metal complexes were reported to have antimicrobial activity [4-5]. Moreover, the enhancement in the biological activity and the decrease in the cytotoxicity of both metal ion and ligand can be achieved by the incorporation of transition metal into the Schiff bases [6]. The interaction of transition metal complexes with DNA have extensively been studied for their usage as probes for DNA structure and their potential application in chemotherapy [7-8]. One of the important DNA related activities of the transition metal complexes is that, some of the complexes show the ability to cleave DNA. Recently, Cu(II) complexes have been reported to be active in DNA strand scission [9-11].

Thermal analyses techniques play an important role in the phase of structural elucidation of metal complexes [12-14]. In particular, thermo-gram gives the ample information regarding coordination of H_2O and Cl, Br etc.

Thiazole heterocycles are present in numerous molecules of interest to the natural product, synthetic organic, agricultural, and medicinal chemistry communities. Examples comprise the Bistratamide natural products [15], the herbicide CMPT and antibiotic Thiostrepton. Thiazole and many substituted thiazoles possess interesting biological activities probably conferred to them by the strong aromaticity of their ring system [16] and also very interesting class of compounds because of their wide range of application as an antimicrobial [17], anti-inflammatory [18], anti-degeneratve [19] and anti-HIV activities [20]. In addition to the thiazole, coumarins and their derivatives portrayed the structural variety and significant biological and pharmacological properties as well. Many of these compounds possess antbacterial, antifungal and insecticidal activities. The hydroxy coumarins are typical phenolic compounds and therefore act as potent metal chelators and free radical scavengers. They are powerful chain-breaking anti-oxidants. Metal complexes of coumarin derivatives have extensively been investigated and reported from our laboratory.

MATERIALS AND METHODS

Instrumentation

All the chemicals purchased were of reagent grade and used without purification, TLC was performed on the TLC plates procured from Merk.

The IR spectra of the ligand and its Co(II), Ni(II) and Cu(II) complexes were recorded on a HITACHI-270 IR spectrophotometer in the 4000-250 cm^{-1} region in KBr disks. The electronic spectra of the complexes were recorded in DMSO on a VARIAN CARY 50-BIO UV-spectrophotometer in the region of 200-1100 nm. The ^1H NMR spectrum of ligand was recorded in DMSO-d_6 on BRUKER 400 MHz spectrometer at room temperature using TMS as an internal reference. ESI mass spectra were recorded on LCMS 2010, SHIMADZU, JAPAN. The mass spectrometer was operated in the +ve ion mode. The electrochemistry of Cu(II) complexes were recorded on CHI1110A-electrochemical (HCH Instruments) analyzer (Made in U.S.A). Thermo gravimetric analysis data were measured from room temperature to 1000°C at a heating rate of 10°C/min. The data were obtained by using a PERKIN-ELMER DIAMOND TG/DTA instrument. Molar conductivity measurements were recorded on a ELICO-CM-82 T conductivity bridge with a cell having cell constant 0.51 and magnetic moment of the complexes was carried out by using Faraday balance. Resorcinol was procured from Sigma Aldrich Chemical Company, further 7-hydroxy-4-methyl-coumarin, 8-formyl-7-hydroxy-4-methyl-coumarin and 2-amino-4-methyl-5-carbethoxythiazole were prepared as described in the literature [21-22].

Synthesis of 2-amino-4-methyl-5-carbethoxythiazole

Ethyl-2-chloro acetoacetate (0.1 mol.) and thiourea (0.1 mol.) are dissolved in ethanol and refluxed for 3-4 hrs. Then, the reaction mixture was cooled to room temperature and made alkaline with conc. ammonium hydroxide. The precipitated 2-amino-4-methyl-5-carbethoxythiozole was filtered and recrystallized from ethanol (Yield 80%, M.P. 175- 177 °C.)

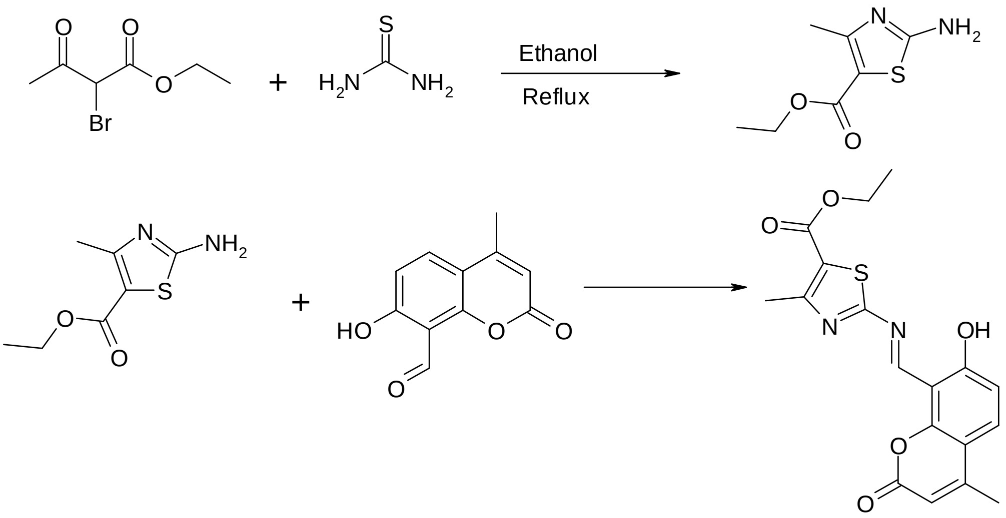


Fig. 1. Synthetic route of ligand

Synthesis of ligand

A mixture of 2-amino-4-methyl-5-carbethoxythiazole and 8-formyl-7-hydroxy-4-methylcoumarin in 1:1 molar proportions was boiled under reflux for 2-3 h in methanol as a medium containing few drops of concentrated HCl. The product was separated, filtered, washed with alcohol and recrystallized from ethanol. Thin layer chromatography (in 1:3 ratio eluent of ethyl acetate and hexane) revealed the presence of single pure Schiff base. (M.P.- 198- 199 ^oC). Flow chart of synthesis of ligand is shown in Fig. 1.

