



Research Article

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Studies on DNA cleavage and antimicrobial screening of Transition Metal Complexes of a Schiff Base Derived from 2-(Aminomethyl)-benzimidazole and *p*-chloro benzaldehyde

Pragathi Jogi^{1*}, Padmaja. M¹, K. V. T. S. Pavan Kumar², Gyanakumari. C¹.

¹Department of Chemistry, Osmania University, Hyderabad, India

²Dept. of plant biotechnology, Dalmia Centre for Research and Development, Hyderabad, India

ABSTRACT

A new series of transition metal Complexes of Cu(II), VO(IV), Mn(II), Co(II), Ni(II) and Zn(II) with a Schiff base derived from 2-AminomethylBenzimidazole and 4-chlorobenzaldehyde(1-(1H-benzimidazol-2-yl)-N-[4-chlorobenzylidene) methanamine-BCTM) were successfully synthesized. The complexes have been characterized using chemical analysis, spectroscopic methods (IR, UV-Visible, ¹H NMR and ESR), conductometric and magnetic data. According to these data, we propose an octahedral geometry to all the metal complexes. Antibacterial activity of the ligand and its metal complexes were studied against two gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa*, and two gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*. It has been found that all the complexes are antimicrobially active and show higher activity than ligand. The nuclease activity of the above metal complexes were assessed by gel electrophoresis assay and the results show that metal complexes can cleave pUC19 DNA in presence of hydrogen peroxide compared to the ligand.

Keywords: Schiff base, 2-aminomethyl benzimidazole, *p*-chloro benzaldehyde.

INTRODUCTION

Compounds containing imines bases have not only found extensive application in organic synthesis, but several of these molecules display significant biological activity [1-4]. In the last decade Schiff base ligands have received more attention mainly because of their wide application in the field of catalysis and due to their antibacterial[5,6], anti-tuberculosis [7], and antitumour activity[8], insecticidal activity[9]. They easily form stable complexes with most transition metal ions. Schiff base complexes derived from heterocyclic compounds have increased interest in the field of bioinorganic chemistry[10]. Benzimidazole and its derivatives are the important class of aromatic heterocyclic compounds with broad spectrum of biological activities such as antimicrobial [11], anti-cancer [12], anti-inflammatory [13], antiviral [14], anticonvulsant [15]. Benzimidazole was confirmed to have moderate *invitro* anti-HIV effect.[16] In last decades, heterocyclic benzimidazoles, their derivatives and transition metal complexes have received considerable attention in coordination chemistry, because of their well-documented biological activities. It was found that such complexes showed larger antimicrobial activities than the free ligands[17,18]. In continuation of our research work[19] on transition metal complexes with Schiff bases, in the present paper we report the synthesis and characterization of Cu(II), Co(II), Ni(II), VO(IV), Mn(II) and Zn(II) complexes of Schiff base derived from 2-aminomethyl benzimidazole.2HCl and *p*-chloro benzaldehyde. These complexes have been characterized by various physicochemical methods.

EXPERIMENTAL SECTION

Materials:-

All the chemicals used were of analytical grade. 2-Aminomethylbenzimidazole.2HCl was of sigma Aldrich chemicals. 4-chloro benzaldehyde, Vanadylsulphate.5H₂O, copper chloride, nickel chloride, zinc acetate, manganese chloride and cobalt chloride were of SD's fine chemicals.

