

## SYNTHESIS, COMPOSITION, GEOMETRY AND ANTIBACTERIAL ASSAY OF BINUCLEAR SCHIFF BASE METAL COMPLEXES DERIVED FROM BENZENE-1,4-DICARBALDEHYDE, 2-HYDROXY-BENZALDEHYDE AND PYRIDINE-2,6-DIAMINE

M. USHARANI<sup>1</sup>, E. AKILA<sup>1</sup>, S. RAMACHANDRAN <sup>2</sup>, G. VELRAJ <sup>2</sup> AND R. RAJAVEL<sup>1\*</sup>

<sup>1</sup>Department of Chemistry, Periyar University, Periyar Palkalai Nagar, Salem, Tamil Nadu, India, <sup>2</sup> Department of Physics, Periyar University, Periyar Palkalai Nagar, Salem, Tamil Nadu, India. Email: sm.usharani@gmail.com

Received: 06 Mar 2013, Revised and Accepted: 24 Apr 2013

### ABSTRACT

A new symmetrical Schiff base ligand was derived from 2-hydroxy-benzaldehyde, pyridine-2,6-diamine and benzene-1,4-dicarbaldehyde and then it is allowed to react with metal salts to form binuclear Schiff base metal complexes.

All the synthesized complexes were characterized based on elemental analyses, IR, <sup>1</sup>H NMR, magnetic moment, molar conductance, ESR, UV, cyclic voltammetry and thermal analysis (TGA). The molar conductance data reveal that the chelates are electrolytes. An IR spectrum shows that the Schiff bases are coordinated to the metal ions in a manner with NNN donor sites of the pyridine ring, oxygen of phenolic OH group and NN donor sites of bipyridyl group. The formation of ligand was also ascertained by <sup>1</sup>H NMR spectra. The electronic spectra and ESR measurements show that Cu(II), Co(II), Mn(II) and Ni(II) complexes has octahedral geometry. The cyclic voltammetric studies of these complexes in N,N-dimethylformamide indicate the structural changes during the course of redox reaction and quasi-reversible nature of the Schiff base binuclear complexes. Thermal stability of the complexes was also evaluated to assess the compatibility of the donating moieties. The synthesized ligands, in comparison to their metal complexes were screened for their antibacterial activity against bacterial species *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia*. The activity data show that the metal complexes are more potent antibacterial than the parent organic ligands against one or more bacterial species. DNA cleavage ability of the synthesized binuclear Schiff base metal complexes were also performed by gel electrophoresis.

**Keywords:** Symmetric Schiff base, Binuclear metal complexes, Antibacterial activity, Thermal stability, DNA cleavage.

### INTRODUCTION

The Designing and synthesis of a new ligand was perhaps the most important step in the development of metal complexes which exhibit unique properties and novel reactivity due to electron donor, electron acceptor properties, structural, functional groups and the position of the ligand in the coordination sphere. This may be the factor for different studies [1,2]. A large number of Schiff base compounds are often used as ligands in coordination chemistry by considering their metal binding ability. Schiff bases have gained importance because of physiological and pharmacological activities associated with them. Schiff base metal complexes have ability to reversibly bind oxygen in epoxidation reactions [3], biological activity [4,5], catalytic activity in hydrogenation of olefins [6,7] nonlinear optical materials [8] and photochromic properties [9]. The Schiff base complexes was also used as drugs and they possess a wide variety of antimicrobial activity against bacteria, fungi and it also inhibits the growth of certain type of tumors [10-13].

In view of these finding, this piece of work has devoted with the aim to synthesize some transition metals complexes with symmetrical Schiff bases ligands, (2-hydroxy-benzaldehyde, pyridine-2,6-diamine and benzene-1,4-dicarbaldehyde) and to examine their physical properties involving spectral behaviours, the electrical conductance values and to determine the efficiency of the synthesized complexes against pathogenic bacteria.

### MATERIALS AND METHODS

All the chemicals were purchased from commercial sources and used without any further purification.

#### Physical measurements

Elemental analysis (C, H and N) was obtained using Perkin Elmer elemental analyzer. The molar conductance of the complexes in DMF (10<sup>-3</sup>) solution was measured at 27±3°C with an Elico model conductivity meter. Infrared spectra were recorded on the Thermo Nicolet, Avatar 370 model FT-IR spectrometer using KBr disc in the range of 4000- 400 cm<sup>-1</sup>. Electronic spectra were recorded at 300 K on a Perkin-Elmer Lambda 40(UV-Vis)

spectrometer using DMF in the range 200-800 nm. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature. NMR signals were obtained from Bruker Avance III, 400MHz model spectrometer. EPR spectra of compounds were recorded on a E-112 ESR Spectrometer with X-band microwave frequency (9.5 GHz). The Redox nature of the complex in DMF was measured using CHI - 760 Electrochemical Analyzer by employing a platinum electrode as working electrode, Ag/AgCl as a reference electrode and platinum wire as auxiliary electrode. The working media consist of DMF containing 0.1M tetrabutylammonium perchloride (TBAP) as supporting electrolyte. TG studies were carried out in the range between 0-500°C using a NETZSCH model thermal analyzer. Molecular modeling studies have been carried out using the density functional theory (DFT) calculations with a hybrid functional B3LYP (Becke's three-parameter hybrid functional using the LYP correlation functional) using the 6-311++G(d,p) basis set [14,15] were performed using the Gaussian 03W software package.

#### Synthesis of Schiff base ligand

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

#### Synthesis of binuclear Schiff base metal complexes

The synthesized ligand (1 mmol) was dissolved in 20 ml of hot ethanol, added drop wise with continuous stirring to a solution of the metal salt (2 mmol) in 20 ml ethanol with constant stirring, followed by the addition of 2,2'-bipyridyl in ethanol and the mixture was boiled under reflux for 5 hrs. Then, the volume of the reaction mixture was reduced to 10 ml by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried *in vacuo*.

