



Studies on Synthetic, Structural Characterization, Thermal, DNA Cleavage, Antimicrobial and Catalytic Activity of Tetradentate (N_4) Schiff Base and Its Transition Transition Metal Complexes

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A novel tetradentate chelate was synthesized by the condensation of cinnamaldehyde and triethylenetetramine. The chelate ligand was used to prepare mononuclear transition metal complexes of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) ions. They were characterized by elemental analyses, conductivity studies, magnetic properties, infrared spectra, UV-visible spectra, ¹H NMR and ¹³C NMR spectra, electron spin resonance spectra, mass spectra and thermogram studies. Their ligand field parameters and LFSE have also been determined. Kinetic studies on the hydrolysis of ethyl acetate have been studied using Co(II) and Ni(II) complexes as homogeneous catalysts and oxidation of pyrocatechol to *o*-quinone using Co(II), Ni(II) and Cu(II) complexes. The deoxyribonucleic acid cleavage activities of pUC19 DNA examined against chelate ligand and its metal complexes have been evaluated by agarose gel electrophoresis method in the presence of H₂O₂. The antimicrobial studies of Schiff base and its metal complexes were tested against the fungi *Candida albicans* and *Aspergillus niger* and bacteria *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*.

Keywords: Tetradentate (N_4) chelate ligand, Spectral characterization studies, Catalytic studies, DNA cleavage, Antimicrobial studies.

INTRODUCTION

The coordination behaviour of bi-, tri- and tetradentate chelating Schiff base ligands has been a subject of study for several years [1]. Some of them with the interest to exhibit a variety of activities like antimicrobial, antimalarial, anti-tubercular, anticancer activities, *etc.* [2-4]. In recent research, aluminum, copper, silver and gold metal based Schiff base complexes have been used to cure the Alzheimer's disease [5]. They exhibit a broad physico-chemical property of corrosion inhibition, hydroformylation, hydrogenation of olefins, oxidation of alcohols, nonlinear optical activity and fluorescence behaviour [6-8]. The iron and cobalt complexes have been employed as homogeneous catalysts in fragmentation of heavy oils. Transition metal complexes with N and O donor Schiff bases were of particular interest of structural ability and sensitive molecular environments. The azomethine or imines highly interact with deoxyribonucleic acid of microorganism which leads to disruption in replication and protein synthesis to resist the growth of microorganisms [9].

Cinnamaldehyde is known to inhibit *E. coli* and *Salmonella typhimurium*'s growth. Possible modes of action include disruption of cell membranes, inhibition of essential enzymes, chelation of essential trace elements such as iron and targeting

of cell membranes [10,11]. In this study, a new series of transition metal complexes has been designed and synthesized using a novel tetradentate Schiff base ligand derived from condensation of cinnamaldehyde and triethylenetetramine. The Schiff base and its metal complexes were characterized involving various spectroscopic studies, which indicate the magnetic properties, geometry and coordination of the metal complexes. The significant activities of DNA cleavage and antimicrobial screening against the Schiff base and its metal complexes is also studied. The Co(II), Ni(II) and Cu(II) metal complexes have behaved as moderate homogeneous catalysts in the oxidation of pyrocatechol to *o*-quinone and hydrolysis of ethyl acetate [12,13].

EXPERIMENTAL

All the reagents were of AR grade. The FT-IR spectra (4000-400 cm⁻¹) had been recorded on KBr pellet using a Perkin Elmer Spectrum ONE-N017-1159 spectrophotometer. The elemental analyses of the synthesized compounds were recorded and the magnetic moments were measured at room temperature on vibrating sample magnetometer EG&G Model: 155 using Hg[Co(CN)₄] as standard. The potential for electrical conductance of the complexes were measured on a systronic conductivity meter, type 304 in DMSO with a dip type cell

fitted with a platinum electrode. The UV-visible spectra were run on a carry-5E spectrometer (200-900 nm). The electrochemical character of the complexes had been studied with the help of cyclic voltammetry obtained on a CHI-600A electrochemical analyzer in the absence of oxygen employing glassy carbon as a working electrode, calomel electrode as a reference electrode and platinum wire as an auxiliary electrode in the presence of NaClO_4 as the background electrolyte. The nuclear magnetic spectra of ^1H and ^{13}C were recorded on JKM-ECS 400 in $\text{DMSO}-d_6$ and CDCl_3 solvents respectively. The electron spray ionization spectrum was recorded using WATERS-Q-TOF of premier-HAB213. The X-band EPR spectrum of Cu(II) complex was recorded at 300 K on Bruker BioSpin GmbH using 2,2-diphenyl-1-picrylhydrazyl as the g-indicators. The thermograms were obtained by tapping NETZCH STA-409C/CD thermal analyzer. Catalytic oxidation of catechol was estimated in 10^{-3} M DMSO solution and reactions were monitored spectrophotometrically with the strongest absorption band of *o*-quinone.

Preparation of quadridentate chelate ligand (L): A hot ethanolic solution of cinnamaldehyde (0.2 M, 0.7 mL) and triethylenetetramine (0.1 M, 0.25 mL) in 2:1 molar ratios and stirred well for about 1 h. Then the mixtures cooled and ice was added. The product was filtered, washed with ethanol and recrystallized from hot solution of ethanol and chloroform [14,15]. The yield was quantitative and the purity of the product was checked by thin layer chromatography.

Preparation of metal chelate complexes: The metal complexes were prepared by a refluxed hot solutions of chelating ligand and the respective metal salts in 1:1 molar ratios [16,17]. The concentrated solution was cooled to room temperature. The coloured metal complexes were filtered, washed with ethanol and recrystallized from hot solution of alcohol and chloroform. The yield was quantitative and the purity of the product was checked thoroughly by TLC.

Hydrolysis of esters: A known amount of Co(II) and Ni(II) complex solutions were prepared by dissolving in a solvent mixture of 20 mL DMSO and 20 mL distilled water (and whose pH was adjusted to 2.5) and then by adding 5 mL of ethyl acetate and the contents mixed thoroughly. The amount of the reaction was determined by pipette out 5 mL of the reaction mixture at regular time intervals and then titrated it against standard NaOH solution using phenolphthalein as an indicator. The reaction was carried out at 25, 35 and 45 °C in a thermostat with an accuracy of ± 0.01 °C. The quantity of catalysts used was in the range 0.01-0.05 g [18].