Synthesis of metal complexes

An alcoholic solution (25mL) of Schiff base (2 m mol.) was refluxed with 1 m mol of metal (II) chlorides [M= Co(II), Ni(II) and Cu(II)] in ethanol (25mL) on steam bath for 1h. Then, 2 m mol. of sodium acetate was added and refluxing was continued for another 3h. The separated complexes were filtered, washed thoroughly with water, ethanol and ether and finally dried in vacuum over fused $CaCl_2$.

RESULTS AND DISCUSSION

All the complexes are colored, stable in air and non-hygroscopic solids. All the complexes are insoluble in water, sparingly soluble in organic solvents and completely soluble in DMSO and DMF. The elemental analyses show that, the cobalt(II), nickel(II) and copper(II) complexes have 1:2 stoichiometry of the type $ML_2.2H_2O$. The molar conductance values are too low to account for any dissociation of the complexes in DMF. Hence, all the complexes are regarded as non-electrolytes in DMF. The metal contents were estimated gravimetrically by the standard methods [23]. Carbon, hydrogen, nitrogen and sulphur were estimated by C, H, and N analyzer. Analytical, magnetic and conductivity data of the complexes are depicted in Table-1.

Table- 1. Elemental analyses of ligand and its metal complexes along with magnetic moment and conductance data

Sl. No.	Compound	C (%)	H (%)	N(%)	S (%)	Metal	μ_{eff} in BM	Molar conductance
1	Ligand(LH)	58.103 (58.064)	4.323 (4.301)	7.633 (7.526)	8.637 (8.602)	----	----	
2	Co(II) Complex	51.899 (58.064)	4.232 (4.062)	6.703 (6.690)	7.901 (7.646)	7.241 (7.048)	4.93	4.3
3	Ni(II) Complex	51.901 (51.674)	4.223 (4.066)	6.599 (6.698)	7.932 (7.655)	6.890 (6.937)	3.29	4.9
4	Cu(II) Complex	52.579 (51.367)	4.443 (4.042)	6.873 (6.658)	7.799 (7.609)	7.452 (7.491)	1.72	3.5

Values given in the parenthesis are calculated values.

FTIR Spectral studies

Infrared frequencies along with their tentative assignments for the ligand and its complexes are presented in Table-2. The FTIR spectrum of ligand exhibits broad weak band in the region of 3053 cm^{-1} could be attributed to the intra-molecular hydrogen bonded –OH. The medium to high intensity band at 1632 cm^{-1} is assigned to ν(C=N) of azomethine group. Two ester groups, one from coumarin lactone has given peak at 1739 cm^{-1} due to ν(C=O) [24] and another carbonyl stretching of carbethoxy group of thiazole moity at 1707 cm^{-1}. The medium stretching band at 1097 cm^{-1} observed in free ligand is ascribed to ν(C-S-C) of thiazole ring [25]. High intensity band present at 1275 cm^{-1-} with an additional band around 1515 cm^{-1} region is due to stretching of phenolic C-O.

The observed changes, in case of Co(II), Ni(II) and Cu(II) complexes, are as under.

A broad and weak band in the region of 3053 cm^{-1}, assigned to H- bonded –OH in Schiff base remained disappeared in all the complexes. The high intensity band due to phenolic C-O, appeared in the region 1275cm^{-1} in the Schiff base, appeared as a medium to high intensity band around 1390 cm^{-1} in complexes. These comparative observations support the formation M-O bond via deprotonation. The band at 1515cm^{-1} in the Schiff base shifted to 1526 cm^{-1}, suggesting the behavior of phenolic oxygen as monodentate. The medium intensity band appeared at 1632 cm^{-1}, due to ν(C=N) in Schiff base, showed lower shift in the complexes. The lower shift in the ν(C=N) of Schiff base indicates that, the imine group is coordinated to the metal (II) ions through nitrogen atom. And is further confirmed by the γ(M-N) in the region of 473-460 cm^{-1}. The band located at 1739 cm^{-1} and 1707 cm^{-1} due to lactonyl γ(C=O) of coumarin moiety and carbethoxy ν(C=O) respectively of Schiff base remained unchanged in complexes, indicating the absence of their involvement in coordination. The same thing is with ν(C-S-C) indicating S atom is not involved in coordination. The presence of coordinated water molecules in the complexes are confirmed by the observed broad band in the region of 3433-3430 cm^{-1} [26] and two weaker bands in the region 836-833 cm^{-1} and 779-759 cm^{-1} due to ν(-OH) rocking and wagging mode of vibrations respectively [27].

Thus, the IR spectral data results provide strong evidences for the coordination of potential bidentate ligand with the metal (II) ions.