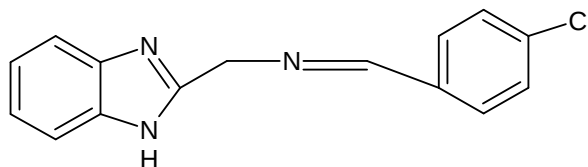
Instrumentation

The percentage compositions of C, H, and N of complexes were determined by using micro analytical methods on Perkin Elmer 240C (USA) elemental analyzer. Infrared spectra the ligand and its complexes were carried out by using KBr pellets in the range (4000-400 cm⁻¹) on Perkin Elmer Infra red model 337. The electronic absorption was carried out by using a Shimadzu UV-1601 using DMSO as solvent. The Mass spectra were recorded by ESI technique on VG AUTOSPEC mass spectrometer instrument. The ¹H NMR spectrum was recorded on Varian Gemini Unity Spectrometer by employing TMS as internal standard. Melting points of the ligand and decomposition temperature of complexes were determined on Polmon instrument (model No.MP-96). The Molar conductance measurements were carried out in DMSO (10⁻³ M) using Digisun Electronic Digital conductivity meter of model: DI-909 having a dip-type cell calibrated with KCl. The Magnetic susceptibilities of complexes were determined on Gouy balance model 7550 at 23 °C. The diamagnetic corrections were made by Pascal's constant and Hg[Co(SCN)₄] was used as a calibrant. ESR spectra were recorded in DMSO solution on a JOEL.TE-3X.X-Band spectrometer equipped with a 100KHZ field modulation unit, central university, Hyderabad.

Synthesis of Schiff base:-

2-aminomethyl benzimidazole.2HCl (1.10g, 5mmol) was dissolved in 15ml of water and neutralized by adding aqueous Na₂CO₃ solution. A stirred solution of 4-chloro benzaldehyde(0.560 g, 5mmol) in 8ml of methanol was added drop wise to the above solution with stirring with in 1 hr. During this period, yellow solid slowly separated out .the solid was filtered off, washed thoroughly with water followed by petroleum ether and dried in vacuo at room temperature. Structure of the Schiff base is shown in the Fig.1.

Fig-I:-Structure of BNCM



Synthesis of metal complexes:-

Methanolic solution of ligand (0.005mol) and methanolic solution of corresponding metal salts(0.005mol)(MX₂, where M= Cu(II), Ni(II), Co(II), Zn(II), Mn(II) and VO(IV); (X=Cl/Acetate/SO₄) were mixed together with constant stirring. The mixture was refluxed for 4 hours at 80 °C. On cooling colored solid metal complexes were precipitated out. The products were filtered, washed with petroleum ether and dried under vacuo over CaCl₂.

Gel electrophoresis:-

The cleavage of pUC¹⁹ DNA was determined by agarose gel electrophoresis[20]. The gel electrophoresis experiments were performed by incubation of the samples containing 40 μM pUC¹⁹ DNA, 50 μM metal complexes and 50 μM H₂O₂ in Tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 μg cm⁻³ ethidium bromide (EB) and photographed under ultraviolet light at 360nm. All the experiments were performed at room temperature unless otherwise mentioned.

RESULTS AND DISCUSSION

The complexes were found to be stable in air and non-hygroscopic. The complexes are soluble in DMSO. The physical properties and analytical data of the complexes are given in Table-1.

Table-1. Physical properties and analytical data of Schiff base and its metal complexes

Compound	mass	MP °C	Molar conductance Ohm ⁻¹ cm ² mol ⁻¹	Found(calculated)%				
				C	H	N	Cl	M
BNTM	270	168	----	66.79 (66.72)	4.48 (4.49)	15.58 (15.51)	13.14 (13.19)	----
[Cu(BNCM)Cl ₃ (H ₂ O)] Cl	475	195	60	37.8 (37.5)	2.94 (2.67)	8.84 (8.7)	29.47 (7.16)	13.26 (13.19)
[Co(BNCM)Cl ₄](Cl ₂ .XH ₂ O)	470.9	228	220	38.21 (38.3)	2.54 (2.49)	8.91 (8.89)	37.15 (7.49)	12.52 (12.49)
[Ni(BNCM)Cl ₄](Cl ₂)	540	>300	290	33.3 (33.2)	2.22 (2.26)	7.77 (7.75)	32.4 (32.1)	10.90 (10.91)
[Zn(BNCM)OAc(H ₂ O) ₂] ⁺²	430	210	18	47.4 (47.8)	4.41 (4.43)	9.76 (9.67)	8.25 (8.16)	15.11 (15.17)
[Mn(BNCM)Cl ₃ (H ₂ O)]Cl	490	292	65	36.7 (36.9)	2.85 (2.84)	8.57 (8.47)	28.57 (28.56)	11.22 (11.18)
[VO(BNCM)SO ₄ (H ₂ O)] ⁺²	451	>300	13	39.91 (39.89)	3.1 (3.91)	9.31 (9.44)	7.87 (7.85)	11.3 (11.29)