The reaction rates of catalyses of metal complexes were compared with the rates obtained by using HCl as a catalyst. 40 mL of HCl of known strength was taken in a stoppered bottle in which 5 mL of ester was added and the degree of reaction was determined as above. The reaction was carried out at 25, 35 and 45 °C using 0.001, 0.01, 0.02 and 0.03 M HCl . The activation energy (E) was measured from the Arrhenius plots. The kinetic parameters, change in the enthalpy of activation ($\Delta H^\ddagger$), entropy of activation ($\Delta S^\ddagger$) and free energy of activation ($\Delta G^\ddagger$) were also determined.

Catecholase activity: The catalytic oxidation of catechol to *o*-quinone by Co(II) , Ni(II) and Cu(II) complexes were

monitored by UV-visible spectrophotometer. The 10^{-3} M metal complex solutions were prepared using DMSO solvent and added to catechol solution together in the spectrophotometric cell at room temperature [19,20]. The reactions were carried out spectrophotometrically with the appearance of absorption band of *o*-quinone at 390 nm for 45 min at time intervals of 5 min by noticed the increase in absorbance. A plot of $\log (A_\infty/A_\infty - A_t)$ vs. 't' was obtained the complex and rate constants were determined.

DNA cleavage studies: The DNA cleavage studies of chelating ligand and its metal complexes were conducted using pUC19 DNA by agarose electrophoresis in the presence of H_2O_2 as an oxidant. The oxidative cleavage of DNA was examined by keeping the concentration of the compound 30 μM and pUC19 DNA as 2 μL to make up the volume to 16 μL with 5 mM tris-HCl/5 mM NaCl buffer solution. The resulting mixture was incubated at 37 °C for 2 h and electrophoresed for 2 h at 50V in tris-acetate-EDTA (TAE) buffer (pH 8.3) using 1 % agarose gel containing 1.0 $\mu\text{g/mL}$ ethidium bromide and photographed under UV light at room temperature [21].

Antimicrobial activity: The *in vitro* biological screening effects of the synthesized chelating ligand and its metal complexes were examined against the bacteria *S. aureus*, *E. coli* and *B. cereus* by the well-diffusion method using agar nutrient as the medium. The holes of 5 mm diameter were punched carefully using a sterile cork borer and were filled with the test solution (1 mg/mL in DMSO). The plates were incubated for 24 h at 37 °C. The zone of inhibition for all the test compounds were measured and the results were compared with the standard drug ciprofloxacin under identical conditions [22].

The antifungal activity of the compounds were estimated by the well-diffusion method against the fungi *C. albicans* and *A. niger* cultured on potato dextrose agar as medium by the above method. The plates were incubated at 37 °C for 72 h. The diameters of the zone of inhibition for chelate ligand and its complexes were estimated and the results were compared with standard fluconazole under similar conditions.

The stock solutions (10^{-6} M) were prepared by dissolving the chelating ligand and its metal complexes in DMSO and the solutions were diluted to find MIC values. In a typical procedure, a well was made on the agar medium inoculated with microorganisms. It was filled with the test solution using a micropipette and the plate was incubated for 24 h in case of bacteria and 72 h for fungi at 37 °C [23]. During this period, it was noticed that the test solution started diffusing, affecting the growth of the inoculated microorganisms. The inhibition zone was developed and its diameter was measured.

RESULTS AND DISCUSSION

The chelating ligand (L) and its metal complexes were insoluble in common organic solvents but soluble in DMSO and DMF. The elemental analyses data of the chelating ligand and its metal complexes were in good agreement with calculated value are given in Table-1.

The electrolytic conductivity studies of the Mn(II) , Fe(III) , Co(II) , Ni(II) and Cu(II) complexes are 140.2, 214.0, 139.5, 149.3 and 134.5 $\text{S cm}^2 \text{mol}^{-1}$, respectively. The molar conductance values were calculated using a DMSO as solvent and

TABLE-1
ELEMENTAL ANALYSIS OF SCHIFF BASE AND METAL COMPLEXES

Compound	m.w.	Elemental analysis (%): Calcd. (Found)				μ_{eff} (BM)	Λ_m (S cm ² mol ⁻¹)
		M	C	H	N		
L = C ₂₄ H ₃₀ N ₄	375.670	—	79.97 (79.94)	8.07 (7.99)	14.96 (14.91)	—	—
[Mn(L)(H ₂ O) ₂]SO ₄	561.556	9.78 (9.72)	51.33 (51.26)	6.10 (6.07)	9.98 (9.94)	5.87	140.2
[Fe(L)(H ₂ O) ₂]Cl ₃	572.760	9.75 (9.69)	50.33 (50.29)	5.98 (5.94)	9.78 (9.72)	5.84	214.0
[Co(L)(H ₂ O) ₂](NO ₃) ₂	593.497	9.93 (9.90)	53.88 (53.84)	5.77 (5.72)	14.16 (14.10)	4.22	139.5
[Ni(L)(H ₂ O) ₂]Cl ₂	540.155	10.86 (10.82)	53.37 (53.33)	6.34 (6.31)	10.37 (10.34)	2.89	149.5
[Cu(L)(H ₂ O) ₂]Cl ₂	545.008	11.66 (11.59)	52.89 (52.83)	6.29 (6.26)	10.28 (10.24)	1.81	134.5

value indicates that these complexes have electrolytic nature [24]. The Fe(III) complex shows the 1:3 electrolyte. The Mn(II), Co(II), Ni(II) and Cu(II) complexes behave as 1:2 electrolyte. Hence, there is an ionic sphere in these complexes.

The magnetic susceptibility studies of the complexes were carried at 300 K. The magnetic moments are in good agreement with the theoretical values. The μ_{eff} values of Mn(II), Fe(III), Co(II) and Ni(II) complexes are 5.86, 5.84, 4.22 and 2.89 BM corresponding to 5, 5, 3 and 2 unpaired electrons respectively [25]. These values suggest a high spin octahedral environment of donor atoms around the metal ions. The magnetic moment of the Cu(II) complex is 1.82 BM due to the presence of one unpaired electron, which suggests a distorted octahedral geometry because of John-Teller effect [26].

Mass spectral and fragmentation of the chelate ligand

(L): The mass spectrum of the Schiff base ligand (Fig. 1) shows a molecular ion peak (M+H)⁺ at m/z 375.52 which is equivalent to its proposed molecular formula (L = C₂₂H₂₅N₃). In the fragmentation of ligand the peaks at m/z 298 (7 %), 216 (17 %), 174 (73 %) and 105 (3 %) are due to the elimination of C₆H₆, C₇H₁₄N₂, C₂H₇N and C₃H₈N₂, respectively (Fig. 2).