Table - 2. Important IR frequencies (in cm^{-1}) of ligand and its metal complexes

Sl. No.	Coordinated water γ(OH)	Lactonyl γ(C=O)	γ(C=N)	H bonded -OH stretching	Phenolic γ(C-O)	γ(M-N)
1	---	1739	1632	3053	1275	----
2	3430	1740	1598	----	1340	460
3	3430	1740	1593	----	1331	470
4	3432	1738	1599	----	1338	473

^{1}H NMR spectral study

The H^{1}NMR spectrum of Schiff base exhibits singlet at 11.89 ppm, 10.43 ppm and 6.28 ppm due to phenolic –OH, azomethine proton (CH=N) and C-3 proton of coumarin ring respectively. And another two singlets at 2.38 ppm to 2.49 ppm are due to methyl protons of coumarin and thiazole rings. Two doublets at 7.91-7.93 ppm and 6.95- 6.98 ppm are attributed to C-5 and C-6 protons of coumarin ring. The quartet and triplet peaks at 4.12- 4.18 ppm and 1.20- 1.25 ppm are assigned to methylene and methyl protons of carbethoxy group of thiazole ring respectively.

Electronic spectral studies

Electronic absorption spectra of all compounds were recorded in DMSO over the range 200- 1100 nm.

The electronic spectra of Co(II) complex shows two absorption bands, one at 549 nm (18213 cm^{-1}) with medium intensity due to $^4T_{1g}$ (F) → $^4T_{2g}$ (F) (ν_1), and another at 910 nm (10989 cm^{-1}) with low intensity due to $^4T_1$g (F) → $^4T_1$g (P) (ν_3), which are the characteristic bands of octahedral Co(II) complexes.

The Ni(II) complex showed three bands at 942 nm (10612 cm^{-1}), 612 nm (16326 cm^{-1}) and 377 nm (26476 cm^{-1}) attributed to the $^3A_{2g}$ →$^3T_{2g}$ (ν_1), $^3A_{2g}$ → $^3T_{1g}$ (F) (ν_2) and $^3A_{2g}$ → $^3T_{1g}$ (P) (ν_3) transitions respectively, which indicate the octahedral geometry around Ni(II) ion.

And Copper (II) complexes showed band in the 684 nm (14619 cm^{-1}) and 561 nm (17,825 cm^{-1}), regions which may be ascribed to 2B$_1$g→2Eg and 2B$_1$g→2B$_2$g transitions, respectively, corresponding to distorted octahedral geometry around Cu(II) ions.

Magnetic studies

The magnetic moments obtained at room temperature are listed in Table- 1. The Co(II) complex shows magnetic moment of 4.93 BM. This value is within the expected range of 4.7-5.2 [28-29] BM for octahedral complexes. Hence, the Co(II) complex has octahedral configuration. The Ni(II) complex shows magnetic moment of 3.29 BM. It is reported that, the octahedral Ni(II) complex exhibits magnetic moment in the range of 2.5-3.5 BM [30]. The Cu(II) complex shows magnetic moment 1.72 BM is slightly higher than the spin-only value, expected for one unpaired electron, which offers possibility of an octahedral geometry [31]. Magnetic moment values suggest the octahedral geometry around metal (II) ions.

ESR spectral studies

The ESR spectral studies of Cu(II) complex provide information regarding metal ion environment. The $g_{\parallel}$ and $g_{\perp}$ values have been found to be 2.1481 and 2.0404 respectively. The g_{av} value calculated comes out to be 2.0763. The trend, $g_{\parallel} > g_{\perp,}$ showed that the electron is localized in d_{x2-y2}.The g_{av} value was calculated to be 2.0763, this deviation g_{av} from that of free electron (2.0023) is due to the covalence property [32]. This is again supported by Kivelson and Neiman, where $g_{\parallel}$ of less than 2.3 indicates covalent environment. The parameter ‘G’, determined as G = ($g_{\parallel}$-2) / ($g_{\perp}$ -2) is found to be 3.6658 which is less than 4 suggesting the considerable interaction in the solid state. The ESR spectral data clearly suggests that, the Cu(II) complex has distorted octahedral geometry around it. The spectrum is showed in Fig. 2.

