

## Research Article

# Synthesis of Mononuclear Schiff Base Cu(II), Ni(II), Co(II) and Mn(II) Complexes and their Application for DNA Cleavage and Antibacterial Agent

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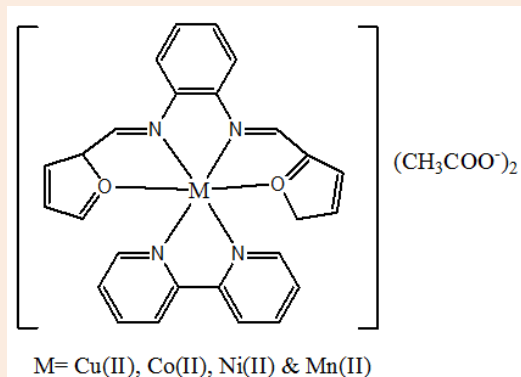
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## Abstract

New series of mononuclear Schiff base Cu(II), Co(II), Ni(II) and Mn(II) complexes have been synthesized with Schiff base derived from *o*-phenylenediamine, furfuraldehyde and 2,2'-bipyridyl. The chelating tendency of the metal complexes have been analysed and elucidated using analytical, spectral (IR, UV, <sup>1</sup>H NMR, ESR) and magnetic studies. These data prove that the Schiff base complexes show the octahedral geometry. Schiff base and their metal complexes have been tested for their antibacterial activities. These complexes show adequate growth inhibitory activity against bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia* than the free ligands. These complexes were determined by the nucleolytic cleavage activities on pUC18 plasmid DNA using gel electrophoresis in the presence of H<sub>2</sub>O<sub>2</sub> and the complexes are to be found to good in nuclease activity.

**Keywords:** Furfuraldehyde, 2, 2' bipyridyl, nuclease activity, *Staphylococcus aureus*.



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## Introduction

The notable developments in the field of bioinorganic chemistry have increased interest in Schiff base complexes, since they have been recognized and considered as biologically significant species [1]. A lucid control of nuclearity of transition metal complexes is important to design systems with the favourite properties, as some of these applications entail the presence of O and N donor atoms in the specific complex [2].

Ligands carrying variable donor functionalities such as, O and N in their structural framework have been studied for many years and continued in the area where active research is going on to understand the properties of donors that offer an idea to design metal complexes for precise purposes and specific applications [3]. The chemistry of Schiff base ligands contains 'strong' donor atoms (like N and O) is quite interesting because of the framework of ligands and will offer insight into competitive coordinating behaviour between the metal centre and the hard donors [4] and also stabilized with low as well as high oxidation states of a central metal atom. Such molecular systems have their own importance in transition metal catalyzed asymmetric synthesis [5]. The polydentate ligands carrying N<sub>4</sub>N<sub>2</sub>,

$N_4O_2$  are well noticed and among them, a few systems have shown excellent catalytic, biological, environmental and material properties [6].

The design and synthesis of a novel ( $N_4O_2$ ) molecular system, carrying both strong donors N and O in the framework of ligand and its chemistry towards Cu(II), Co(II), Ni(II) and Mn(II) ions are dealt with. The findings on a DNA cleavage and antibacterial activities using the synthesized complexes are reported.

## Materials and Methods

### Measurements

Elemental analyses for C, H and N were carried out using a Perkin-Elmer 2400-II elemental analyzer. UV-Vis spectra were obtained in DMF on a Perkin-Elmer Lambda 40(UV-Vis) spectrometer in the range 200-800nm. FT-IR data were recorded as KBr disc using Thermo Nicolet, Avatar 370 model spectrometer in the range 4000-400 $cm^{-1}$ . Molar conductance of the complexes in DMF was measured using an Elico model conductivity meter. Magnetic susceptibility measurements were carried out by employing the Gouy method at room temperature. NMR signals were got from Bruker Avance III, 400MHz model spectrometer. EPR spectra of compounds were recorded on an E-112 ESR Spectrometer with Xband microwave frequency (9.5GHz).

### Synthesis of Schiff base

The Schiff base ligand was synthesized by adding o- phenylenediamine (1mM) in 20ml of ethanol and furfuraldehyde (2mM) in 20ml ethanol. The mixture was refluxed for 2-3hrs. Then solution of the ligand was kept for slow evaporation and coloured precipitate was collected and then dried in air.