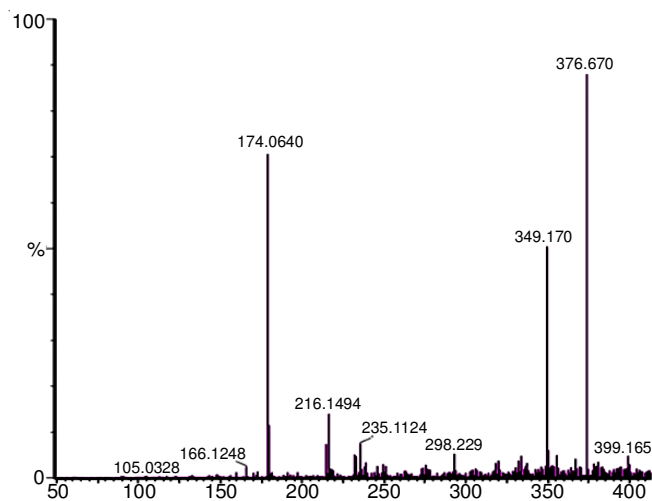


Fig. 1. Mass spectrum of Schiff base (L)

FT-IR spectra: The FT-IR spectral data of chelating ligand and its metal complexes are given in Table-2. In the ligand the peak at 1656 cm⁻¹ is due to the appearance of azomethine group (-CH=N-) and is shifted to lower frequency region around 1641-1635 cm⁻¹ in all the metal complexes [27]. This shows that the azomethine nitrogen of the ligand is complexed with metal ions.

A band at 3312 cm⁻¹ in the ligand is due to N-H group. On complexation this group is shifted to lower frequency indi-

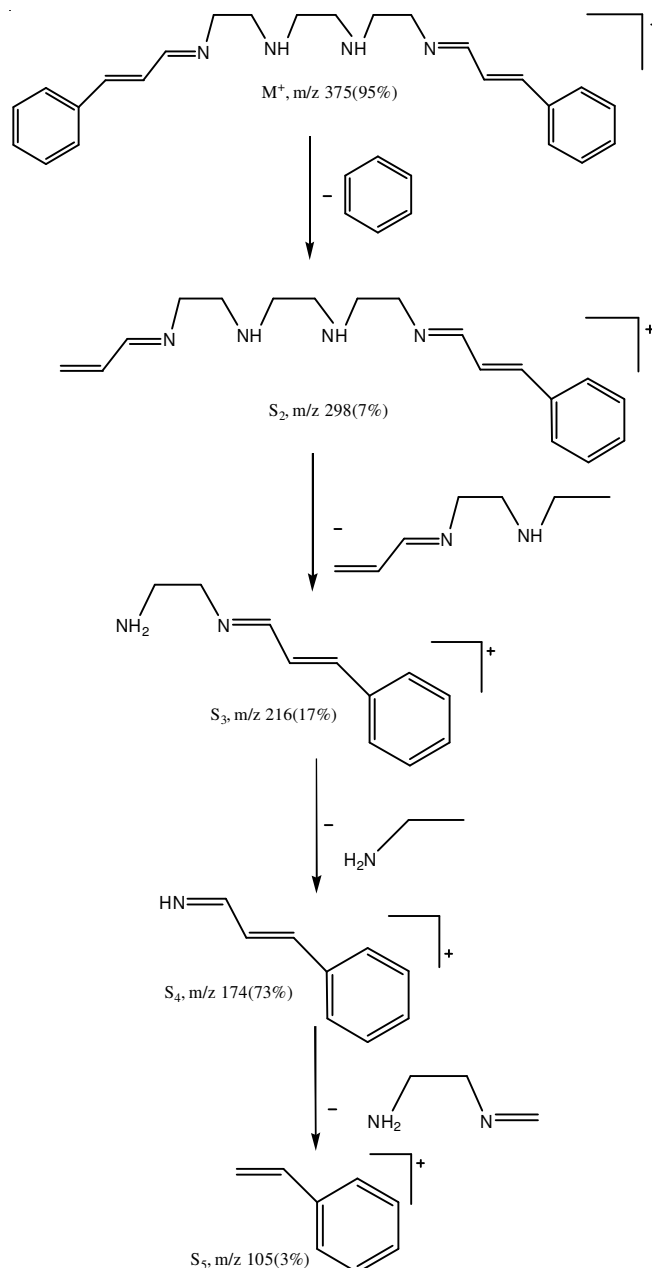


Fig. 2. Mass fragmentation of Schiff base

cating the coordinate of nitrogen of N-H group. The appearance of new bands in the spectra of the complexes in the regions 3440-3414, 531-514 and 491-464 cm⁻¹ were assigned to $\nu(\text{M-OH}_2)$, $\nu(\text{M-N})$ and $\nu(\text{M-O})$ respectively, which are absent in the free Schiff base [28]. The presence of these band give evidence for the bonding of nitrogen atoms with the metal ions in tetradentate chelating environment.

TABLE-2
KEY INFRARED SPECTRAL BAND (cm⁻¹) OF SCHIFF BASE AND ITS METAL COMPLEXES

Compound	$\nu(\text{M-OH}_2)$	$\nu(\text{N-H})$	$\nu(\text{C=N})$	$\nu(\text{N-H})$ (in-plane bending)	$\nu(\text{M-N})$	$\nu(\text{M-O})$
L = C ₂₄ H ₃₀ N ₄	—	3312	1656	1494	—	—
[Mn(L)(H ₂ O) ₂]SO ₄	3414	3280	1641	1450	514	461
[Fe(L)(H ₂ O) ₂]Cl ₃	3416	3275	1633	1413	531	481
[Co(L)(H ₂ O) ₂](NO ₃) ₂	3435	3279	1639	1449	514	469
[Ni(L)(H ₂ O) ₂]Cl ₂	3436	3278	1636	1461	518	474
[Cu(L)(H ₂ O) ₂]Cl ₂	3414	3276	1635	1416	521	474