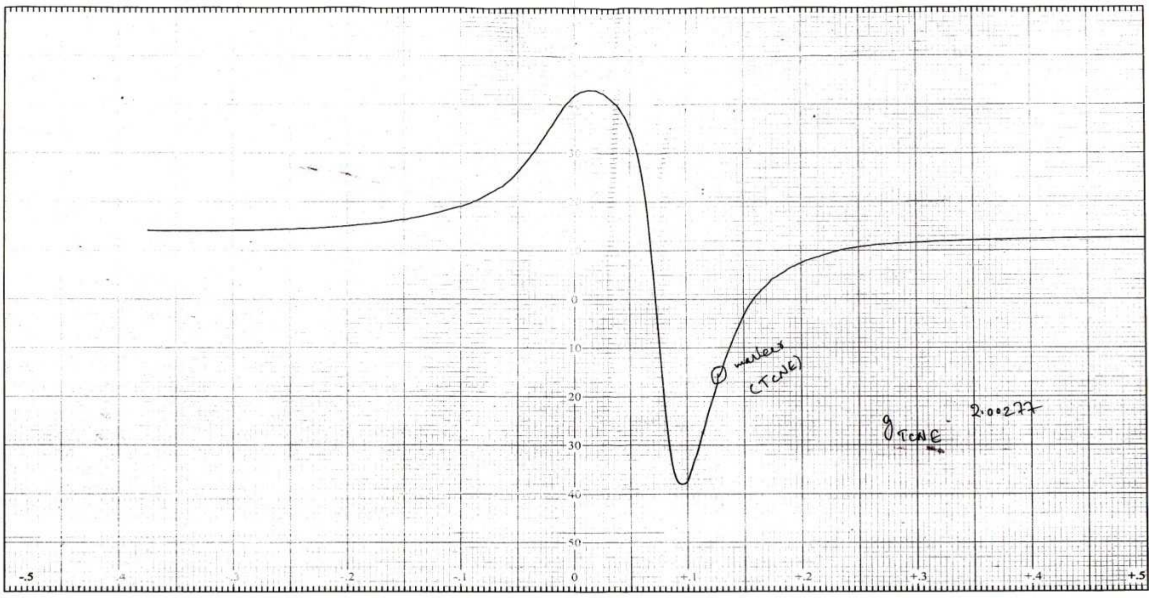


Fig. 2. ESR spectrum of Cu(II) complex

ESI- mass spectral study

The ESI- mass spectrum of Cu(II) complex, as a representative, has been studied and depicted in Fig. 3. The spectrum shows a molecular ion peak M^+ at m/z 841 which is equivalent to its molecular weight $[ML_2. 2H_2O]^+$. And the peak at 873 is due to the methanol adduct of complex molecule.

The same pattern has been observed in case of all the complexes. The ESI mass spectrum of Ni(II) and Co(II) complexes gave molecular ion peaks at m/z 836 and 837.So, the ESI- mass spectra of all the complexes provide one of the supportive data for the formation of octahedral complexes.

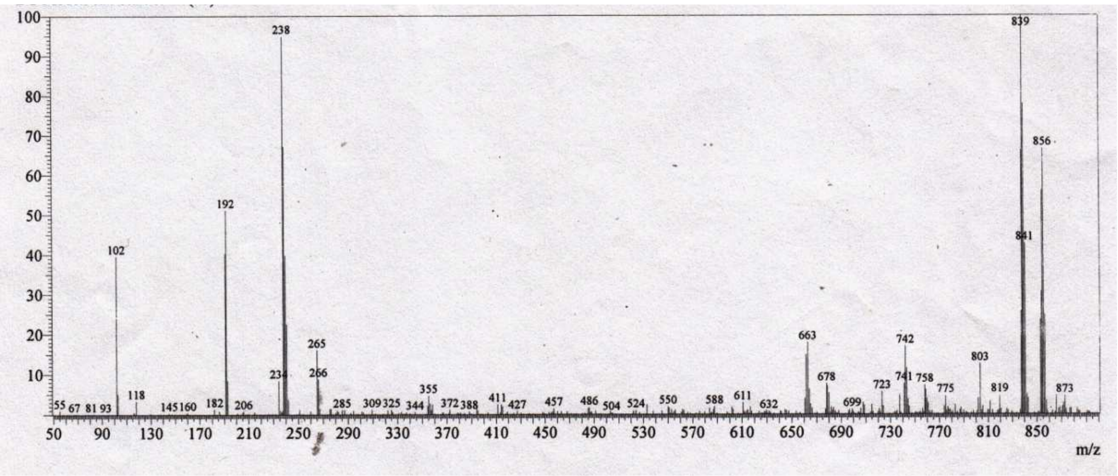


Fig. 3. ESI-mass spectrum of Cu(II) complex

Thermal study

All the prepared complexes were studied for their thermal behavior over the temperature range of 10-1000 °C under nitrogen atmosphere. The thermograms of Co(II), Ni(II) & Cu(II) complexes are shown in Fig. 4, 5 & 6. All the three metal complexes underwent decomposition in three considerable steps. As a representative, Co(II) complex is discussed in detail.

Step- 1. Mass loss in the first step, as depicted in the thermogram of Co(II) complex, is due to two water molecules coordinated to the metal ion in the temperature ranging from 150-200 ^{0}C. The peak is exothermic [33]. Empirically, it can be written as under.

$Co(C_{36}H_{30}N_4O_{10}S_2).2H_2O$ ----------> $Co(C_{36}H_{30}N_4O_{10}S_2)$-$2H_2O$

Step- 2. The exothermic peak in the temperature range of 260- 350 °C shows the decomposition of thiazole moieties from the ligand. The peak value centered at 275 °C. Empirically [34], it can be written as under.

$Co(C_{36}H_{30}N_4O_{10}S_2)$ ----------> $Co(C_{22}H_{14}O_6)$-$C_{14}H_{16}N_4O_4S_2$

Step- 3. The remaining coumarin part of ligand ligand underwent decomposition in the temperature ranging from 390- 490 °C and the exothermic peak value is centered at 468 °C [35]. And this decomposition can empirically be shown as under.

$Co(C_{22}H_{14}O_6).2H_2O$ ----------> Metal oxide-$C_{14}H_{16}N_4O_4S_2$

TG-DTA curves of Ni(II) complex show weight losses in three steps. In the first step, in the temperature range 155-180 °C is attributed to the combined mass loss of one lattice celled two coordinated water molecules. The mass loss at second step is observed in the temperature range 350- 370 °C is attributed to the loss of thiazole moiety. The mass loss at third step at 399 °C is attributed to the elimination of coumarin moiety. Thus, the TG/DTA study of complexes gave strongest evidence in the elucidation of geometry of metal complexes.