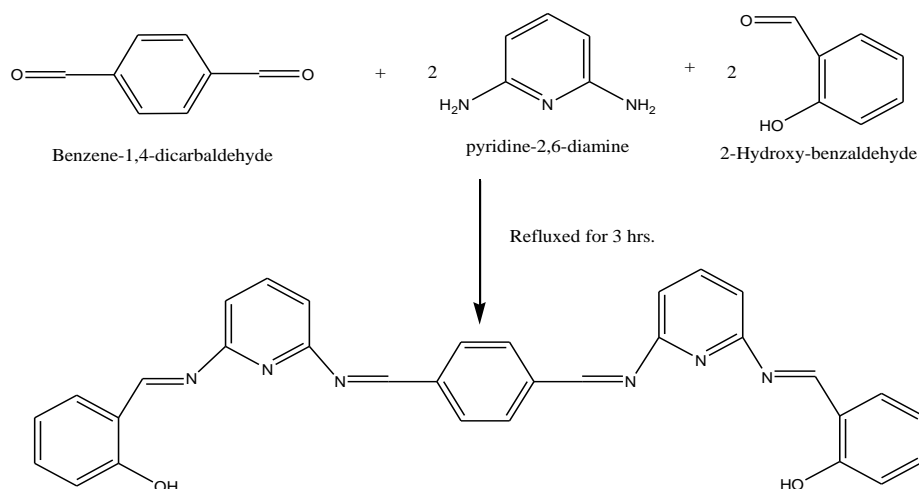


Fig. 1: Synthesis of Schiff base ligand

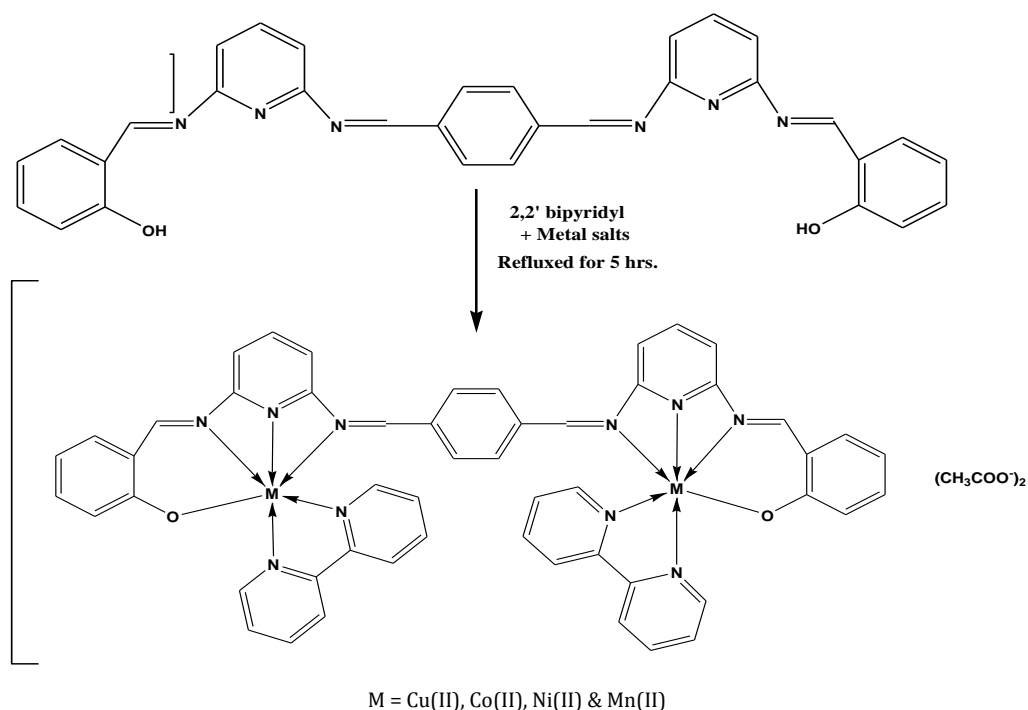


Fig. 2: Synthesis of Binuclear Schiff base Metal complexes

### ***In-vitro* antibacterial assay**

The biological activities of the newly synthesized Schiff base and its metal complexes have been studied for their antibacterial and antifungal activities by disc diffusion method [16,17]. The antibacterial activities were done by using the following organisms (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Klebsilla pneumonia*). These bacterial strains were chosen as they are the known pathogens of human body and used at 25, 50, 75 and 100  $\mu\text{mL}^{-1}$  concentrations in DMF. The bacteria were sub cultured in agar medium. The Petri dishes were incubated for 24 hrs at 37°C. Standard antibacterial drug (streptomycin) was also screened under similar conditions for comparison.

### **DNA cleavage activity**

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis [18]. The gel electrophoresis experiments were performed by incubation of the samples containing 40  $\mu\text{M}$  pUC18 DNA, 50  $\mu\text{M}$  metal complexes and 50  $\mu\text{M}$  H<sub>2</sub>O<sub>2</sub> in Tris-HCl buffer (pH

7.2) at 37°C for 2 hrs. After incubation, 1 $\mu\text{L}$  of loading buffer (bromophenol blue in H<sub>2</sub>O) was added to each tube and the mixed samples were loaded on 1% agarose gel. The electrophoresis was carried out for 2 hrs at 50V in Tris-acetic acid-EDTA buffer (pH 7.2). After electrophoresis, the gel was stained with 1 $\mu\text{g}/\text{cm}^3$  ethidiumbromide (EB) for 30 min prior to being photographed under ultraviolet light at 360 nm. The cleavage efficiency was measured by determining the ability of the complex to convert the supercoiled DNA (Form I) to nicked circular form (Form II) or linear form (Form III). All the experiments were performed at room temperature.

### **RESULTS AND DISCUSSION**

Analytical data of the Schiff base ligands and their binuclear metal complexes are given in Table.1.1 and are in well agreement with the expected values. All the Cu(II), Co(II), Ni(II) and Mn(II) complexes are colored non hygroscopic solids, stable in air. They are sparingly soluble in common organic solvents, but soluble in DMF and DMSO.

Table 1.1: Analytical data of the Schiff base ligands and its binuclear metal complexes

Compound	Molecular Formula	colour	Yield %	Melting Point (°)	% of Nitrogen		% of Metal	
					Cal	Exp	Cal	Exp
L	C <sub>32</sub> H <sub>24</sub> N <sub>6</sub> O <sub>2</sub>	Yellow	80	136	16.02	16.01	-	-
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>56</sub> H <sub>44</sub> Cu <sub>2</sub> N <sub>10</sub> O <sub>6</sub>	Dark green	70	>200	12.97	12.95	11.76	11.73
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>56</sub> H <sub>44</sub> Co <sub>2</sub> N <sub>10</sub> O <sub>6</sub>	Brown	75	>200	13.07	13.06	11.00	11.01
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>56</sub> H <sub>44</sub> Ni <sub>2</sub> O <sub>6</sub>	Brown	70	>200	13.07	13.08	10.96	10.98
[Mn <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	C <sub>56</sub> H <sub>44</sub> Mn <sub>2</sub> N <sub>10</sub> O <sub>6</sub>	Black	75	>200	13.18	13.16	10.33	10.31

### Molar conductance

One of the measurements commonly employed in the determination of charge type of the complexes is to determine the equivalent conductance at infinite dilution of those complexes. The molar conductance of binuclear Schiff base complexes were recorded in DMF 10<sup>-3</sup>M at room temperature (Table 1.2). The complexes showed molar conductance (135-145 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>). It was concluded from the results that complexes were electrolytic in nature. The molar conductance value suggested that the anions were outside the coordination sphere and not bonded to the metal ion therefore, these complexes may be formulated as [M<sub>2</sub>LY<sub>2</sub>]Z<sub>2</sub> where Y=2,2'-bipyridyl and Z = acetate ion.