UV-visible spectra: The electronic absorption spectral data of chelating ligand and its metal complexes were recorded in DMSO. The absorption region, ligand field parameters and ligand field stabilization energy (LFSE) of the complexes are shown in Table-3. They have been studied with the view to obtain more information on stereochemistry of the complexes and procure more support for the conclusion. The electronic absorption spectrum of the Schiff base shows a band at 29850 cm⁻¹ which attributes to $n \rightarrow \pi^*$ transition of the azomethine (-C=N-) chromophore. This transition can be found again in the complexes but shifts towards lower frequencies which indicates the coordination of the ligand with metal ions. The electronic spectrum of Mn(II) complex exhibits three bands at 11682 cm⁻¹ (ν_1), 16103 cm⁻¹ (ν_2) and 19121 cm⁻¹ (ν_3) which are assigned to the transitions ${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{1g}(\text{G})$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$ and ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g(\text{G})$, respectively. The ligand field parameters, ligand field splitting energy (Dq), inter electronic repulsion parameter (B'), nephelauxetic ratio (β), $\beta\%$ and LFSE have been calculated using the equations given by Konig [29]. The values of ligand field parameters Dq, B', β , $\beta\%$ and LFSE for this complex are 1168.2 cm⁻¹, 865.3 cm⁻¹, 0.901, 9.86 and 139.81 KJ mol⁻¹, respectively. The inter electronic repulsion parameter (B') is less than free metal ion (960 cm⁻¹) and the nephelauxetic ratio (β) is less than one which indicates a moderate covalent character of the metal-ligand bonds with high spin octahedral environment.

The absorption spectrum of Fe(III) complex obtained three bands at 13440 cm⁻¹ (ν_1), 17790 cm⁻¹ (ν_2) and 19520 cm⁻¹ (CT) due to the transitions ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{G})$ (ν_1), ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$ (ν_2),

respectively. The values of ligand field parameters Dq and LFSE value for this complex are 1344 cm⁻¹ and 160.85 KJ mol⁻¹, respectively. These values suggest a high spin octahedral environment for the central metal ion.

The absorption spectrum of Co(II) complex shows three bands at 10764 cm⁻¹ (ν_1), 19305 cm⁻¹ (ν_2) and 26815 cm⁻¹ (ν_3) which are assigned to the transitions ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$, ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{P})$, respectively. The appearance of intense band around 31400 cm⁻¹ may be a charge transfer band. The ligand field parameters Dq, B', β , $\beta\%$ and LFSE for Co(II) complex have been calculated and their respective values correspond to 1076.4 cm⁻¹, 839.90 cm⁻¹, 0.864, 13.50 and 128.83 KJ mol⁻¹. The inter electronic repulsion parameter (B') is less than free metal ion (971 cm⁻¹) and the nephelauxetic ratio (β) is less than one which indicates a moderate covalent character of the metal-ligand bonds with octahedral geometry.

The electronic spectrum of Ni(II) complex gives two bands at 15673 cm⁻¹ (ν_2) and 24937 cm⁻¹ (ν_3) assignable to ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$ and ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$ transitions, respectively in an octahedral environment. The lowest band $\nu_1 \approx (10\text{ Dq})$ was not observed due to limited range of the instrument used. The ligand field parameters Dq, B', β , $\beta\%$ and LFSE for Ni(II) complex have been calculated and their respective values correspond to 1170.4 cm⁻¹, 838.80 cm⁻¹, 0.805, 19.42 and 140.80 KJ mol⁻¹. The inter electronic repulsion parameter (B') is less than free metal ion (1040 cm⁻¹) and the nephelauxetic ratio (β) is less than one which indicates a moderate covalent character of the metal-ligand bonds in an octahedral environment [30].

TABLE-3
UV-VISIBLE SPECTRA OF SCHIFF BASE AND ITS METAL COMPLEXES

Compound	Frequency (cm ⁻¹)	Assignment	Dq (cm ⁻¹)	B' (cm ⁻¹)	β	$\beta\%$	LFSE (KJ mol ⁻¹)
L = C ₂₄ H ₃₀ N ₄	29850	$n \rightarrow \pi^*$	—	—	—	—	—
Mn(II) Complex	11682 (ν_1)	${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{1g}(\text{G})$	1168.2	865.30	0.901	9.86	139.81
	16103 (ν_2)	${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$					
	19121 (ν_3)	${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g(\text{G})$					
Fe(III) Complex	13440 (ν_1)	${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{G})$	1344.0	-	-	-	160.85
	17790 (ν_2)	${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$					
	19520 (CT)	LMCT					
Co(II) Complex	10764 (ν_1)	${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{F})$	1076.0	839.90	0.864	13.50	128.83
	19305 (ν_2)	${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}(\text{F})$					
	26815 (ν_3)	${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{F})$					
Ni(II) Complex	9363 (ν_1)	${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}(\text{F})$	1170.4	838.80	0.805	19.42	140.08
	15673 (ν_2)	${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$					
	24937 (ν_3)	${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$					
Cu(II) Complex	14365 (ν_1)	${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$	1436.5	—	—	—	171.93
	25157 (CT)	LMCT					

The electronic spectrum of Cu(II) complex exhibits three bands, a low intense broad band in the region 16411-12320 cm^{-1} with maxima at 14365 cm^{-1} due to ${}^2E_g \rightarrow {}^2T_{2g}$ transition, the high intense band in the region 22900-27100 cm^{-1} with maxima at 25157 cm^{-1} due to symmetry forbidden ligand to metal charge transfer transition (LMCT). The band above 27150 cm^{-1} was assigned for the ligand. A distorted octahedral geometry around Cu(II) ion due to John-Teller effect is proposed [31]. The ligand field splitting energy (Dq) and LFSE values are 14365.0 cm^{-1} and 171.93 KJ mol^{-1} , respectively.

${}^1\text{H}$ and ${}^{13}\text{C}$ nuclear spin resonance spectra of the chelate ligand (L): The ${}^1\text{H}$ NMR spectrum of chelating ligand was recorded in $\text{DMSO}-d_6$ solvent using tetramethylsilane (TMS) as an internal standard. The Schiff base showed broad signal at 7.3 ppm due to aromatic protons, while the azomethine group protons was observed at 7.71 ppm. The N-H proton at 3.43 ppm indicates the presence of secondary amine in the Schiff base [32].

The ${}^{13}\text{C}$ NMR spectrum of chelating ligand was recorded in CDCl_3 solvent. The chemical shift for aromatic carbons region was a set of peak at 128.78 ppm, while the chemical shift of $-\text{C}=\text{N}$ -carbons were seen in the range 162.73 ppm. The chemical shift for aliphatic carbons was a set of multiples in the range 56.91-57.55 ppm were given Table-4.