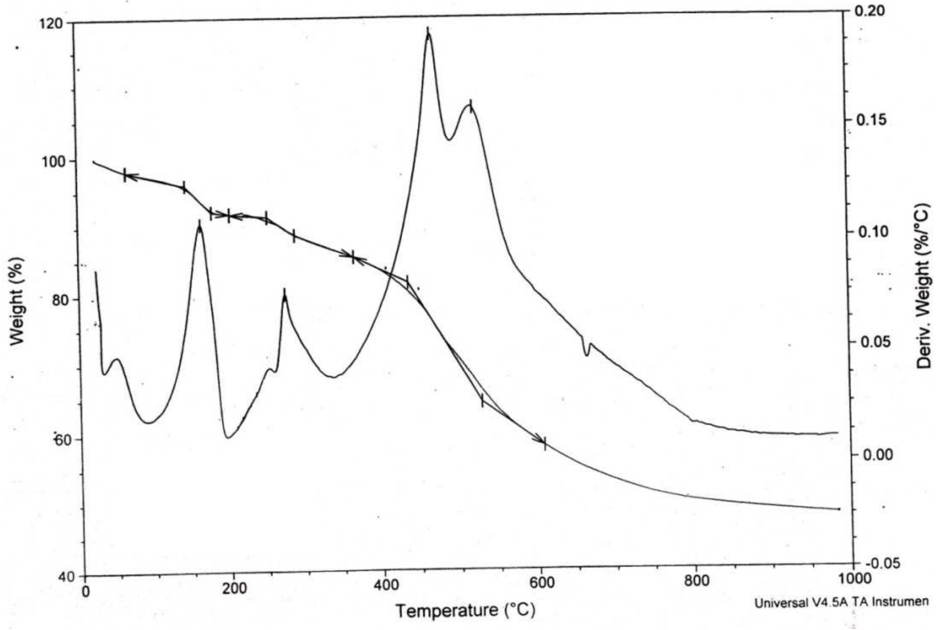


Fig. 4. Thermogram ofCo(II) complex

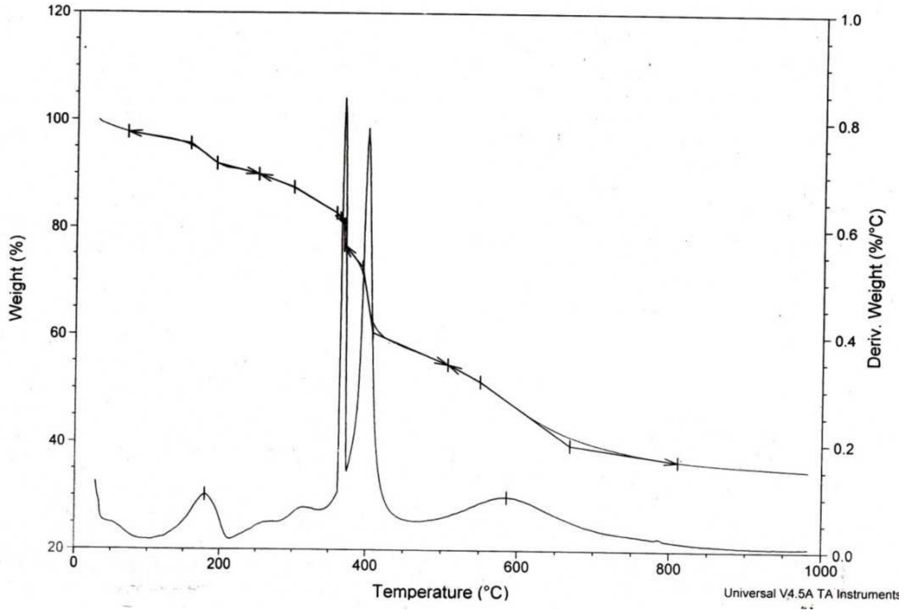


Fig. 5. Thermogram of Ni(II) complex

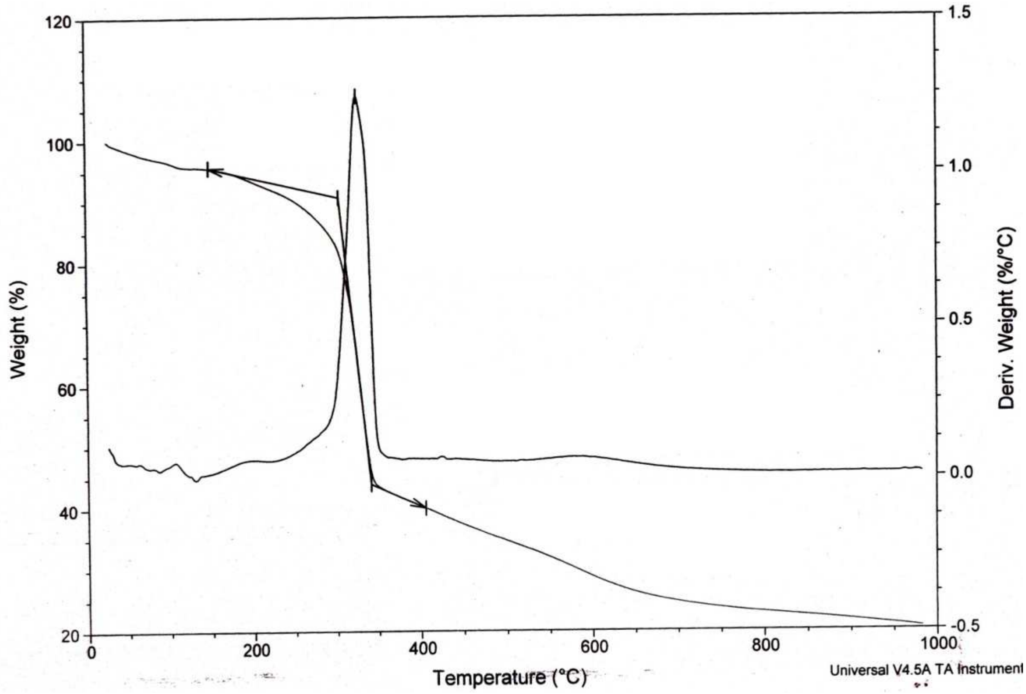


Fig. 6. Thermogram of Cu(II) complex

Electrochemistry

Electrochemical properties of the complexes were studied in dimethyl sulfoxide (DMSO) containing 0.05 M n-Bu_4NClO_4 as the supporting electrolyte.

The ligand was found to be electro-inactive, hence it is called is an ‘‘innocent’’ ligand. And Cu(II) complex, in its cyclic voltammogram, has displayed a reduction peak at E_{pc} = 0.5831 V with a corresponding oxidation peak at E_{pa} = 0.3119 V. The peak separation of this couple (ΔE_p) is 0.2712 V at 0.1V and increases with scan rate.

Fluorescence study

The emission spectra of the ligand and its Co(II), Ni(II) and Cu(II) complexes were investigated in DMSO and reported in Fig 7.

The ligand was characterized by an emission band around 500 nm. The Co(II) complex has shown an emission band at 452 nm, Ni(II) complex at 467 nm and Cu(II) complex at 449 nm. In case of complexes, the intensity of the emission band is decreased. This decrease in the intensity of the emission is due to the quenching property of metal ions.