Table 1.2: Molar conductance data of the Schiff base binuclear metal complexes

Compounds	Solvent	Molar conductance scm <sup>2</sup> mol <sup>-1</sup>	Type of electrolyte
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	DMF	135	1:2 electrolyte
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	DMF	142	1:2 electrolyte
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	DMF	147	1:2 electrolyte
[Mn <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	DMF	139	1:2 electrolyte

### IR spectra

The important infrared frequencies of Schiff base and its Cu(II), Co(II), Ni(II) and Mn(II) complexes are tabulated in table 1.3. The extent of shift depends on bond strength between the atoms. For the synthesized binuclear Schiff base complexes, we observed the following changes; the high intense band due to phenolic -OH appeared in the region at 3372 cm<sup>-1</sup> in the Schiff base was disappeared in the complexes [19,20]. The band at 1496 and 1462 cm<sup>-1</sup> are due to the CH deformation mode of vibrations of CH bond [21]. The band observed in the region 1274 cm<sup>-1</sup> for ν(C-O) stretching vibrations undergoes shift to higher wave number at

1287-1298 cm<sup>-1</sup> compared with free ligand band [22]. These observations support the formation of M-O bonds via deprotonation. So the H-bonded -OH groups have been replaced by the metal ion. The medium intense band in the range 1610 cm<sup>-1</sup> are observed due to ν(C=N) which has been shifted towards lower region at around 1604 cm<sup>-1</sup> in the complexes indicating the participation of the azomethine group in the complexes formation this shift is also due to the reduction of double bond character of carbon-nitrogen bond of azomethine group and indicates that C=N of the ligand coordinates to the metal through nitrogen and is further reflected by the appearance of new band at 430-490 cm<sup>-1</sup> (lower wave number region) due to ν(M-N). The bands near 1600 cm<sup>-1</sup> are assigned to aromatic ring, which are found to overlap with strong absorption band due to ν(C=N) azomethine group. The complex formation was further evidenced by the appearance of non-ligand band in the complexes around 535-570 cm<sup>-1</sup> assigned as ν(M-O) bands [23,24].

On the other hand, from the spectroscopic behaviour of metal complexes of pyridine, it is known that after complexation the ring deformation found at 567 and 402 cm<sup>-1</sup> in the free pyridine is shifted to higher frequencies [25], indicating coordination via the pyridine nitrogen, as previously reported for pyridine complexes [26]. Therefore, this shift is very useful in establishing the participation of pyridine in complex formation.

The band at 1444 cm<sup>-1</sup> and 1521 cm<sup>-1</sup> were due to symmetric stretching frequency and asymmetric frequency of acetate ion. This result predicts that the acetate ions were coordinated outside the coordination sphere.

These significant shifts of free ligand ν(C=N) to lower wave number region and ν(C-O) towards higher wave number region in metal complexes point to the bonding of the ligand to metal ion through phenolic oxygen and azomethine nitrogen and support for the coordination sites of the ligand.

Table 1.3: Infrared spectroscopic data of the Schiff base ligands and its binuclear metal complexes

Compound	Free-OH	-C=N	In-plane & Out-of-plane Pyridine ring deformations	C-O	M-O	M-N
L	3362.39	1620.39	567, 402	1274.78	-	-
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1606.13	633, 413.34	1288.46	537.55	446.22
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1605.86	643.56, 413.80	1293.14	507.42	439.25
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1604.09	638. 42, 422.51	1298.82	534.32	493.56
[Mn <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-	1604.74	652.36, 416	1287.68	575.66	435.69

### Electronic Spectra

In the spectra of the Schiff base ligand, the absorption band observed at 294 nm were assigned to intra-ligand π→π\* transition and the band at 386 nm were assigned due to n→π\* transition associated with the azomethine chromophore (-C=N) [27].

The dark green colour complexes of Cu(II) show the d-d transition bands in the range 690 nm<sup>-1</sup>. This spin allowed transitions are assigned due to <sup>2</sup>E<sub>g</sub>→<sup>2</sup>T<sub>2g</sub> [28,29]. This confirms distorted octahedral geometry around the metal ion.

The reaction of cobalt salt with the ligand yielded a binuclear Co(II) complex. The electronic spectrum of the obtained complex showed a band at 695 nm corresponding to <sup>4</sup>T<sub>1g</sub>(F)→<sup>4</sup>A<sub>2g</sub>(F)

transition which is consistent with the octahedral geometry of the complex [30].

Based on the electronic spectrum, nickel(II) complex, has the octahedral geometry with its characteristic features. The electronic spectrum of the complex (Table 1.4) showed two bands, the first band occurs at 570 nm which is corresponding to the <sup>3</sup>A<sub>2g</sub>→<sup>3</sup>T<sub>1g</sub>(P) transition. The other band occurs at 660 nm which is corresponding to the <sup>3</sup>A<sub>2g</sub>→<sup>3</sup>T<sub>2g</sub> electronic transition. The two transitions are consistent with an octahedral geometry of the complex [31].

The Mn(II) binuclear complex shows bands at 555, 694 nm respectively corresponds to <sup>6</sup>A<sub>1g</sub>→<sup>4</sup>E<sub>g</sub>(4D), <sup>6</sup>A<sub>1g</sub>→<sup>4</sup>T<sub>2g</sub>(4G) transitions which are compatible to an octahedral geometry around manganese(II) ion [32].

Table 1.4: Electronic Spectral data of Schiff base ligand and its binuclear metal complexes.

Compound	Electronic spectra (nm)			Geometry of the complex	
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L→M	d-d	
L	294	386	-	-	-
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	285	374	480	690	Octahedral
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	292	390	440	695	
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	281	342	445	570	
				660	
[Mn <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	293	346	430	555	
				694	

### Electron Spin Resonance

The ESR spectrum of the binuclear Schiff base Cu(II) complex exhibits two signals at  $g_{\perp}$ ,  $g_{\parallel}$  and  $g_{\text{aver}} = 1/3(g_{\parallel} + 2g_{\perp}) = 2.206$ , these values follows the same trend  $|g_{\parallel}| > g_{\perp} > g_e$  which suggest that the presence of unpaired electron  $dx_{2-y_2}$  orbital giving octahedral geometry[33].

In the axial spectra, the g-values are related with exchange interaction coupling constant (G) by the expression,  $G = |g_{\parallel}| - 2 / |g_{\perp}|$

- 2 reflects the spin-interaction between Cu(II) centers of the binuclear Cu(II) complex. According to Hathaway and Billing [34], if  $G > 4$ , the spin-exchange interaction is negligible and if it is less than 4 considerable spin-exchange interaction prevails. In the present case G value comes out to be 4.8, which indicated negligible exchange interaction between the two copper ions and evidenced the monomeric nature of the complex [35]. These data are in agreement with those obtained from the electronic spectra and confirm the octahedral geometry for Cu(II) Schiff base complexes.

Table 1.5: Electron Spin Resonance data of binuclear Cu(II) complexes.

Complex	$g_{\parallel}$	$g_{\perp}$	$g_{\text{av}}$	$\Delta g$	G
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	2.438	2.090	2.206	0.348	4.86

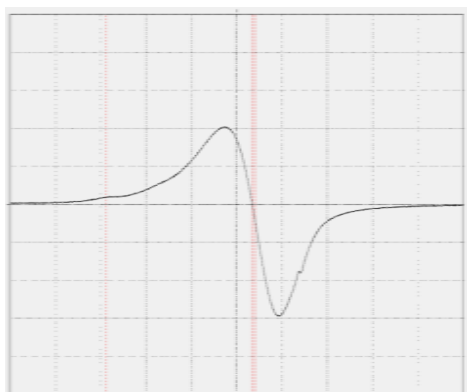


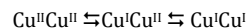
Fig. 3: ESR Spectra of binuclear Schiff base Cu(II) complex

### Cyclic voltammetry

The electrochemical behavior of Cu(II) complex was examined in DMF and TBAP as supporting electrolyte. The cyclic voltammogram of Cu(II) complex in  $10^{-3}$  M solution was recorded at room temperature in the potential range -2.0 V to +1.2 V with a scan rate of  $100 \text{ mVs}^{-1}$ .