IR spectral studies:-

The most important infrared spectral bands that provide conclusive structural evidence for the Coordination of the ligands to the central metal ions are given in the Table2 and IR spectrum of Co-BNCM shown in the Figure-3. The ligand shows a strong band at 1643cm⁻¹ characteristic of ν (C=N) stretching vibration[21], this band undergoes a negative shift in the complexes indicating the participation of the azomethine nitrogen in coordination [22].. Generally the IR spectra of the free ligands show a broad band around 3450cm⁻¹ which can be attributed to NH stretching vibration of benzimidazole moiety. The position of this band remains at nearly the same frequency in the spectra of the metal complexes suggesting the non-coordination of this group[23]. Appearance of a broad band in 3000 to 3300cm⁻¹ region was observed in the spectra of metal complexes indicating the presence of coordinated or lattice water molecules[24]. Water molecules are coordinated, confirmed by the occurrence of additional band at 815-845cm⁻¹ arising due to OH rocking vibrations[25]. Other band of M-N appears at around 430cm⁻¹[26]. In addition, vanadyl complex shows a band at 980cm⁻¹ attributed to ν V=O frequency[27-30]. The IR spectrum of the complex 1 shows a band at 1591cm⁻¹ which may be attributed to $\nu_{\text{asym}}(\text{COO}^-)$ stretching Vibration. A band at 1402 cm⁻¹ in the IR spectrum of the complex may be assigned to $\nu_{\text{sym}}(\text{COO}^-)$ stretching vibration [31,32]. the band appearing at 1591 cm⁻¹ in the spectrum of the zn complex can be considered as an overlapped band due to two stretching vibrations of C=N and asymmetric coordinated COO⁻.

¹H NMR:-

The ¹H NMR spectral data of the ligand is summarized in the Table-4 and shown in the figure-2. A sharp singlet at 8.55 assignable to azomethine proton (-CH=N-) confirms the formation of the ligand as proposed

Table.2 Characteristic IR bands (cm⁻¹) of the compounds studied

Compound	ν CH=N	ν M-N	ν M-OH	ν V=O
BNCM	1643	---	---	---
CuBNCM	1593	434	3202,836	---
CoBNCM	1602	433	3195,841	---
NiBNCM	1628	427	---	---
MnBNCM	1605	432	3191,820	---
VOBNCM	1625	461	3054,844	980
Zn-BNCM	1591	434	3353,838	---

Electronic spectra and magnetic moment:-

The magnetic susceptibility and electronic spectral data of the metal complexes are given in Table-3. The magnetic susceptibility data showed that all the metal complexes, except zinc complex, are paramagnetic. The values show that the manganese and cobalt complexes are high spin and the zinc complex is diamagnetic. The values are consistent with octahedral geometry, which is further supported by electronic spectral data. The Cu(II) complex showed one broad band at 13568cm⁻¹ assignable to the ²Eg→²T_{2g} transition that is characteristic of an octahedral environment[33]. The electronic spectrum of Co(II) complex showed bands at 14970,20366