### Synthesis of mononuclear Schiff base Metal complexes

The ethanolic solution of synthesized ligand (1mM) was added drop wise in to an ethanolic solution of the metal salt (1mM) with constant stirring, followed by the addition of 2, 2' bipyridyl (1mM). The mixture was boiled under reflux for 3-5hrs. Then, the volume of the reaction mixture was reduced by evaporation. The precipitated complexes were filtered off, washed with ethanol and then dried in *vacuo*.

### Anti-bacterial activity

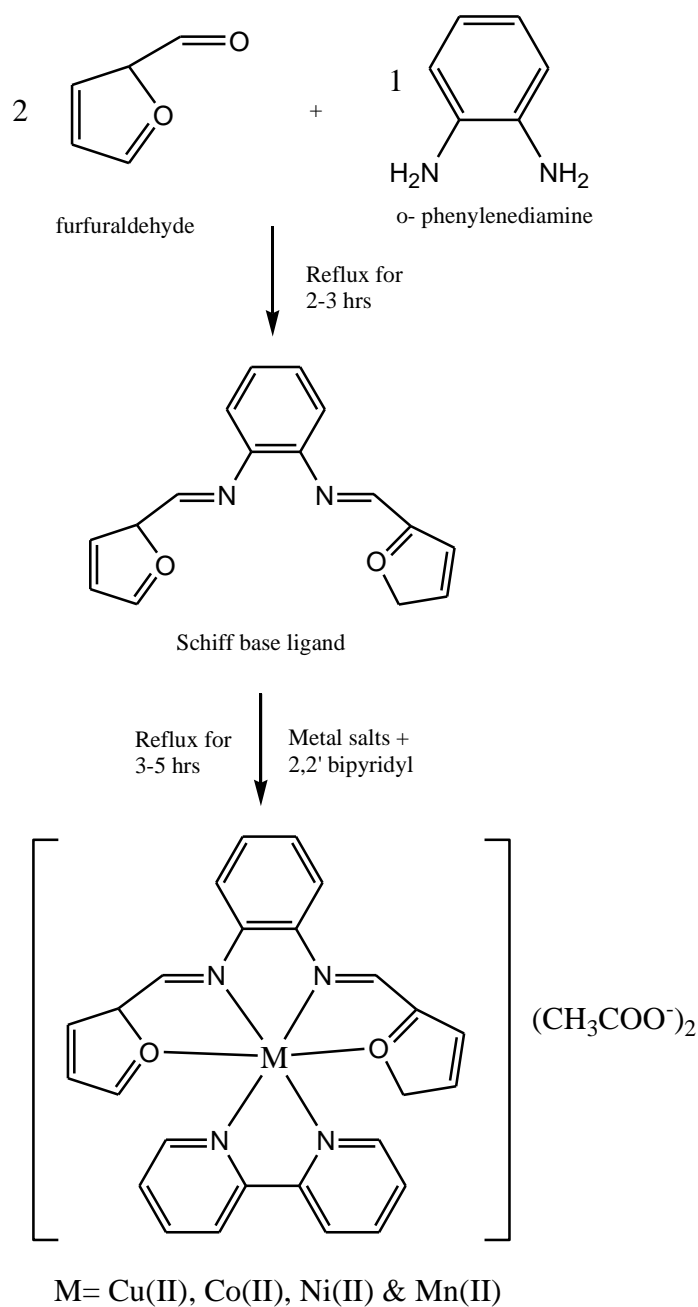
The anti-bacterial activities were carried out at Progen Lab at Salem, India. For the determination of the activity of the synthesized metal complexes against the sensitive organisms standard disc-agar diffusion method [7] is adopted. After testing, compounds were dissolved in DMF (which have no inhibition activity), to get concentration of 100 $\mu g/mL$ . The test was performed using medium potato dextrose agar contains infusion of 200g potatoes, 6g dextrose and 15g agar. Uniform size filter paper disks (three disks per compound) were impregnated by equal volume from the specific concentration of dissolved tested compounds and carefully placed on incubated agar surface. After incubation for 36hrs at 27°C inhibition of the organisms evidenced by clear zone surrounding each disk was measured and used to calculate mean of inhibition zones. *Streptomycin* was used as a standard.