The symmetrical binuclear Schiff base Cu(II) complexes are associated with two quasireversible reduction waves. The first reduction potential ranges from -1.3 to -1.4 V and the second redox potential lies in the ranges from -1.0 to -0.94 V [36,37]. This indicates that each process corresponds to single electron-transfer process, and this can be assigned to the redox couple  $\text{Cu}^{\text{II}}\text{Cu}^{\text{II}}/\text{Cu}^{\text{II}}\text{Cu}^{\text{I}}$ . The second quasi-reversible wave can be attributed to the

formation of the mixed valent  $\text{Cu}^{\text{II}}\text{Cu}^{\text{I}}/\text{Cu}^{\text{I}}\text{Cu}^{\text{I}}$  species. Based on these observations, it is reasonable to suggest that the reduction process may involve the stepwise redox processes depicted.



Similarly the waves of oxidation potential show a quasireversible. The  $E_{1/2}$  values indicate that each couple corresponds to one electron transfer process. On comparing the cyclic voltammograms, we observed that the variation in oxidation and reduction potential may be due to geometry of the complex which arises due to different organic ligand moieties coordinated to the metal ion [38].

The cyclic voltammograms of the nickel(II) complexes were recorded in the potential range 1.2 to -2.0 V. The dinickel complexes typically undergo two well-separated one electron reductions [39,40]. Controlled potential electrolysis was also carried out and the experiment reports that each couple corresponds to one-electron transfer process. The two reduction processes are assigned as follows:



All the nickel(II) complexes show two oxidation potential in the range of 0.3–1.10 V. The data of cyclic voltammograms of the complexes are summarized in Table 1.5.2. Controlled potential electrolysis experiment indicates that the two oxidation peaks are associated with stepwise oxidation process at nickel(II) center.

The data of cyclic voltammogram of binuclear Mn(II) and Co(II) complexes are tabulated in table 1.6.1 and 1.6.2. The  $\Delta E_p$  values suggest the existence of quasireversible couple. The  $E_{1/2}$  values indicate that each couple corresponds to one electron transfer process.

Table 1.6.1: Electrochemical data of binuclear Schiff base metal complexes in DMF medium (reduction)

Complexes	$E_{\text{pc}}$ (V)	$E_{\text{pa}}$ (V)	$E_{1/2}$ (V)	$\Delta E$ (mV)
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-1.3	-1.4	-1.35	100
	-1.0	-0.9	-0.95	100
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-1.1	-1.3	-1.2	200
	-0.9	-0.6	-0.75	300
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-1.1	-1.4	-1.25	200
	-0.8	-0.9	0.85	100
[Mn <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	-1.7	-1.8	-1.75	100
	-0.8	-0.5	-0.65	300

Table 1.6.2: Electrochemical data of binuclear Schiff base metal complexes in DMF medium (oxidation)

Complexes	E <sub>pc</sub> (V)	E <sub>pa</sub> (V)	E <sub>1/2</sub> (V)	ΔE (mV)
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	0.3	0.6	0.45	300
	0.9	1.0	0.95	100
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	0.3	0.5	0.4	200
	0.9	1.0	0.95	100
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	0.3	0.4	0.4	100
	1.05	1.1	1.075	50
[Mn <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	0.2	0.3	0.25	100
	0.9	1.15	1.02	250

### <sup>1</sup>H NMR spectra

The <sup>1</sup>H NMR Schiff base was recorded in DMSO at room temperature. Three different type of protons were identified i) resonance exhibits due to phenolic -OH protons around 10.26 ppm [41], ii) characteristic resonance due to azomethine proton in the Schiff base appears at 8.126 ppm was observed and iii) the other signals in the region 6.95–7.95 ppm exhibits due to aromatic protons. All these observations support the infrared conclusions.

### Magnetic studies

The magnetic moments of Cu(II), Co(II), Ni(II) and Mn(II) complexes were measured at room temperature. The magnetic moment for Cu(II) complex is 1.69 B.M. this clearly shows that there is no major spin interactions (spin only value-1.73 B.M.) [42]. Thus the present Cu(II) complex is devoid of any spin interaction with octahedral geometry. This finding is further confirmed from the clear resolution of the ESR spectra. The Co(II) complex showed the magnetic moment at 4.80 B.M. further corroborated the electronic spectral findings [43]. The observed magnetic moment of Ni(II) Complex at 2.94 B.M. which indicates the presence of two unpaired electrons. This further confirms the proposed geometry around the Ni(II) ion [44]. The Mn(II) complex show 5.89 B.M. at room temperature corresponding to five unpaired electrons which suggest octahedral geometry [45].

### Thermal analysis

The thermal decomposition of all complexes was investigated (Table 1.7). The TG curves indicate that the decomposition is a multiple step process and that the final residual weight corresponds to the metal oxide. The copper(II) complex decomposed in three steps with the temperature ranges from 210–425°C corresponding to the loss of 2CH<sub>3</sub>COO, 2 bipyridyl and five aromatic moiety with six nitrogen atoms, respectively.

The thermal decomposition of the complex with the molecular formula [Ni<sub>2</sub>(C<sub>52</sub>H<sub>38</sub>N<sub>10</sub>O<sub>2</sub>)]<sub>2</sub>·2OAc proceeds with three degradation step. In the estimated mass loss of the first step 10.99 % (Calcd. 11.06 %) could be attributed to the loss of acetate group. Whereas, in the second step the liberation of two bipyridyl unit respectively, shown in table 1.6. These decomposition takes place within the temperature range from 225–440°C and the decomposition is in progress from 440°C.

Observations of thermogram of Co(II) complex indicate that they are stable up to 200°C. Thereafter, they start decomposing and the weight loss observed in the temperature range 230–375°C roughly corresponds to elimination of two acetate molecules, two bipyridyl groups and ligand moieties.

Table 1.7: Thermogravimetric Data of binuclear Schiff base metal complexes.

Complexes	TG-range(°C)	Estimated (Calculated) in (%)	Assignment
		Mass loss	
[Cu <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	Stage-1 (210-225)	10.89 (10.92)	Loss of 2OAc ions
	Stage-II (225-275)	8.85 (8.89)	2 Bipyridyl group
	Stage-III (275-425)	48.52 (48.54)	Five aromatic moiety with six nitrogen atom.
	Above 425		Decomposition is in progress.
[Ni <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	Stage-1 (220-260)	10.99 (11.06)	Loss of 2OAc ions
	Stage-II (260-275)	8.91 (8.97)	2 Bipyridyl group
	Stage-III (275-440)	48.92 (48.98)	Five aromatic moiety with six nitrogen atom.
	Above 440		Decomposition is in progress.
[Co <sub>2</sub> L <sub>1</sub> X <sub>2</sub> ]Y <sub>2</sub>	Stage-1 (230-255)	11.049 (11.056)	Loss of 2OAc ions
	Stage-II (255-300)	8.92 (8.97)	2 Bipyridyl group
	Stage-III (300-375)	48.93 (48.96)	Five aromatic moiety with six nitrogen atom.
	Above 375		Decomposition is in progress.