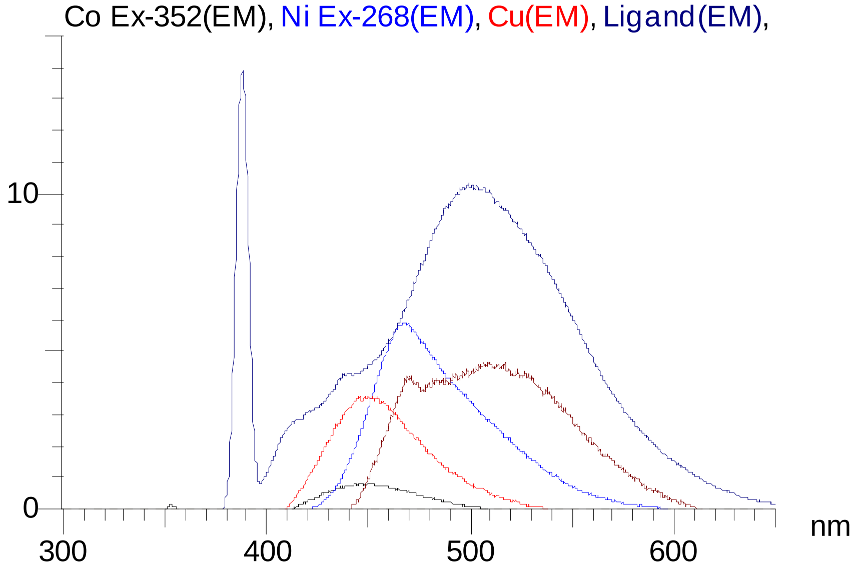


Fig. 7. Fluorescence spectra of ligand and its metal complexes in DMF solution

RESULTS

In vitro antimicrobial activity

Many authors have studied [36-38] biological properties of transition metal compounds of coumarin derivatives.

Antimicrobial results of ligand and its metal complexes are presented in Table- 3. Schiff base showed some activity against *Staphylococcus aureus (MRSA), Escherichia coli, Salmonella typhi.* We could notice the enhancement in the activity when it is stitched with Co(II), Ni(II) and Cu(II). Cu(II) complex, in particular, has showed better activity against both bacteria and fungi. This higher antimicrobial activity of metal complexes, compared to ligand, is probably due to change in structure due to coordination and chelating tends to make metal complexes act as more potent and powerful bacteriostatic agent. Thus, inhibiting growth of micro-organisms [39].