Compound	Chemical shift (δ , ppm)	Assignment
$\text{L} = \text{C}_{24}\text{H}_{30}\text{N}_4$	1.2509	(m, 2H, $-\text{CH}_2-\text{CH}_2-$)
	2.3712	(s, 1H, $-\text{NH}-$)
	2.715-2.5059	(m, 2H, $-\text{NH}-\text{CH}_2-$)
	3.4345	(m, 1H, $-\text{CH}=\text{CH}-$)
	5.3712	(s, 1H, $-\text{CH}-\text{Ar}$)
	7.3097	(m, 5H, ArH)
	7.7127	(d, 1H, $-\text{N}=\text{CH}-$, azomethine H)
	57.91-57.55	$-\text{C}-\text{C}$ -(aliphatic)
	128.78	$-\text{C}=\text{C}$ -(aromatic)
	162.73	$-\text{C}=\text{N}$ -(azomethine)

Electron spin resonance spectrum: The EPR spectrum of the Cu(II) complex was recorded at 300 K using DMSO, on the X-band at the frequency 9.27 GHz under a magnetic field strength about 3360G. The EPR spectrum of Cu(II) complex showed an anisotropically broad signal as given in Fig. 3. The analysis of spectrum gives $A_{\parallel}$, $g_{\parallel}$ and $g_{\perp}$ values as $140 \times 10^{-4} \text{ cm}^{-1}$, 2.263 and 2.101, respectively. These values indicate that the ground state of Cu(II) is predominantly $d_{x^2-y^2}$. The trend $g_{\parallel} > g_{\perp} > 2.0023$ that has been observed for the complex indicates that the unpaired electron is localized in the $d_{x^2-y^2}$ orbital of the Cu(II) ion and is a characteristic of the axial symmetry. The g_{av} value has been calculated as 2.155 using the equation $g_{\text{av}} = 1/3[g_{\parallel} + 2g_{\perp}]$. The deviation of g_{av} from that of free electron (2.0023) is due to the covalence character. The parameter 'G' is calculated by using expression $G = g_{\parallel} - 2/g_{\perp} - 2$. The G value indicates the interaction between metal centers in solid complex consistent with Hathaway approach [33]. The covalency parameter α^2 has been calculated and given in Table-5 as:

$$\alpha^2 = (A_{\parallel}/0.036) + (g_{\parallel} - 2.0023) + (3/7)(g_{\perp} - 2.0023) + 0.04$$

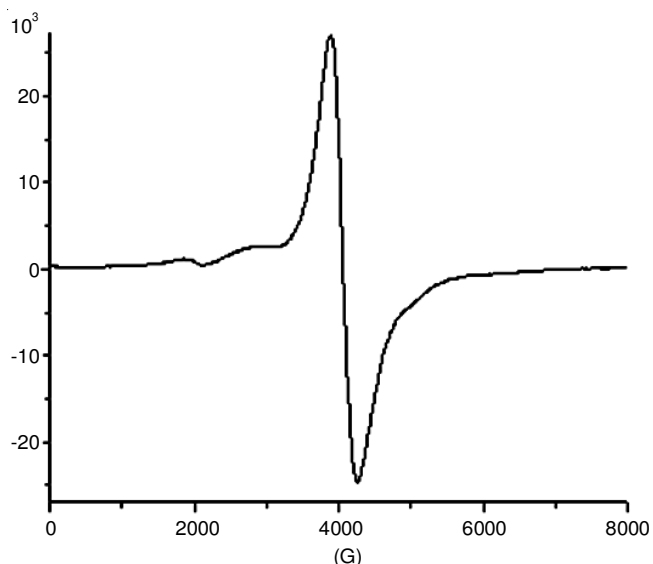


Fig. 3. ESR spectrum of Cu (II) complex

$A_{\parallel} \times 10^{-4}$	$g_{\parallel}$	$g_{\perp}$	g_{av}	G	α^2
140	2.263	2.101	2.155	2.64	0.732

If the value of $\alpha^2 = 0.5$, it indicates complete covalent bonding while the value of $\alpha^2 = 1.0$ suggests complete ionic bonding. The observed value of α^2 (0.732) of the complex is less than unity, which indicates that the complex has some covalent character in the ligand environment. The EPR spectral study thus suggests the distorted octahedral geometry of the Cu(II) complex [34].

TGA and DTA analysis: The TGA and DTA studies of Mn(II), Fe(III), Co(II), Ni(II) and Cu(II) complexes were carried out from room temperature to 1400 $^{\circ}\text{C}$. In the first stage all the metal complexes have shown (Figs. 4 and 5) an endothermic peak at 100-160 $^{\circ}\text{C}$. This indicates the presence of two coordinated aqua molecules in these complexes [35]. The second and third stages are found in the temperature range 180-600 $^{\circ}\text{C}$ and above 600 $^{\circ}\text{C}$ the formation of metal oxides as final product. The data reveals that all the metal complexes are of octahedral geometry formed by a tetradentate (N_4) Schiff base chelate ligand and two coordinated aqua molecules.