### Molecular Modelling

The modern major advances in the computational chemistry tools provides an alternative, approximate, approach for obtaining the three dimensional structures of the complexes in the case of absence of X-ray crystal structure.

The optimized geometry of the Binuclear Schiff base Cu(II) complexes is represented (fig.4) with some of selected structural

parameters (bond length and angles). The values of the bond length (Å) and angles (°) are shown in table 1.8.

Four NNNO donor atoms from the approximately planar Schiff base ligand (binding via two imine nitrogen, N atom of pyridine ring, phenolic oxygen) and the two nitrogen atoms from the bipyridyl molecule forms octahedral arrangement around the two Cu atoms.

Metal-azomethine nitrogen N64-Cu73, N49-Cu74, N41-Cu73 and N42-Cu74 distances are 1.8889 Å, 1.9361 Å, 2.0166 Å and 2.000 Å respectively. The bond angles around Cu atom is 93.972°, 91.1264°, 73.7023°, 157.9182°, 83.594°, 146.2494°, 100.6826° and 99.3517° corresponds to N10-Cu73-O72, N9-Cu73-O72, N9-Cu73-N64, N9-Cu73-N41, N10-Cu73-N41, N27-Cu74-N42, N27-Cu74-O57, N49-

Cu74-O57 respectively and the remaining angles are shown in table 1.8. According to these parameters we could determine each Cu atom is surrounded by an octahedral formed by the two azomethine Nitrogen, one pyridine N atoms, one hydroxyl group and two nitrogen of bipyridyl group respectively also proves to have no M-M bonding mode in this binuclear complexes.

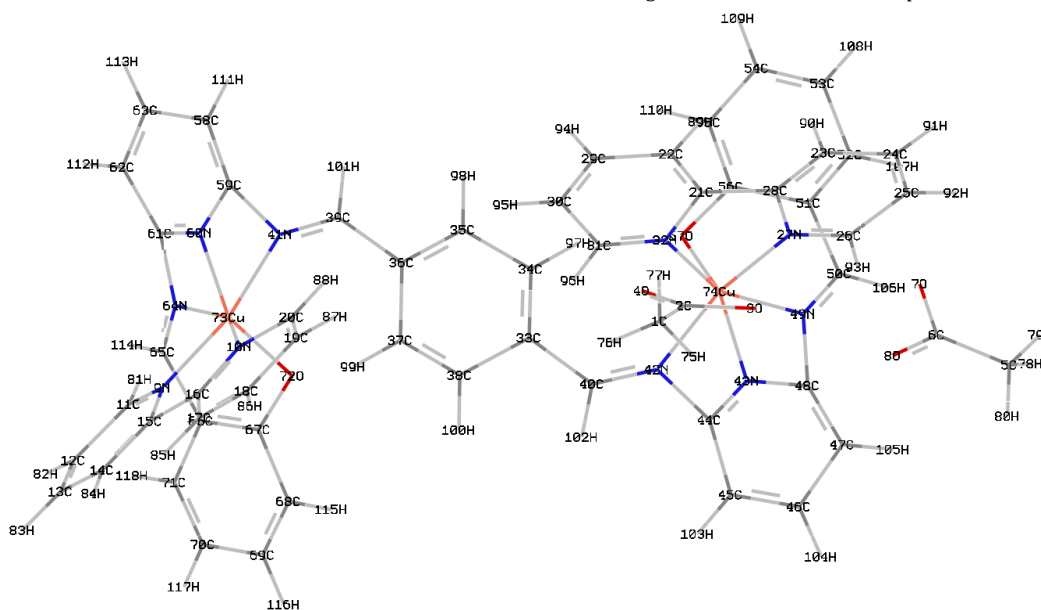


Fig. 4: Optimized structure of Binuclear Cu(II) Schiff base complex

Table 1.8: Optimized geometric data for  $[Cu_2L_1X_2]Y_2$  using B3LYP/6-311++G(d,p).

Bond angle	Value (°)	Bond angle	Value (°)	Bond length	Value (Å)
N9-Cu73-N10	81.123	N32-Cu74-N42	86.2601	N9-Cu73	1.8928
N9-Cu73-N41	157.9182	N32-Cu74-N43	133.4611	N27-Cu74	1.8909
N9-Cu73-N60	116.9223	N32-Cu74-N49	153.819	N10-Cu73	1.9253
N9-Cu73-N64	73.7023	N42-Cu74-O57	109.922	N32-Cu74	1.9097
N9-Cu73-O72	91.1264	N43-Cu74-N49	61.0228	N42-Cu74	2.0000
N10-Cu73-N41	83.594	N43-Cu74-O57	131.9915	N41-Cu73	2.0166
N10-Cu73-N60	123.5471	N49-Cu74-O57	99.3517	N43-Cu74	1.8353
N10-Cu73-N64	151.3803	N42-Cu74-N49	116.1234	N49-Cu74	1.9361
N10-Cu73-O72	93.972	N42-Cu74-N43	57.0508	O57-Cu74	1.8204
N41-Cu73-N60	60.1424	N27-Cu74-O57	100.6826	N60-Cu73	1.8137
N41-Cu73-N64	115.9621	N32-Cu74-O57	84.1892	N64-Cu73	1.8889
N41-Cu73-O72	105.7914	N27-Cu74-N49	70.8976	O72-Cu73	1.8103
N60-Cu73-N64	60.088	N27-Cu74-N32	82.9404		
N60-Cu73-O72	134.6155	N27-Cu74-N42	146.2494		
N64-Cu73-O72	99.8272	N27-Cu74-N43	110.9985		

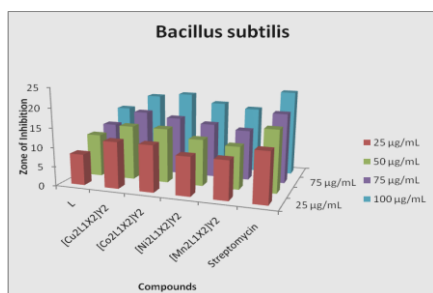
#### Antibacterial activity

The biological activity of the Schiff base ligands, their binuclear complexes and streptomycin (as a standard compound) were tested against bacteria because bacteria can achieve resistance to antibiotics through biochemical and morphological

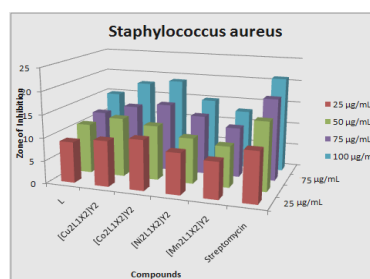
modifications [46-47]. The remarkable activity of the two Schiff base ligands and their complexes may be due to pyridyl-N and the hydroxyl groups, which may play an important role in the antibacterial activity [48], as well as the presence of two imine groups which imports in elucidating the mechanism of transformation reaction in biological system.