24881cm⁻¹, which are assignable to $^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ and $^4T_{1g}(P) \rightarrow ^4T_{1g}(F)$ transitions respectively, that are characteristic of octahedral configuration[33]. The Ni(II) complex exhibited 3 transitions (shown in the figure-4) at 14858, 24390, 30864cm⁻¹, which are assignable to $^3A_{2g} \rightarrow ^3T_{2g}(F)$, $^3A_{2g} \rightarrow ^3T_{1g}(F)$ and $^3A_{2g} \rightarrow ^3T_{1g}(P)$ transitions. Electronic spectra of Mn(II) complex display absorption bands at 24813, 25241, 25188, 28490cm⁻¹, characteristic of octahedral geometry corresponding to $^6A_{1g} \rightarrow ^4T_{1g}(^4G)$, $^6A_{1g} \rightarrow ^4E_g(^4D)$, $^6A_{1g} \rightarrow ^4T_{1g}(^4P)$ and $^6A_{1g} \rightarrow ^4E_g(^4G)$ transitions respectively. The VO(IV) complex exhibited three transitions at 12658, 17857, 23809cm⁻¹, which are assignable to $^2E \rightarrow ^2B_2$, $^2B_1 \rightarrow ^2B_2$ & $^2A_1 \rightarrow ^2B_2$ respectively, that are characteristic of octahedral geometry[33]. The diamagnetic Zn(II) complex did not show any d-d bands and their spectra are dominated by charge transfer bands 23500-25000cm⁻¹ was assigned due to transition possibly in an octahedral environment.