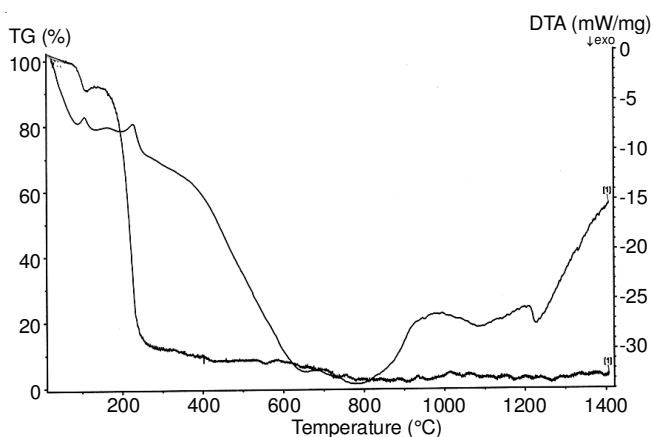


Fig. 4. TGA-DTA thermogram of Co(II) complex

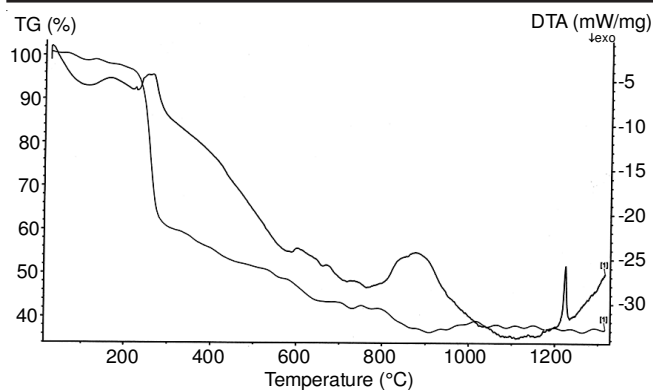


Fig. 5. TGA-DTA Thermogram of Cu(II) complex

Electrochemical studies: The electrochemical behaviour of the Fe(III) and Cu(II) complexes have been examined in DMSO with 0.1 M NaClO_4 about the range of +2.0 V to -2.0 V using cyclic voltammetry with glassy carbon as working electrode (Fig. 6). The electrochemical data of the complexes are given in Table-6. The Fe(III) and Cu(II) complexes shows that one electron transfer process it gives a quasi-reversible redox behaviour [36].

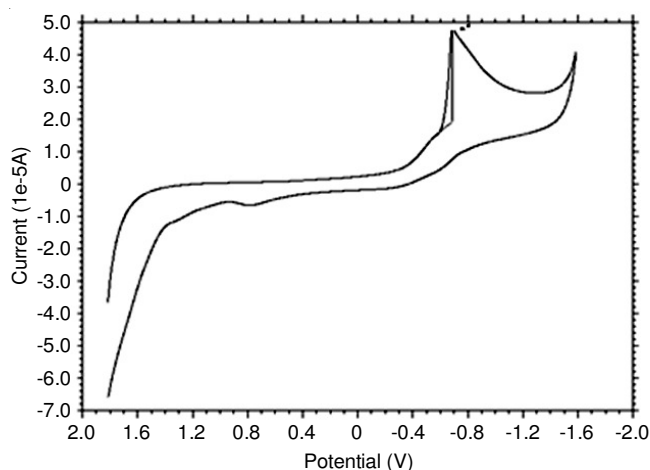


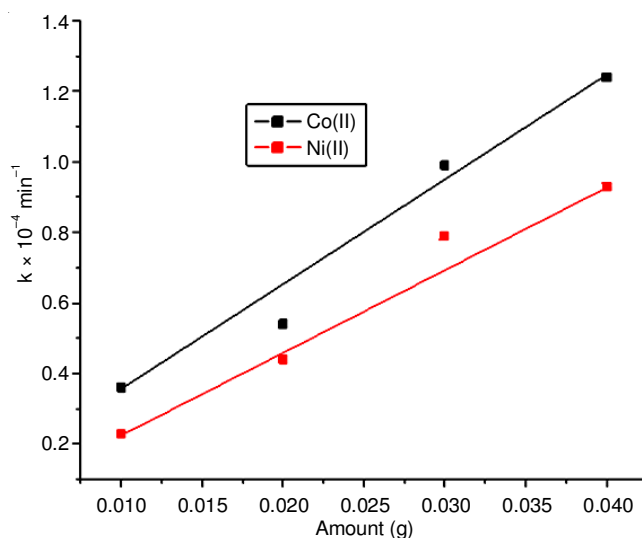
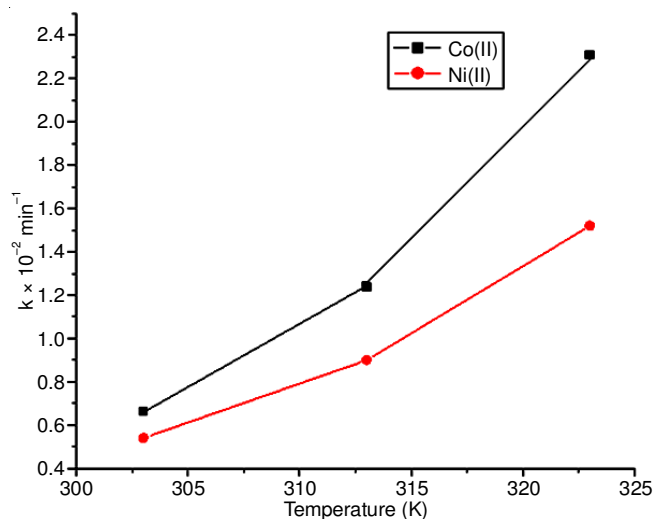
Fig. 6. Cyclic voltammogram of Fe(III) complex

TABLE-6
ELECTROCHEMICAL DATA OF
SCHIFF BASE METAL COMPLEXES

Complexes	E_{pc} (V)	E_{pa} (V)	$E_{1/2}$ (V)	ΔE (mV)	i_{pa} (μA)	i_{pc} (μA)
Fe(III)/Fe(II)	0.628	-0.776	0.702	148	30.82	-0.625
Cu(II)/Cu(I)	0.215	0.510	0.363	295	14.26	10.12

Hydrolysis of esters: The ester hydrolysis of ethyl acetate were studied by complexes as homogeneous catalysts. The concentrations of ester, the amount of the catalyst has been varied in the range 0.01-0.04 g. The obtained value of k is proportional to the amount of the catalyst [37].

The plot of rate constant *versus* the quantity of catalyst is linear showing that the rate constant is directly proportional to the quantity of catalyst used in the reaction (Fig. 7). Hydrolysis of ethyl acetate in presence of HCl is first order. In the present study, it is found that Co(II) and Ni(II) complexes show first order kinetics as shown by the linear plot of $\log(a-x)$ against ' t ' (Fig. 8).