Table 1.9: Antimicrobial activity of ligand and its binuclear metal complexes

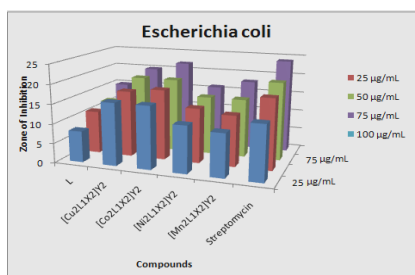
Compound	Diameter of inhibition zone (mm)															
	Bacillus subtilis				Staphylococcus aureus				E-Coli				Klebsilla pneumonia			
	Concentrations (µg/mL)															
L	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
$[Cu_2L_1X_2]Y_2$	8	11	12	15	9	11	12	15	8	11	12	15	7	7	9	11
$[Co_2L_1X_2]Y_2$	12	14	16	19	10	13	14	18	16	17	19	20	10	12	15	16
$[Ni_2L_1X_2]Y_2$	12	14	15	20	11	12	15	19	16	18	19	22	11	13	13	18
$[Mn_2L_1X_2]Y_2$	10	12	14	18	9	10	13	15	12	14	15	16	9	11	12	17
Streptomycin	10	11	13	17	8	9	11	13	11	13	15	18	8	10	13	16
	13	16	18	22	11	15	18	21	14	18	20	24	12	16	19	24



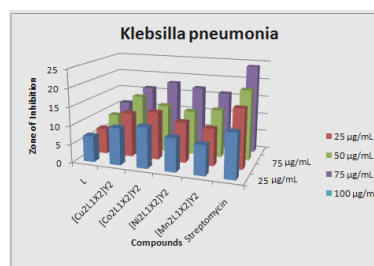
**Fig. 5:** Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Bacillus subtilis*



**Fig. 6:** Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Staphylococcus aureus*



**Fig. 7:** Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Escherichia coli*



**Fig. 8:** Antibacterial activity of Schiff base ligand and its binuclear Schiff base metal complexes against *Klebsilla pneumonia*

The activity of Schiff base ligand and their complexes increases as the concentration increases because it is a well known fact that concentration plays a vital role in increasing the degree of inhibition [49]. Against all the organisms, ligand did not exhibit any remarkable activity compared with standard streptomycin, whereas all the complexes showed moderate to high activities. It is suggested that the complexes having antimicrobial activity may act either by killing the microbe or by inhibiting multiplication of the microbe by blocking their active sites [50].

#### DNA Cleavage

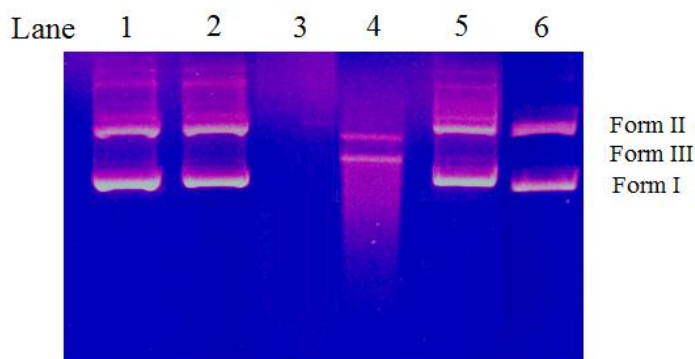
The cleavage activity of complexes has been assessed by their ability to convert super coiled pUC18 DNA from Form I to Form II and Form III by gel electrophoresis. Figure 6 shows the cleavage pattern of pUC18 DNA. The DNA cleavage efficiency of the complexes was due to the different binding affinity of the complex to DNA.

The control experiments did not show any apparent cleavage of DNA (lane 1 & 2).  $[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2$  complex in the presence of  $\text{H}_2\text{O}_2$  at higher concentration (50  $\mu\text{M}$ ) shows more cleavage activity. The supercoiled plasmid DNA was completely degraded (lane 3). This shows that a slight increase in the concentration over the optimal value led to extensive degradations, resulting in the disappearance of bands on agarose gel [51,52].  $[\text{Co}_2\text{L}_1\text{X}_2]\text{Y}_2$  complex in the presence

of  $\text{H}_2\text{O}_2$  resulting the conversion of open circular form (Form II) into linear form (Form III) (lane 4).  $[\text{Ni}_2\text{L}_1\text{X}_2]\text{Y}_2$  complex in the presence of  $\text{H}_2\text{O}_2$  (lane 5) at higher concentration (50  $\mu\text{M}$ ) shows cleavage activity in which supercoiled DNA (Form I) cleaved and converted to open circular form (Form II). The  $[\text{Mn}_2\text{L}_1\text{X}_2]\text{Y}_2$  complex in the presence of  $\text{H}_2\text{O}_2$  (lane 6) at higher concentration (50  $\mu\text{M}$ ) shows cleavage activity in which supercoiled DNA (Form I) cleaved and supercoiled form converted to open circular form (Form II).

The general oxidative mechanisms proposed account of DNA cleavage by hydroxyl radicals via abstraction of a hydrogen atom from sugar units predicts the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed. It has been shown earlier that the cleavage is inhibited by free radical scavengers, implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metallo complexes bound hydroxyl radical or a peroxy species generated from the co-reactant  $\text{H}_2\text{O}_2$ . This results in oxidative attack on the deoxyribose moiety at the C-1 hydrogen.

These results indicate that the metal ions play an important role in the cleavage of isolated DNA. As the compound was observed to cleave DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome [53].



**Fig. 9:** Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by  $\text{H}_2\text{O}_2$  and metal complexes. From left to right: Lane 1- DNA alone; Lane 2- DNA alone +  $\text{H}_2\text{O}_2$ ; Lane 3- DNA +  $[\text{Cu}_2\text{L}_1\text{X}_2]\text{Y}_2$  +  $\text{H}_2\text{O}_2$ ; Lane 4- DNA +  $[\text{Co}_2\text{L}_1\text{X}_2]\text{Y}_2$  +  $\text{H}_2\text{O}_2$ ; Lane 5- DNA +  $[\text{Ni}_2\text{L}_1\text{X}_2]\text{Y}_2$  +  $\text{H}_2\text{O}_2$ ; Lane 6- DNA +  $[\text{Mn}_2\text{L}_1\text{X}_2]\text{Y}_2$  +  $\text{H}_2\text{O}_2$ .

## CONCLUSION

Single crystals of the complexes could not be isolated from any common solvent. However, the analytical, spectroscopic, magnetic and TG data enabled the prediction of the possible structures.

## ACKNOWLEDGEMENTS

Financial support from DST INSPIRE Fellowship, New Delhi, is gratefully acknowledged. We are also thankful to our Supervisor and professors of department of chemistry, Periyar University - Salem for further support.