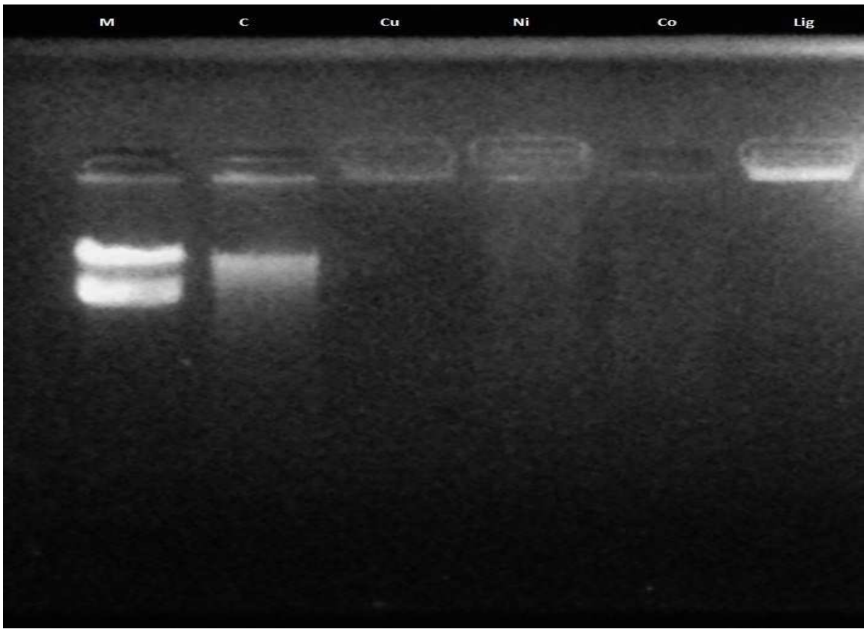
DNA cleavage

Fig. 8 shows results of oxidative DNA cleavage experiments were carried out with the ligand and its metal complexes. Control experiments categorically revealed that, untreated DNA does not show any cleavage (Fig.8, Lane C), whereas all the metal complexes exhibited cleavage activity on DNA.

The difference in the migration is observed in all the lanes of our synthesized compounds compared to the control DNA of E. coli (Lane C). This shows the control DNA alone does not show any apparent cleavage, whereas Co(II), Ni(II) and Cu(II) complexes have shown. The results indicated the important role of metal ions in isolated DNA cleavage reaction. From these results, we infer that Co(II), Ni(II) and Cu(II) complexes act as potent nuclease agent. And with this, we can conclude that, the compounds inhibit the growth of pathogens by cleaving the genome.

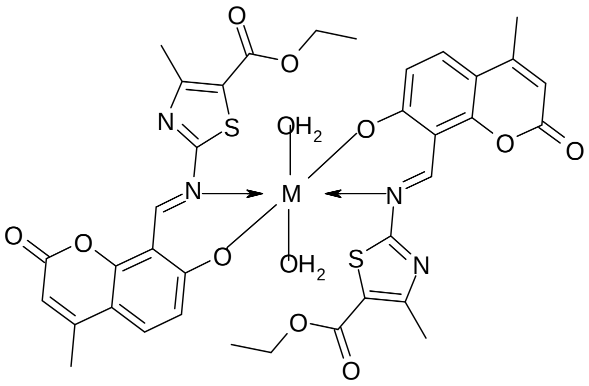
Table- 3. Antibacterial and Anti fungal activities of ligand and its metal complex

Compounds	Conc. ($\mu g\ ml^{-1}$)	Growth inhibition against bacteria (in mm)			Growth inhibition against Fungi (in mm)		
		S. aureus	*E. coli*	*S. typhi*	*A. niger*	*C. albicans*	*A. flavus*
Ligand(LH)	100	9	14	8	4	3	2
	200	9	16	9	5	4	3
	400	12	17	11	5	4	3
Co complex	100	9	11	8	3	3	3
	200	10	11	9	4	3	4
	400	12	11	10	4	4	5
Ni complex	100	10	11	9	4	4	4
	200	10	12	10	5	6	5
	400	13	12	11	5	6	6
Cu complex	100	14	15	11	6	8	3
	200	15	16	12	6	9	4
	400	18	19	14	7	9	4
Gentamycin(Std)	100	20	23	16	---	---	---
	200	24	26	21	---	---	---
	400	25	28	25	---	---	---
Amphotericin(Std)	100	---	---	---	9	11	7
	200	---	---	---	11	12	9
	400	---	---	---	12	14	12



M: standard molecular weight marker, C: control DNA of E. coli, Other lanes are of respective metal complexes treated with DNA of E. coli.

Fig.8. DNA cleavage activity on genomic DNA of E. coli



Where M= Co(II), Ni(II) and Cu(II)
Fig. 9. Proposed structure of Metal complexes

CONCLUSION

The synthesized ligand acts as a bidentate ligand which coordinates through the azomethine nitrogen atom and the oxygen atom of phenolic group via deprotonation to metal ion. The bonding of ligand to metal ion was confirmed by the analytical, IR, electronic, magnetic, ESR, ESI- mass studies. Electrochemical study of Cu(II) complex can provide the degree of the reversibility of one electron transfer reaction and it has quasi-reversible character. Among the Co(II), Ni(II) and Cu(II) complexes, Cu(II) shows better antimicrobial activity towards *Staphylococcus aureus (MRSA), Escherichia coli, Salmonella typhi*. From DNA cleavage study it can be concluded that, the compound inhibits the growth of the pathogenic organism by cleaving the genome.

The observations of all the analytical and spectral data put together made us to propose a most probable structure as shown in Fig. 9, in which the complex has the stoichiometry of the type [$ML_2.2H_2O$] {where M = Co(II), Ni(II), and Cu(II)}