Fig. 7. Plot of rate of reaction *versus* amount of catalysts at 313 K, Volume of ethyl acetate: 5 mLFig. 8. Plot of rate of reaction *versus* temperature

The rate constants for the hydrolysis of ethyl acetate obtained by using different catalysts at various temperatures are given in Tables 7 and 8. Keeping the quantity of the catalyst and ester as constant it has been observed that when the temperature is varied in the range 303-313 K, the value of k increases with increase in temperature. HCl is more efficient catalyst than the Co(II) and Ni(II) complexes. It is observed that $\Delta H^\ddagger$ values are closer to E values in accordance with absolute

TABLE-7
INFLUENCE OF THE AMOUNT OF CATALYST ON THE
HYDROLYSIS OF ETHYL ACETATE BY REPRESENTATIVE
COMPLEXES $[\text{Co}(\text{L})(\text{H}_2\text{O})_2](\text{NO}_3)_2$ AND $[\text{Ni}(\text{L})(\text{H}_2\text{O})_2]\text{Cl}_2$

Complexes	Amount of catalyst (g)	Specific reaction rate ($k \times 10^{-4}, \text{min}^{-1}$)
$[\text{Co}(\text{L})(\text{H}_2\text{O})_2](\text{NO}_3)_2$	0.010	0.36
	0.020	0.54
	0.030	0.99
	0.040	1.24
$[\text{Ni}(\text{L})(\text{H}_2\text{O})_2]\text{Cl}_2$	0.010	0.23
	0.020	0.46
	0.030	0.79
	0.040	0.93

TABLE-8
KINETICS OF HYDROLYSIS A OF ETHYL ACETATE IN PRESENCE OF SCHIFF BASE METAL COMPLEXES AS
HOMOGENEOUS CATALYSTS (VOLUME OF ESTER: 5 mL, AMOUNT OF CATALYST: 50 mg)

Complexes	T (K)	$k \times 10^{-2} (\text{min}^{-1})$	E (kJ mol ⁻¹)	$\Delta H^\ddagger$ (kJ mol ⁻¹)	$\Delta S^\ddagger$ (J K ⁻¹ mol ⁻¹)	$\Delta G^\ddagger$ (kJ mol ⁻¹)
Co(II)	303	0.63	52.852	50.250	-121.48	88.274
	313	1.24				
	323	2.31				
Ni(II)	303	0.54	42.171	39.569	-157.84	88.976
	313	0.90				
	323	1.52				

reaction rate theory for solution. The value of $\Delta G^\ddagger$ is found to be higher in all the cases. The values of $\Delta S^\ddagger$ were found to be negative in all the cases indicating that molecules in transition state are more ordered than the reactants in the ground state.

Catecholase activity: The oxidation of catechol to the corresponding quinone formation is known as catecholase activity. In catecholase activity studies pyrocatechol has been employed as substrate [38]. The catecholase activity of the Co(II), Ni(II) and Cu(II) complexes were carried out using pyrocatechol as the substrate. Solutions ($10^{-3} \text{ mol dm}^{-3}$) of complexes in dimethyl sulfoxide were treated with 100 equivalents of pyrocatechol in the presence of air. The reaction was carried out spectrophotometrically at 390 nm for 45 min at time intervals of 5 min are given in Table-9. The slope was determined by the method of initial rates by monitoring the growth of the product *o*-quinone at the absorption band 390 nm. Plots of $\log (A_\infty/A_\infty - A_t)$ versus time for catecholase activity results drawn for the metal complexes (Figs. 9 and 10).

The observed kinetics show a first order dependence on the complex concentration and the initial rate constant values show that Cu(II), Ni(II) and Co(II) complexes were $5.72 \times 10^{-3} \text{ min}^{-1}$, $0.33 \times 10^{-3} \text{ min}^{-1}$ and $0.40 \times 10^{-3} \text{ min}^{-1}$, respectively [39]. The Cu(II) complex exhibits higher activity than the other metal complexes due to redox potential of the Cu(II) metal ion and the binding of the catechol with ligand molecules.

TABLE-9
KINETICS STUDIES OF OXIDATION OF PYROCATECHOL
IN PRESENCE OF Co(II), Ni(II) AND Cu(II) COMPLEXES
AS HOMOGENEOUS CATALYSTS

Time (min)	$\log (A_\infty/A_\infty - A_t)$		
	Co(II) complex	Ni(II) complex	Cu(II) complex
0	0	0	0
5	0.0007	0.0009	0.013
10	0.0015	0.0018	0.029
15	0.0022	0.0026	0.042
20	0.0030	0.0033	0.050
25	0.0038	0.0042	0.057
30	0.0043	0.0050	0.068
35	0.0050	0.0058	0.081
40	0.0056	0.0065	0.094
45	0.0061	0.0072	0.104

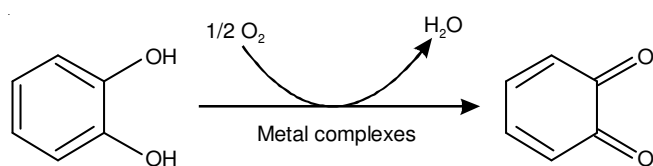


Fig. 9. Catalytic oxidation of catechol to *o*-quinone

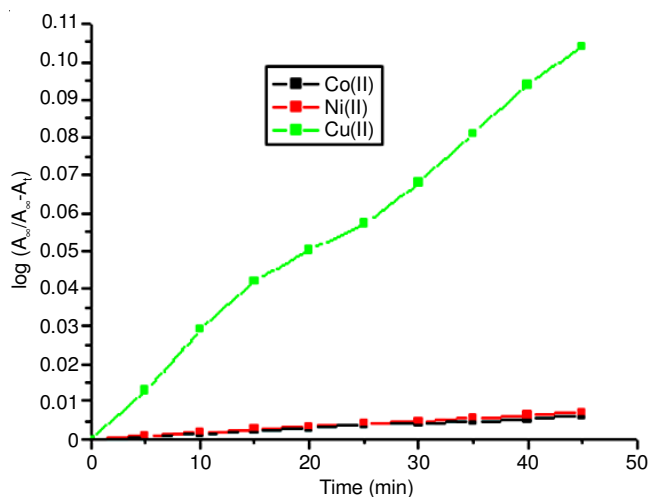
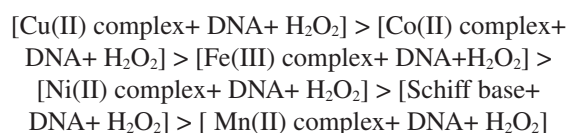


Fig. 10. Plot of $\log (A_\infty/A_\infty - A_t)$ versus time

The metal having negative reduction potential to decreased the catalytic efficiency of metal complexes due to a more difficult to reduction of metal ions.