## REFERENCES

- Balsells J, Mejorado L, Phillips M, Ortega F, Aguirre G, Somanathan R, Walsh P.J, Synthesis of Chiral Sulfonamide / Schiff Base Ligands, *Tetrahedron Asymm* 1998; 9: 4135.
- Sima J, Mechanism of Photoredox Reactions of Iron(III) Complexes Containing Salen-type Ligands, *Crao. Chem. Acta* 2001; 74: 593.
- Viswanathamurthi P, Natarajan K, Ruthenium(II) Carbonyl Complexes Containing Tetradentate Schiff Bases and Their Catalytic Activities, *Synth. React. Inorg. Met.-Org. Chem* 2006; 36:415-418.
- Ren S, Wang R, Komastu K, Krause P.B, Zyrianov Y, McKenna C. E, Csipke C, Tokes Z. A, Lien E. J, Synthesis, biological evaluation, and quantitative structure-activity relationship analysis of new Schiff bases of hydroxysemicarbazide as potential antitumor agents, *J. Med. Chem* 2002; 45: 410.
- Tarafder M. T. H, Ali M. A, Saravana N, Weng W. Y, Kumar S, Tsafe N. U, Crouse K. A, Coordination chemistry and biological activity of nickel(II) and copper(II) ion complexes with nitrogen-sulphur donor ligands derived from S-benzylthiocarbamate (SBDTC), *Transition Met Chem* 2001; 26: 613.
- Colman J, Hegedu L. S, Principles and Applications of Organotransition Metal Chemistry, University Science Book, California, 1980.
- E. Akila, M. Usharani and R. Rajavel, Design, synthesis and interpretation of binuclear schiff base metal complexes and their application; antibacterial activity, *International Journal of Inorganic and Bioinorganic Chemistry*, 2012;2 (2): 15-19.
- Mahajan K, Swami M, and Singh R.V, Microwave synthesis, spectral studies, antimicrobial approach and coordination behavior of antimony(III) and bismuth(III) compounds with benzothiazoline, *Russ. J. Coord. Chem* 2009; 35: 179-185.
- Liu J, Zhang T.X, Ji L.N, DNA-binding and cleavage studies of macrocyclic copper(II) complexes, *J. Inorg. Biochem* 2002; 91:269-276.
- Mehmet Tumer, Duygu Ekinci, Ferhan Tumer, Akif Bulut, Synthesis, characterization and properties of some divalent metal(II) complexes: Their electrochemical, catalytic, thermal and antimicrobial activity studies, *Spectrochim. Acta Part A* 2007; 67: 916-929.
- Sartaj Tabassum, Mehvash Zaki, Farukh Arjmand, Iqbal Ahmad, Synthesis of heterobimetallic complexes: In vitro DNA binding, cleavage and antimicrobial studies, *Journal of Photochemistry and Photobiology B: Biology* 2012; 114: 108-118.
- Debojyoti Lahiri, Ritankar Majumdar, Ashis K Patra And Akhil R Chakravarty, Anaerobic DNA cleavage in red light by dicopper(II) complexes on disulphide bond activation, *J. Chem. Sci* 2010;122 (3) 321-333.
- Costamagna J, Vargas J, Latorre R, Alvarado A, Mena G, Coordination compounds of copper, nickel and iron with Schiff bases derived from hydroxynaphthaldehydes and salicylaldehydes, *Coord. Chem. Res* 1992;119: 67-88.
- Schlegel H.B., Optimization of equilibrium geometries and transition structures, *J. Comput. Chem* 1982;3: 214-218.
- Peng B, Chao H, Sun B, Li H, Gao F, Ji L.N, Synthesis, DNA-binding and photocleavage studies of cobalt(III) mixed-polyppyridyl complexes: [Co(phen)<sub>2</sub>(dpta)]<sup>3+</sup> and [Co(phen)<sub>2</sub>(amtp)]<sup>3+</sup>, *J. Inorg. Biochem* 2007; 101: 404-411.
- Hassan A. M, Nassar A. M, Hussien Y. Z & Elkmash A. N, Synthesis, Characterization and Biological Evaluation of Fe (III), Co (II), Ni(II), Cu(II), and Zn(II) Complexes with Tetradentate Schiff Base Ligand Derived from Protocatechualdehyde with 2-Aminophenol, *Appl Biochem Biotechnol* 2012; 167:581-594.
- Collee J. G, Duguid J. P, Farser A. G, Marmion B. D (Eds.), *Practical Medical Microbiology*, Churchill Livingstone, New York, 1989.
- Raman N, Mitu L, Sakthivel A, and Pandi M. S. S, Studies on DNA cleavage and antimicrobial screening of transition metal complexes of 4-aminoantipyrine derivatives of N<sub>2</sub>O<sub>2</sub> type, *J. Iran. Chem. Soc*, 2009; 6(4):738-748.
- Magdy Shebl, Saied M.E. Khalil, Saleh A. Ahmed, Hesham A.A. Medien, Synthesis, spectroscopic characterization and antimicrobial activity of mono-, bi- and tri-nuclear metal complexes of a new Schiff base ligand, *J. Mol. Struct* 2010; 980: 39-50.
- Adel A. A. Emara, Azza A. A. Abou-Hussen, Spectroscopic studies of bimetallic complexes derived from tridentate or tetradentate Schiff bases of some di- and tri-valent transition metals, *Spectrochim. Acta Part A* 2006;64: 1010-1024.
- Makode J. T and Aswar A. S, Synthesis, characterization, biological and thermal properties of some new Schiff base complexes derived from 2-hydroxy-5-chloro-acetophenone and S-methylthiocarbamate, *Indian J. chem* 2004; 43(A):2120-2125.
- Ravanasiddappa M, Sureshg T, Syed K, Radhavendray S. C, Basavaraja C and Angadi S. D, "Transition Metal Complexes of 1, 4 (2-Hydroxyphenyl -1-yl) Diimino Azine, Synthesis, Characterization and Antimicrobial Studies, *E-J. chem* 2008;5(2):395-403.
- Raju M. Patil, Synthetic, structural and biological Properties of binuclear complexes with some Schiff bases, *Acta Poloniae Pharmaceutica Drug Research* 2007; 64(4):345-353.
- Tahir Ali Khan and Minnat Shagufta, Transition metal ion directed bimetallic macrocyclic complexes, *Transition Met. Chem* 1999; 24: 669-671.
- Nakamoto K, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, fourth ed., Wiley, New York, 1980.
- West D. X, Swearingen J. K, Martinez J. V, Ortega S. H, El-Sawaf A. K, Meurs F. V, Castineiras A, Garcia I, Bermejo E, Spectral and structural studies of iron(III), cobalt(II,III) and nickel(II) complexes of 2-pyridineformamide N(4)-methylthiosemicarbazone, *Polyhedron* 1999;18: 2919.
- Lakhdar Sibous, Embarek Bentouhami, Amor Maiza, Gilles M. Bouet, Mustayeen Ahmed Khan, Synthesis, Characterization and Electrochemical Behavior of CoII, NiII and CdII Complexes with N2O2 Donor Ligands Derived from 4,4'-Diaminobiphenyl and 2-Hydroxybenzaldehyde or 2,4-Dihydroxybenzaldehyde, *J Solution Chem* 2010; 39:511-521.
- Shriodkar S.G., Mane P.S. and Chondhekar T.K., synthesis and fungi toxic studies of Mn(II),Co(II),Ni(II) and Cu(II) with some heterocyclic Schiff base ligands, *Ind. J. Chem* 2001; 40A: 1117.
- Chandra S, Gupta L.K, Agrawal S, Synthesis spectroscopic and biological approach in the characterization of novel [N4] macrocyclic ligand and its transition metal complexes, *Trans. Met. Chem* 2007;32(5): 558-563.
- Salib K.A.R, Stefan S.L, Abu El-Wafa S.M, El-Shafiy H.F, Metal Complexes Of Novel Symmetrical Schiff Base Ligands, *Synth. React. Inorg. Met.-Org. Chem* 2001; 31(5): 895.
- Khalil S. M. E, Mashaly M. M, Emara A. A. A, Copper(II), Nickel(II), Oxovanadium(IV) and Dioxouranium(VI) Complexes of Novel Asymmetric Tetradentate Schiff Base Ligands Derived from 6-Methyl-3-Formyl-4-Hydroxy-2-(1H)-Quinolone, *Synth. React. Inorg. Met.-Org. Chem* 1995; 25(8):1373.
- Mohamad jabber Al-Jeboori, Hasen ahmed Hasan, Worood A. Jafer Al-Sa'idi, Formation of polymeric chain assemblies of transition metal complexes with a multidentate Schiff-base, *Transition Met. Chem* 2009; 34(6):593-598.
- Mishra A. P, Mishra R. K and Shrivastava S. P, Structural and antimicrobial studies of coordination compounds of VO(II), Co(II), Ni(II) and Cu(II) with some Schiff bases involving 2-amino-4-chlorophenol, *J. Serb. Chem. Soc* 2009; 574: 523-535.
- Sulekh Chandra, Lokesh Kumar Gupta, Electronic, EPR, magnetic and mass spectral studies of mono and homo-binuclear Co(II) and Cu(II) complexes with a novel macrocyclic ligand, *Spectrochim. Acta Part A* 2005; 62:1102-1106.