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REFERENCES

[1] A. Hills, D. L. Hughes, G.J. Leigh, J.R. Sanders, *J. Chem. Soc. Dalton Trans.*, **1991**, 325-329.
[2] R. Knoch, A. Wilk, K.J. Wannowius, D. Reinen, H. Elias, *Inorg. Chem.*, **1990**, 29, 3799-3805.
[3] F.A. Bottino, P. Finocchiaro, E. Libbertini, *J. Coord. Chem.*, **1988**, 16, 341-345
[4] U. El-Ayaan, A.A.-M. Abdel-Aziz, *Eur. J. Med. Chem.*, **2005**, 40, 1214–1221.
[5] M. Sonmez, I. Berber, E. Akbas, *Eur. J. Med. Chem.*, **2006**, 41, 101–105
[6] Z. Travnicek, M. Malon, Z. Sindelar, K. Dolezal, J. Rolcik, V. Krystof, M. Strnad, J. Marek, *J. Inorg. Biochem.*, **2001**, 84, 23–32.
[7] Y. Li, Y. Wu, J. Zhao, P. Yang, *J. Inorg. Biochem.*, **2007**, 101, 2, 283-290.
[8] X. Wang, H. Chao, H. Li, X. Hong, L. Ji, X. Li, *J. Inorg. Biochem.*, **2004**, 98, 3, 423-429.
[9] J. Liu, T. Zhang, T. Lu, L. Qu, H. Zhou, Q. Zhang, L. Ji, *J. inorg. Biochem.*, **2002**, 91, 1, 269-276
[10] V.G. Vaidyanathan, B.U. Nair, *J. Inorg. Biochem.*, **2003**, 93, 3-4, 271.
[11] P.R. Reddy, K.S. Rao, B. Satyanarayana, *Tetrahedron Lett.*, **2006**, 47, 41, 7311-7315.
[12] M.S. Refat, *J Therm Anal Calorim.*, **2010**, 102, 3, 1095-1103
[13] S. M. Mostashari, S. Baie, *J Therm Anal Calorim.*, **2010**, 99, 2, 431-436

[14] A. M. Garrido Pedrosa, M. J. B. Souza, D. M. A. Melo and A. S. Araujo, *J Therm Anal Calorim.*, **2007**, 87, 2, 351-355

[15] S. You, J.W. Kelly, *Chem. Eur. J.*, **2004**, 10, 71-75.

[16] G. Kornis, “Comprehensive Heterocyclic Chemistry”, A.R. Katritzky Ed., Pregamon press, **1984**, Vol 6, Part B, 545-578.

[17] Z.H. Chohan, A. Scozzafava, C.T. Supuran, *J. Enzyme. Inhib. Med. Chem.*, **2003**, 18, 259–263

[18] A. Geronikaki, D. Hadjipavlou-Litina, M. Amourgianou, *IL Farmaco.*, **2003**, 58, 489–495

[19] A.M. Panico, A. Geronikaki, R. Mgonzo, V. Cardile, B. Gentile, I. Doytchinova, *Bioorg. Med. Chem.*, **2003**, 11, 2983–2989.

[20] G. Maass, U. Immendoerfer, B. Koenig, U. Leser, B. Mueller, R. Goody, E. Pfaff, *Antimicrob. Agents Chemother.*, **1993**, 37, 2612–2617

[21] K.M. Boy and J.M. Guernon, *Tetrahedron Lett.*, **2005**, 46, 13, 2251-2252.

[22] G.B. Bagihalli, P.G. Avaji, P.S. Badami and S.A. Patil, *J. Coord. Chem.*, **2008**, 61, 2793–2806

[23] Vogel A.I. A Text Book of Quantitative Inorganic Analyses, ELBS Longman’s Green and Co. Ltd., London, 3rd Edn. **1962**.

[24] W.J. Geary, *Coord. Chem. Rev.*, **1971**, 7, 81-122.

[25] T. Karabasanagouda, A. V. Adhikari, R. Dhanwad & G. Parameshwarappa, *Indian J. Chem.*, **2008**, 47B, 144-152

[26] S.A. Patil, S.N. Unki, P.S. Badami , Med Chem Res. DOI 10.1007/s00044-011-9932-6.

[27] P.R. Shukla, V.K. Singh, A.M. Jaiswal, J. Narain, *J. Ind. Chem. Soc.*, **1983**, 60, 321-324.

[28] S. Chandra, R. Kumar, R. Singh, A.K. Jain, Spectrochim. Acta Part A, **2006**, 65, 852-858

[29] F.A. Cotton, G. Wilkinson, Advanced Inorganic Chemistry 5th Edn. Wiley, New York **1988**.

[30] F.A. Cotton, G. Wilkinson, Advanced Inorganic Chemistry 5th Edn. Wiley, New York **1988**.

[31] P. Akilan, M. Thirumavalavan, M. Kandaswamy, *Polyhedron*, **2003**, 22, 3483-3492

[32] T. Rosu, E. Pahontu, C. Maxim, R. Georgescu, N. Stanica, A. Gulea, *Polyhedron*, **2011**, 30, 154-162

[33] S.A. Patil, S.N. Unki, P.S. Badami, *J Therm Anal Calorim.*, **2013**, 111, 1281–1289

[34] M. G. Abd El Wahed, E. M. Nour, S. Teleb and S. Fahim, *J Therm Anal Calorim.*, **2004**, 76, 343-348

[35] G.B. Bagihalli, P.G. Avaji, S.A. Patil, P.S. Badami, *Eur. J. Med. Chem.*, **2008**, 43, 2639.

[36] A. Kukarni, P.G. Avaji, G.B. Bagihalli, S.A. Patil, P.S. Badami, *J. Coord., Chem.*, **2009**, 62, 481-492

[37] S.A. Patil, V.H. Naik, A.D. Kulkarni, P.S. Badami, *Spectrochim. Acta Part A*, **2010**, 75, 347-354.

[38] Z.H. Chohan, A. Scozzafava, C.T. Supuran, *J. Enzym. Inhib. Med. Chem.*, **2003**, 18, 259-263.