DNA cleavage studies: The DNA cleavage activity of the chelating ligand and its metal complexes has been compared with that of the control. The chelating ligand and its metal complexes could able to convert super coiled DNA into open circular DNA [40]. The oxidative cleavage involves the oxidation of deoxyribose by absorption of sugar hydrogen or oxidations of nucleobases in the presence of an oxidative agent. The oxidative cleavage implies by hydroxyl radical species of $\text{O}_2(\text{OH})$. In the present study, the pUC19 DNA gel electrophoresis experiment has been studied in the presence of the oxidant H_2O_2 (50 $\mu\text{g/mL}$) with chelating ligand and its metal complexes [41]. The results show that Fe(III), Co(II), Ni(II) and Cu(II) complexes have effectively degraded the pUC19 DNA in presence of the oxidant and chelating ligand and Mn(II) complex does not show any significant cleavage. The descending order of DNA cleavage results are:



Antimicrobial activity: The *in vitro* antimicrobial activity of the synthesized chelating ligand and its metal complexes had been tested against bacteria and fungi and the zone of inhibition values are given in the Table-10. The minimum inhibitory concentration (MIC) values of the synthesized compounds were given in Table-11. The synthesized chelating ligand shows inhibitory effect (MIC values of 50-100 $\mu\text{g/mL}$)

TABLE-10
ANTIMICROBIAL ACTIVITY OF SCHIFF BASE AND ITS METAL COMPLEXES

Compound	Antibacterial inhibition (mm)			Antifungal inhibition (mm)	
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>C. albicans</i>	<i>A. niger</i>
L	10	09	10	09	10
Mn(II)	11	12	11	10	12
Fe(III)	14	15	13	12	14
Co(II)	12	11	12	12	13
Ni(II)	11	10	11	11	11
Cu(II)	21	24	22	24	20
Ciprofloxacin	28	32	28	—	—
Fluconazole	—	—	—	36	30

TABLE-11
DETERMINATION OF MIC FOR ANTIMICROBIAL ACTIVITIES

Compound	Concentration (µg/mL)				
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. cereus</i>	<i>C. albicans</i>	<i>A. niger</i>
L	50.0	100.0	50.0	100.0	100.0
Mn(II)	25.0	25.0	50.0	50.0	25.0
Fe(III)	12.5	25.0	12.5	25.0	12.5
Co(II)	12.5	12.5	25.0	25.0	12.5
Ni(II)	25.0	25.0	50.0	25.0	25.0
Cu(II)	12.5	12.5	12.5	12.5	12.5
Ciprofloxacin	6.25	6.25	6.25	—	—
Fluconazole	—	—	—	12.5	6.25

on growth of the tested bacterial strains. All the metal complexes have shown greater bactericidal activities against *E. coli* (MIC 12.50-25 µg/mL), *S. aureus* (MIC 12.50-50 µg/mL) and *B. cereus* (MIC 12.50-50 µg/mL) than the Schiff base. In the fungal studies, the Schiff base had an inhibitory effect (MIC values in range 50-100 µg/mL) on the growth of the tested strains [42]. The metal complexes also have shown greater fungicidal activities against *C. albicans* (MIC 12.50-50 µg/mL) and *A. niger* (MIC 12.50-50 µg/mL).

The values indicate that the metal complexes possess higher antimicrobial activity than the free ligand. Such increased activity of the metal chelates can be explained by Overtone's [43] and Tweedy chelation theory [44]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid soluble materials due to which liposolubility is an important factor, which controls the antifungal activity.

On chelation theory the polarity of metal ion will be reduced due to the positive charge of the metal ion with donor groups. The delocalization of π -electrons increases the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and restricts further multiplicity of the microorganisms. The variation in the effectiveness of complexes against different microorganisms, depends either on the impermeability of the microbe's cells. The Cu(II) complex has exhibited better activity when compared to Schiff base and other metal complexes due to higher polarity reduction and partial sharing with donor groups which enhances the lipophilic character.

Conclusion

A novel tetradentate (N₄) chelate ligand and its mononuclear metal complexes were synthesized by the condensation

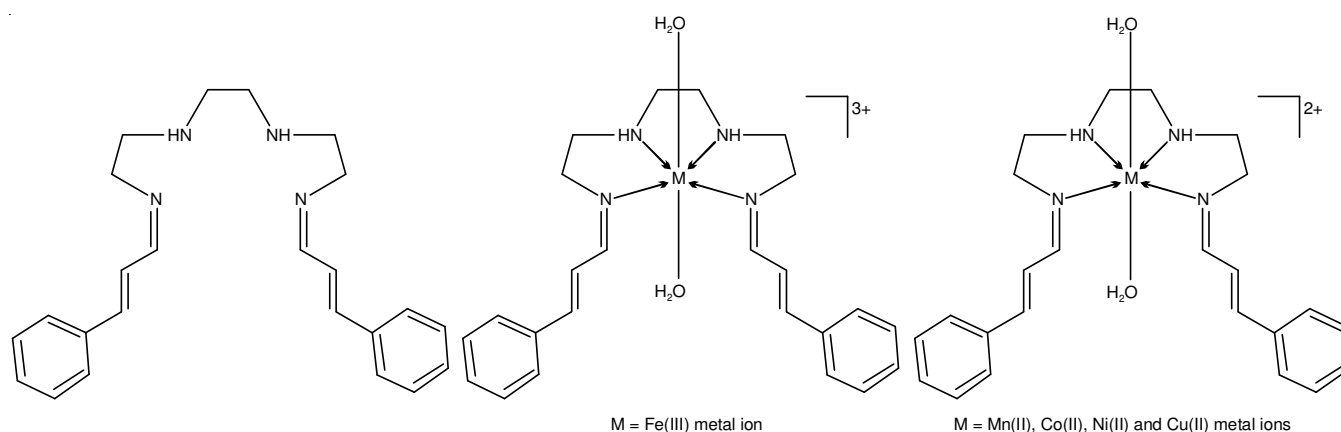


Fig. 14. Proposed structure of Schiff base (L) and its metal complexes

of cinnamaldehyde and triethylenetetramine. The spectral characterization and results of analytical techniques reveals that the chelate ligand acts as a N₄ donor. The results showed that the chelate ligand would coordinate in a tetradentate manner with the metal ions and the geometry of Mn(II), Fe(III), Co(II) and Ni(II) complexes were octahedral geometry and the Cu(II) complex showed a distorted octahedral geometry. All the metal complexes are of paramagnetic in nature. The TGA-DTA study had shown the presence of two coordinated water molecules. The proposed structure of the chelating ligand and its metal complexes was shown in Fig. 11. The catalytic study of the metal complexes followed a first order kinetics with higher $\Delta G^\ddagger$ in mild acidic medium. The copper complex shows higher catalytic activity against oxidation of catechol than the other complexes. All the metal complexes except Mn(II) had shown strong DNA damage. The Cu(II) complex exhibited powerful antimicrobial activity where as the other molecules showed a moderate activity.

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