### Agarose Gel electrophoresis

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis. The gel electrophoresis experiments were achieved by incubation of the samples containing 40 $\mu M$  pUC18 DNA, 50 $\mu M$  metal complexes and 50 $\mu M$   $H_2O_2$  in tris-HCl buffer (pH 7.2) at 37°C for 2hrs. After incubation, the samples were electrophoresed for 2hrs at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 $\mu g/cm^3$  ethidium bromide (EB) and photographed under ultraviolet light at 360nm. All the experiments were carried out at room temperature [8].

## Results and Discussion

The data collected from various analytical methods of the newly prepared Schiff base ligands and their mononuclear metal complexes are listed in Table 1 and well agree with the predicted values. All the complexes from Cu(II), Co(II), Ni(II) and Mn(II) are coloured, non-hygroscopic solids and very stable in air. They are slightly soluble in common organic solvents but soluble in DMF and DMSO. The analytical data show that the complexes may be expressed as [MLX], where L = Schiff base X= 2, 2' bipyridyl and M = Cu(II), Co(II), Ni(II) and Mn(II). The solubility of the complexes in DMF acceptable calculation of the molar conductivity ( $\Lambda_m$ ) of  $10^{-3}$  M solutions at 25°C shows that all the complexes are observed as 1:2 type electrolyte in DMF solution.



**Figure 1** Proposed structure of Schiff base metal complexes

**Table 1** Analytical data of the Schiff base ligand and its mononuclear metal complexes

Com- pound	Molecular Formula	colour	Yield %	Melt- ing Point (°)	% of Nitrogen		% of Metal		Molar conductan- ce $\Lambda_m$ ( $\text{s cm}^2$ $\text{mol}^{-1}$ )
					Cal	Exp	Cal	Exp	
L	$\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_2$	Yellow	80	140	9.88	9.86	-	-	-
[CuLY]Z <sub>2</sub>	[Cu(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	Dark Green	70	>200	11.15	11.14	12.64	12.63	132
[NiLY]Z <sub>2</sub>	[Ni(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	Brown Black	75	>200	11.26	11.23	11.80	11.79	145
[CoLY]Z <sub>2</sub>	[Co(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	Brown	70	>200	11.25	11.23	11.83	11.82	148
[MnLY]Z <sub>2</sub>	[Mn(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	Black	75	>200	11.34	11.33	11.12	11.10	151

Where L= Ligand, Y= 2, 2' bipyridyl and Z = acetate ion.

### IR spectra

The IR spectra provide appreciated information regarding the coordinating sites of Schiff base ligand which has been previously discussed by Raman et al [9]. The IR spectra of the complexes were compared with that of the free ligand to determine the changes that might have taken place during the chelation. A comparative study of the IR spectra of ligand and its metal complexes discloses that some peaks are common and therefore, only important peaks, which have either shifted or have newly appeared, are discussed. Table 2 shows that  $\nu(\text{C-O})$  and  $\nu(\text{C=N})$  modes appear at  $1224\text{-}1332\text{cm}^{-1}$  and  $1595\text{-}1620\text{cm}^{-1}$  respectively. The shifting of (C-O) to higher frequency as compared to the ligand ( $1224\text{cm}^{-1}$ ) is owing to the conversion of hydrogen bonded structure into a covalent metal bonded structure. Lowering of (C=N) in the complexes as compared to the ligand ( $1620\text{ cm}^{-1}$ ) is due to reduction of double bond character of carbon-nitrogen bond of the azomethine group. This result expects that the acetate ions were coordinated outside the coordination sphere. Metal-ligand bond is further confirmed by the appearance of a medium intensity band in the range  $444\text{-}468$  and  $530\text{-}560\text{cm}^{-1}$  in the spectra of the complexes allotted to stretching frequencies of (M-N) bond and (M-O) bond formation respectively [10].