Fig-3:-IR spectrum of Co-BNCM:-

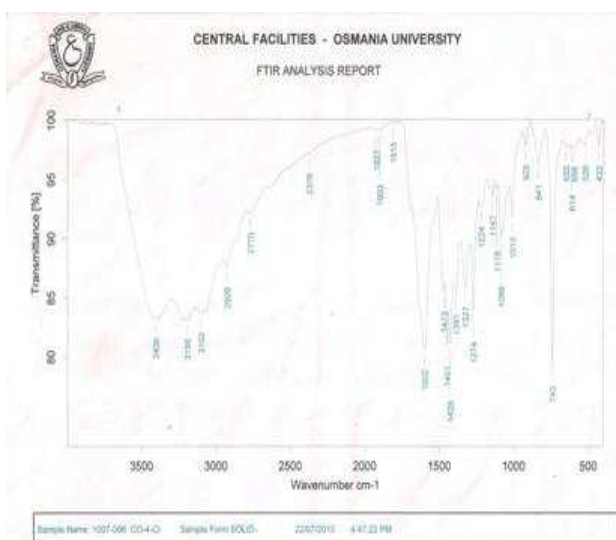


Fig-4:- UV_Visible spectrum of Ni-BNCM

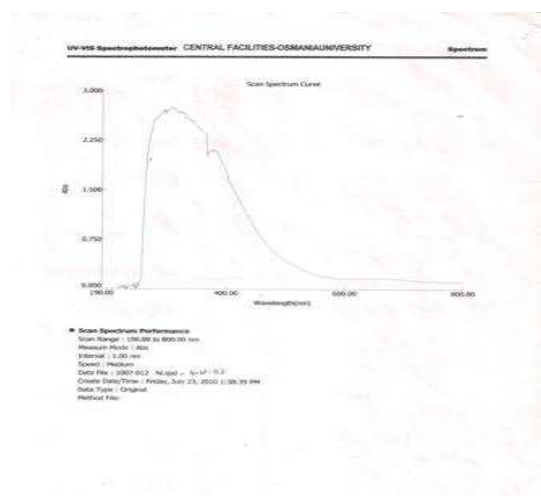


Table-3 Electronic spectral data and magnetic moment data

Complex	μ_{eff}	Frequency(cm ⁻¹)	Assignments
Cu-BNCM	1.72	13568	$^2E_g \rightarrow ^2T_{2g}$
		14970	$^4T_{1g}(F) \rightarrow ^4T_{2g}(F)$
		20366	$^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$
		24881	$^4T_{1g}(P) \rightarrow ^4T_{1g}(F)$
Ni-BNCM	2.8	14858	$^3A_{2g} \rightarrow ^3T_{2g}(F)$
		24390	$^3A_{2g} \rightarrow ^3T_{1g}(F)$
		30864	$^3A_{2g} \rightarrow ^3T_{1g}(P)$
VO-BNCM	1.67	12658	$^2E \rightarrow ^2B_2$
		17857	$^2B_1 \rightarrow ^2B_2$
		23809	$^2A_1 \rightarrow ^2B_2$
Mn-BNCM	4.82	24813	$^6A_{1g} \rightarrow ^4T_{1g}(^4G)$
		25241	$^6A_{1g} \rightarrow ^4E_g(^4D)$
		25188	$^6A_{1g} \rightarrow ^4T_{1g}(^4P)$
		28490	$^6A_{1g} \rightarrow ^4E_g(^4G)$
Zn-BNCM	Diamagnetic	-	-

ESR spectra of Cu(II) complex:-

The ESR spectral studies of Cu (II) complex provide information of the metal ion environment. The ESR spectrum of the Cu (II) complex was recorded in DMSO at liquid nitrogen Temperature(LNT). This complex in the frozen state at 77 K shows four slightly resolved peaks of low intensities in the low field region and one intense peak in the

high field region. In distorted octahedral and square planar complexes, the unpaired electron lies in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with $g_{\parallel} > g_{\perp}$ ($2.18 > 2.05$) & g_{avg} is calculated as 2.097, which suggests That the complex is having distorted octahedral geometry . Also it is supported by the fact that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital. ESR spectrum of Cu-BNTM is given in the Figure.5. The spin-orbit coupling constant, λ value (-330 cm^{-1}) calculated using the relations, $g_{\text{avg}} = 1/3[g_{\parallel} + 2g_{\perp}]$ and $g_{\text{avg}} = 2(1 - 2\lambda/10Dq)$, is less than the free Cu(II) ion (-832 cm^{-1}) which also supports covalent character [34] of M-L bond in the complex.

Fig-2:- NMR spectrum of BNCM

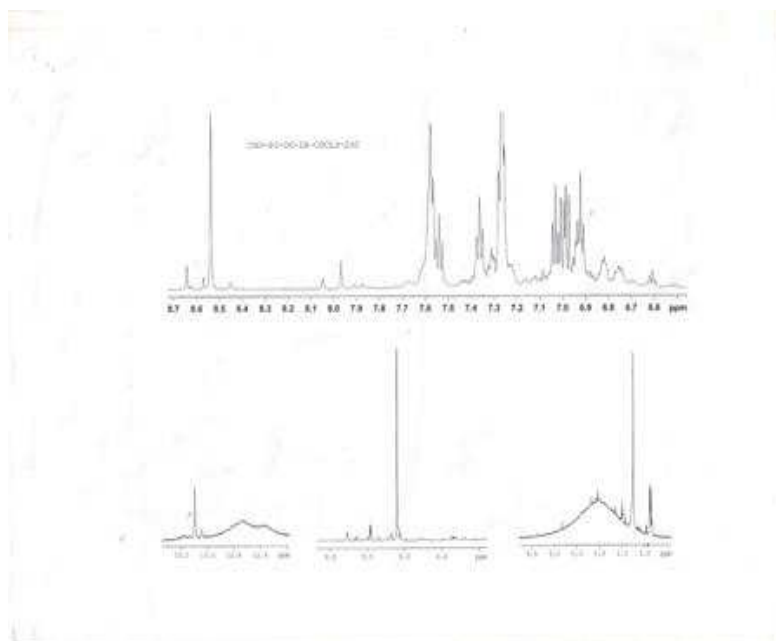


Table-4. ^1H NMR data of the Schiff base

^1H	
δ ppm	Assignment
8.55(1H,s)	-CH=N-
5.1(2H,s)	-CH ₂ -
13.1(1H,s)	-NH-
7.65(m)	Benzimidazole & p-chloro benzaldehyde rings protons

Molecular modeling studies:-

The possible geometries of metal complexes were evaluated using the molecular calculation with Arguslab 4.0.1 version software. The metal complexes were built and geometry optimization was done using molecular mechanics uniform force field (UFF) method. The molecular modeling pictures and the energies of metal complexes are shown in the Figure6.