35. Hathaway B.J, Billing D.E, The electronic properties and stereochemistry of mono-nuclear complexes of the copper(II) ion, *Coord. Chem. Rev* 1970; 5: 143-207.
36. Shirin Z, Mukherjee R. M, Synthesis, spectra and electrochemistry of ruthenium(III) complexes with cage-like Schiff-base ligands, *Polyhedron* 1992;11(20): 2625.
37. Shyamala A, Chakravarty A.R, Synthesis, structure and electrochemical properties of complexes with a ( $\mu$ -oxo)bis-( $\mu$ -carboxylato)diruthenium(III) core, *Polyhedron* 1993;12(12):1545.
38. Chandra S, Gupta L. K, Sangeetika, Synthesis, Physicochemical and Electrochemical Studies on Mn(II), Co(II), Ni(II), and Cu(II) Complexes with an N-Donor Tetradentate (N4) Macrocyclic Ligand Derived from Ethyl Cinnamate, *Synth. React. Inorg. Met.-Org. Chem* 2004; 34(9):1591.
39. Wada H, Aono T, Moudo K, Ohba M, Makstumoto N, Okawa H, Macrocyclic heterodinuclear NiMn and CuMn complexes: crystal structure and electrochemical behaviour, *Inorg. Chim. Acta* 1996;246:13-21.
40. Manonmani J, Kandaswamy M, Synthesis, characterization and electrochemical studies of unsymmetrical macrocyclic mono and binuclear nickel(II) complexes, *Polyhedron* 2003; 22: 989-996.
41. Raman N, Ravichandran S and Thangaraja C, Copper(II), cobalt(II), nickel(II) and zinc(II) complexes of Schiff base derived from benzil-2,4-dinitrophenylhydrazone with aniline, *J. Chem. Sci* 2004;116 (4) 215-219.
42. El-Dissouky A, Hindawey A.M and Abdel-Salam A, Metal chelates of heterocyclic nitrogen-containing ketones. XIX. Some acetato- and nitrate-copper(II) complexes of phenyl-2 picolyl ketone hydrazone and phenyl-2-picolyl ketone phenyl hydrazone, *Inorg. Chem. Acta* 1986; 118: 109.
43. Cotton F.A, Wilkinson G, *Advanced Inorganic Chemistry: A Comprehensive Text*, 4th ed., John Wiley and Sons, New York, 1986.
44. Singh D.P, Vandna Malik, Krishan Kumar, Chetan Sharma, Aneja K.R, Macrocyclic metal complexes derived from 2,6-diaminopyridine and isatin with their antibacterial and spectroscopic studies, *Spectrochim. Acta Part A* 2010;76: 45-49.
45. Toshi Nagata, Takayuki Nagasawa, Sergei K. Zharmukhamedov, Vyacheslav V. Klimov, Suleyman I. Allakhverdiev, Reconstitution of the water-oxidizing complex in manganese depleted photosystem II preparations using synthetic binuclear Mn(II) and Mn(IV) complexes: production of hydrogen peroxide, *Photosynth Res* 2007;93:133-138.
46. Mishra A. P, Mishra R. K, and Mrituanjay D. Pandey, Synthetic, Spectral, Structural and Antimicrobial Studies of Some Schiff Bases 3-d Metal Complexes, *Russ. J. Inorg. Chem* 2011;56(11): 1757-1764.
47. Keerthi kumar. C.T, Keshavayya J, Rajesh T and Peethambar S.K, Synthesis, characterization and biological activity of heterocyclic azo dyes derived from 2-aminobenzothiazole, *Int J Pharm Pharm Sci* 2013;5(1): 296-301.
48. Ispir E, Toroglu S, Kayraldrz A, Syntheses, characterization, antimicrobial and genotoxic activities of new Schiff bases and their complexes, *Transition Met Chem* 2008; 33:953-960.
49. Alka Choudharya, Renu Sharma, Meena Nagar, Synthesis, characterization and antimicrobial activity of mixed ligand complexes of Co (II) and Cu (II) with N,O/S donor ligands and amino acids, *Int Res J Pharm Pharmacol* 2011;1(6):172-187.
50. Tagenine Jeewoth, Minu G. Bhowon and Henri Li Kam Wah, Synthesis, characterization and antibacterial properties of Schiff bases and Schiff base metal complexes derived from 2,3-diamino-pyridine, *Transition Met Chem* 1999; 24: 445-448.
51. Rakesh sahu, sonal tiwari and gunjan kalyani, Thiazolidinone based 2,5-disubstituted-1,3,4-thiadiazole: synthesis and Antimicrobial evaluation, *Int J Pharm Pharm Sci* 2013; 5(1): 290-291.
52. P.Jayaseelan, S. Prasad, S.Vedanayaki and R. Rajavel, Synthesis, Spectral Characterization, anti-microbial activities, DNA binding and cleavage studies of new binuclear Schiff base metal complexes derived from 3,3' diaminobenzidine, *Arabian Journal of Chemistry*, doi;10.1016/j.arabjc.2011.07.029.
53. Kulkarni A, Patil S A & Badami P S, Synthesis, characterization, DNA cleavage and in vitro antimicrobial studies of La(III), Th(IV) and VO(IV) complexes with Schiff bases of coumarin derivatives, *Eur J Med Chem* 2009;44:2904.