**Table 2** Infrared Spectroscopic Data of the Schiff Base Ligand and its mononuclear metal complex

Compounds	(C=N) ( $\text{cm}^{-1}$ )	(C-O) ( $\text{cm}^{-1}$ )	(M-N) ( $\text{cm}^{-1}$ )	(M-O) ( $\text{cm}^{-1}$ )
$\text{C}_{16}\text{H}_{12}\text{N}_2\text{O}_2$	1620	1244	--	--
[Cu(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	1605	1288	460	530
[Ni(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	1609	1320	454	544
[Co(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	1595	1332	468	560
[Mn(C <sub>26</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub> )]	1600	1324	444	553

### Electronic spectra and magnetic moment

The electronic spectral data of the metal complexes in DMF solution are showed in Table 3. The nature of the ligand field around the metal ion was inferred from the electronic spectra. The electronic spectrum of Co(II) complex revealed three bands in the region of 660, 570 and 530nm which were tentatively allotted to  ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{F})$  ( $\nu_1$ ),

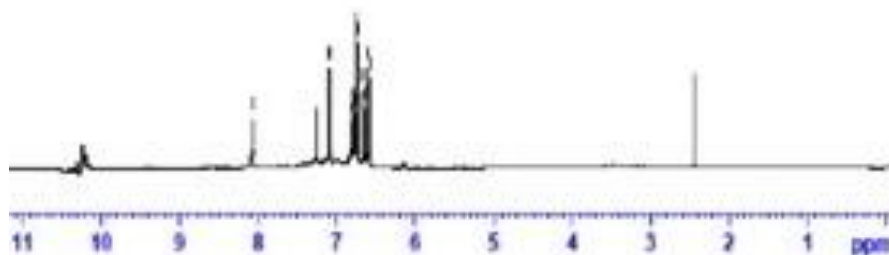
${}^4T_{1g} \rightarrow {}^4A_{2g}(F)$  ( $\nu_2$ ) and  ${}^4T_{1g} \rightarrow {}^4T_{1g}(P)$  ( $\nu_3$ ) transitions, respectively. The value of magnetic moment was 5.12 B.M. which specifies the presence of Co(II) complex in octahedral geometry [11]. The electronic spectrum of the Ni(II) complex showed three bands at 695, 542 and 575nm assignable to  ${}^3A_{2g} \rightarrow {}^3T_{2g}(F)$  ( $\nu_1$ ),  ${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$  ( $\nu_2$ ) and  ${}^3A_{2g} \rightarrow {}^3T_{1g}(P)$  ( $\nu_3$ ) transitions, respectively. The value of magnetic moment was 3.42BM; therefore octahedral geometry is suggested for this complex [12]. The  ${}^2E_g$  and  ${}^2T_{2g}$  states of the octahedral Cu(II) ( $d^9$ ) split under the effect of the tetragonal distortion and the distortion can be such as to cause the three transitions  ${}^2B_{1g} \rightarrow {}^2B_{2g}$ ;  ${}^2B_{1g} \rightarrow {}^2E_g$  and  ${}^2B_{1g} \rightarrow {}^2A_{1g}$  to remain unresolved in the spectra. It is determined that, all three transitions 690, 570 and 535nm lie within the single broad envelope centered at the same range previously mentioned. This assignment is in agreement with the general observation that Cu(II) d-d transitions are normally close in energy. The magnetic moment of 1.97BM falls within the range normally observed for octahedral Cu(II) complexes [13]. The electronic spectra of Mn(II) complexes show the absorption bands in the range 694, 555 and 522nm. These absorption bands may be assigned to the  ${}^6A_{1g} \rightarrow {}^4A_{1g}$ ,  ${}^6A_{1g} \rightarrow {}^4A_{2g}$ , and  ${}^6A_{1g} \rightarrow {}^4E_g$ ,  ${}^4A_{1g}$  transitions, respectively. These bands suggest that the complexes possess an octahedral geometry. The Mn(II) complex show magnetic moments is 5.92BM at room temperature corresponding to five unpaired electrons which suggest octahedral geometry [14]. In the spectra of the Schiff base ligand, the absorption band observed at 281-294nm is assigned to intra-ligand  $\pi \rightarrow \pi^*$  transition and the band at 342-390nm is assigned due to  $n \rightarrow \pi^*$  transition associated with the azomethine chromophore ( $-C=N$ ).