Antibacterial studies:-

The new Schiff base and the complexes were tested for in vitro antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* using diffusion method.[35] The diffusion method requires filter paper disks, the medium used is muller-hinton agar with 2% of glucose and the diameter of inhibition zone is visually read at 24hrs after incubation at 37°C. The compounds are added on to the filter paper containing this medium. The antimicrobial activity was estimated on the basis of the size of inhibition zone formed around the paper disks on the seeded agar plates. Streptomycin was used as standard.

Fig-5:-ESR spectrum of Cu-BNCM

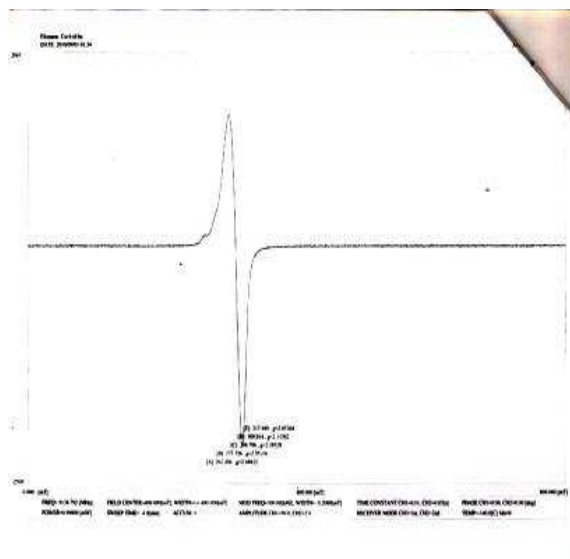
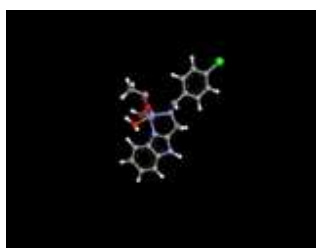
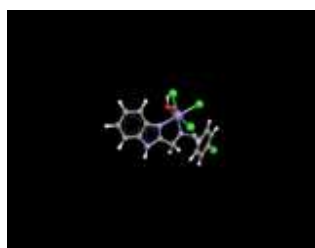
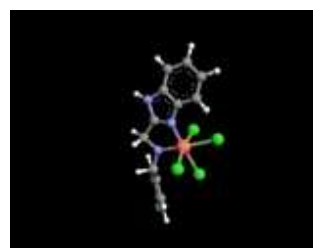
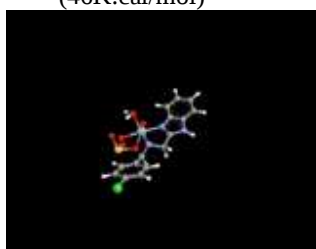
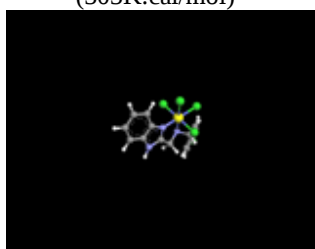
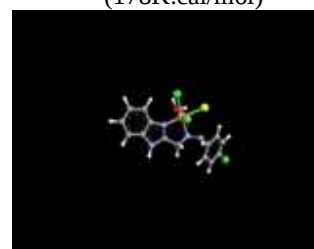


Fig-6:- Molecular modelling structures for metal complexes

[Zn (BNCM)(H₂O)₂OAc]
(46K.cal/mol)[Mn(BNCM)(Cl)₃H₂O]
(303K.cal/mol)[Ni(BNCM)(Cl)₄]
(178K.cal/mol)[VO(BNCM)(H₂O)₂SO₄]
(267K.cal/mol)[Co(BNCM)(Cl)₄]
(21.08K.cal/mol)[Cu(BNCM)(H₂O)(Cl)₃]
(97K.cal/mol)

DMSO was used as solvent control for antimicrobial activities. The Schiff base and the complexes exhibited varying degrees of inhibitory effect on the growth of the tested bacterial species. The values reveal that the Schiff base became more effective when coordinated to the metal ions. Such increased activity of the metal chelates is due to lipophilic nature of the metal ions in complexes.[36] Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through the azomethine nitrogen with the active centres of the cell constituents resulting in interferences with the normal cell process[37]. The results are presented in the Table 5.