**Table 3** Electronic Spectral data of Schiff base ligand and its complexes.

Compound	Electronic spectra (nm)				Geometry of the complex
	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	L→M	d-d	
$C_{16}H_{12}N_2O_2$	294	386	-	-	-
[Cu( $C_{26}H_{20}N_4O_2$ )]	285	374	480	690,570,535	Octahedral
[Ni( $C_{26}H_{20}N_4O_2$ )]	292	390	440	695,542,575	
[Co( $C_{26}H_{20}N_4O_2$ )]	281	342	445	660,570,530	
[Mn( $C_{26}H_{20}N_4O_2$ )]	293	346	430	694,555,522	

### ${}^1H$ NMR spectra

The  ${}^1H$  NMR Schiff base was recorded in DMSO-  $d_6$  at room temperature. Various types of protons were identified: (i) resonance shows because of phenolic  $-OH$  protons around 10.26ppm (ii) characteristic resonance because of azomethine proton in the Schiff base is observed at 8.227ppm and (iii) the other signals in the region 6.54–7.42ppm appear due to presence of aromatic protons. All these observations corroborate the infrared results.



**Figure 2** NMR spectra of the ligand

### EPR spectra

The EPR spectra of complexes offer information of status in studying the metal ion environment. The EPR spectra of the  $[\text{Cu}(\text{C}_{26}\text{H}_{20}\text{N}_4\text{O}_2)]$  Schiff base complexes recorded as powder samples with room temperature, on X-band at frequency 9.3GHz under the magnetic field strength 4000 G.

The EPR spectrum of the  $[\text{Cu}(\text{C}_{26}\text{H}_{20}\text{N}_4\text{O}_2)]$  (Figure 3) complexes show a broad signal with  $g_{\text{iso}}$  at 1.9998 thus, proving it be an octahedral geometry [15].

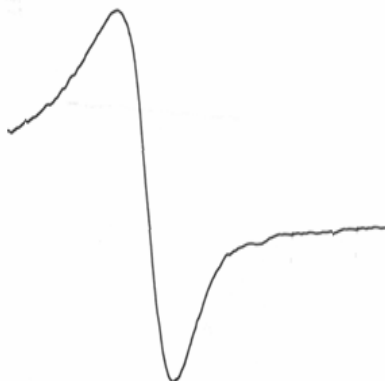


Figure 3 EPR spectra of  $[\text{Cu}(\text{C}_{26}\text{H}_{20}\text{N}_4\text{O}_2)]$  complex

### Antibacterial activity

Biological activity of the ligand and a sequence of its metal complexes  $[\text{Cu}(\text{II})]$ ,  $[\text{Ni}(\text{II})]$ ,  $[\text{Co}(\text{II})]$  and  $[\text{Mn}(\text{II})]$  were separated for anti-bacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia* by using disc-agar diffusion method (Figure 4). The results show that the complexes display more activity and the ligand have less activity against same microorganisms under same experimental conditions. This would suggest that the chelation of the complex easily cross a cell membrane. This phenomenon can be explained by Tweedy's chelation theory. The polarity of the metal ion is considerably reduced by Chelation because of fractional sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation enhance the lipophilic character of the central metal atom, which later favors its infusion through the lipid layer of the cell membrane. The copper (II) complex shows higher anti-fungal activity than other complexes. The change in the effectiveness of various compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells [16].

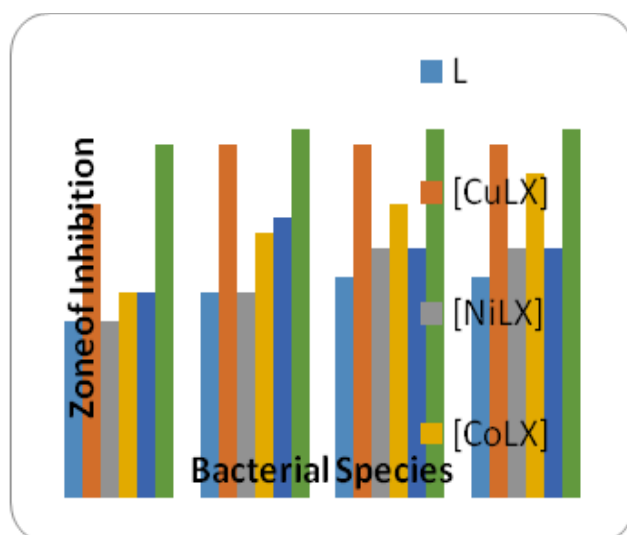


Figure 4 Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against Bacterial Strains