Table-5:-Antibacterial activity data for Schiff base and its metal complexes

Compound	B.Subtilis		S.Aureus		E.coli		P. Aeruginosa	
	30µl	60µl	30µl	60µl	30µl	60µl	30µl	60µl
Ligand	---	---	+	++	+	++	+	+++
Cu complex	+	++	+	++	+	++	+	++
Co complex	---	---	---	---	---	---	---	---
Ni complex	++	++++	+	+++	+	+++	+	+++
Zn complex	+	+++	+++	++++	++	+++	+++	++++
OV complex	++	+++	+	+++	++	+++	++	+++
Mn complex	++	++++	+	++	++	++	++	+++

*C = 10mg/L. Inhibition zone diameter : +=8-10mm; ++=10-14mm; +++= 14-18mm; ++++=18-26mm; --=no activity

DNA cleavage studies:-

DNA cleavage is measured by relaxation of supercoiled pUC¹⁹ DNA to nicked circular conformation and linear conformation. During electrophoresis process supercoiled DNA will migrate faster when compared with DNA in nicked and linear confirmations. Figure 7 illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC¹⁹ DNA induced by the metal complexes interest of the study. The control experiments did not show any apparent cleavage of DNA (lane 1 & 2). Cu, Zn & VO complexes in the presence of H₂O₂ (lane 6, 12 & 14) at 50µM concentration shows more cleavage activity when compared in absence of H₂O₂. Ni complex has shown good comparable activity with other compounds and is evident by complete degradation of DNA resulting in the disappearance of bands on gel. The supercoiled plasmid DNA was completely degraded resulting in the disappearance of bands on agarose gel [38]. Further the presence of a smear & decreased intensity of bands in the gel diagram indicates the presence of radical cleavage [39].

Fig-7:-DNA Cleavage activity



Lane1-DNA alone, 2 - DNA + H₂O₂, 3 - DNA + BCTM, 4-DNA + H₂O₂ + BCTM, 5-DNA + Cu(II) complex, 6-DNA + H₂O₂ + Cu(II) complex, 7 - DNA + Ni(II) complex, 8 - DNA + H₂O₂ + Ni(II) complex, 9 - DNA + Co(II) complex, 10 - DNA + H₂O₂ + Co(II) complex, 11 - DNA + Zn(II) complex, 12 - DNA + H₂O₂ + Zn(II) complex, 13 - DNA + OV(IV) complex, 14 - DNA + H₂O₂ + OV(IV) complex, 15 - Marker

CONCLUSION

Cu(II), Co(II), Ni(II), Mn(II), OV(IV) and Zn(II) complexes of the Schiff base derived from 2-Amino methyl benzimidazole and p- chloro benzaldehyde were prepared and characterized. The study reveals that

- Zn(II), VO(IV) complexes are non-electrolytes and Cu(II), Mn(II) are 1:1 electrolytes and Co(II), Ni(II) complexes are 1:2 electrolytes;
- The Schiff base behaves as a neutral bi dentate ligand and is coordinated through the azomethine nitrogen, pyridyl nitrogen of benzimidazole moiety;
- All the metal complexes have octahedral geometry;
- The biological activity of all the complexes is higher than that of the free schiff base ligand;

(v) The interaction of these complexes with pUC19 DNA was investigated by gel electrophoresis. From the observation, Cu, Zn and OV complexes cleave DNA as compared to control DNA and other complexes in the presence of H₂O₂.

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