### DNA cleavage

Gel electrophoresis experiments were achieved using pUC18DNA with ligand, complexes in presence and absence of  $H_2O_2$ . Complexes exhibit cleavage ability at low concentration ( $40 \mu M$ ). When calf-thymus DNA is subjected to electrophoresis, comparatively fast migration will be noticed for the complete super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated [17]. From Figure 5 the complexes show more activity in the presence of oxidant which may be due to the reaction of hydroxyl radical with DNA. The results of DNA cleavage studies have been shown in Figure 5. All metal complexes were able to convert DNA (Form I) into open circular (Form II). The mononuclear Cu (II) complex was found to be highly active in cleaving DNA in the presence of hydrogen peroxide.

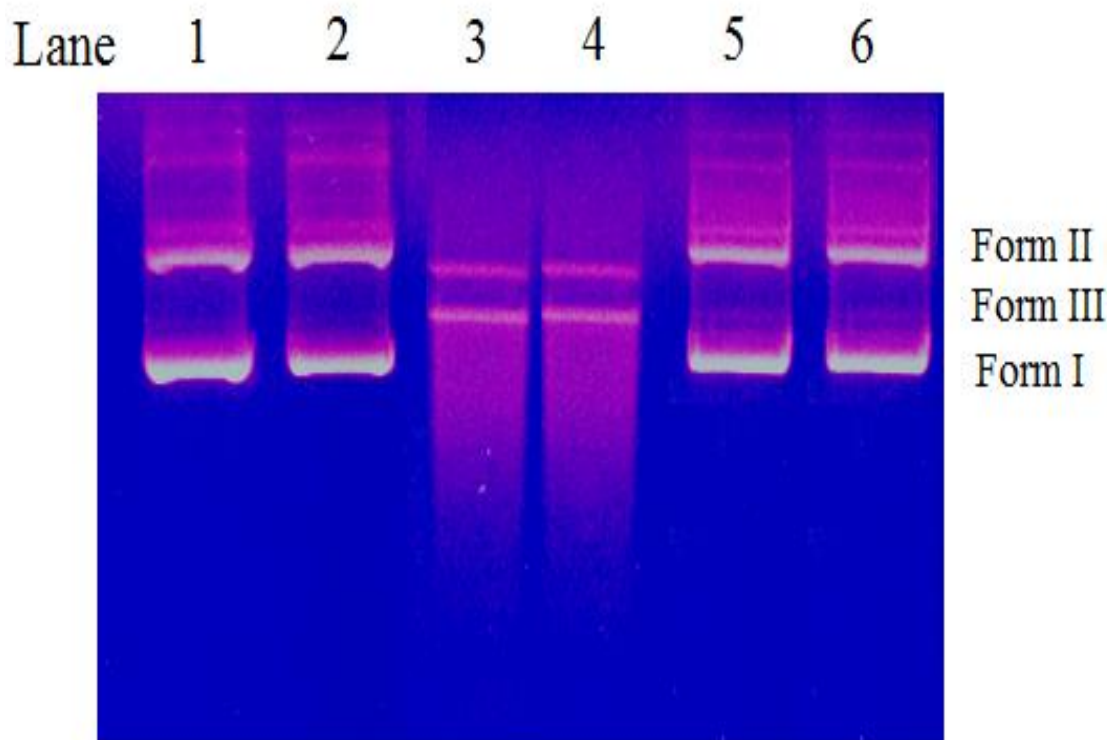


Figure 5 Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by  $H_2O_2$  and metal complexes. Lane 1-DNA alone; Lane 2- DNA alone +  $H_2O_2$ ; Lane 3-DNA + Cu complex +  $H_2O_2$ ; Lane 4-DNA + Ni complex +  $H_2O_2$ ; Lane5-DNA + Co complex; Lane 6-DNA+ Mn complex +  $H_2O_2$ .

### Conclusions

The present study proves that octahedral geometry around Cu(II), Co(II), Ni(II) and Mn(II) complexes coordinating through nitrogen and oxygen atoms. The observations of antimicrobial activity prove that the compounds exhibit antimicrobial properties and it is important to note that the metal chelates show more inhibitory effects than the parent ligand. DNA cleavage studies notice that all the complexes significantly cleaves DNA molecule.

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