
Synthesis, Characterization, DNA Binding and DNA Cleavage Studies of Transition Metal Complexes of Schiff Base Derived from 2-Amino Methyl Benzimidazole and P-Methyl Benzaldehyde

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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-Aminomethyl Benzimidazole and 4-methyl benzaldehyde(1-(1H-benzimidazol-2-yl)-N-[4- methyl benzylidene) methanamine-BMBM) 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. DNAbinding properties of these metal complexes with CT-DNA in a potassium phosphate buffer (pH 7.2) were investigated using UV-Vis absorption spectroscopy. Experimental studies suggest good DNA binding ability of Co(II) complexes through intercalation. 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: DNA binding, Cleavage activity, Metal complexes, 2-amino benzimidazole, p-methyl benzaldehyde

INTRODUCTION

Benzimidazole derivatives play an important role in medicinal field with many biological and pharmacological activities related to it. Compounds containing a benzimidazole moiety are important chemical classes as a result of their significant biological activities¹. The structural similarities of benzimidazole nucleus to biological compounds, such as, the purine base of the DNA and the occurrence of this nucleus in vitamin B₁₂ have been of great interest to pharmaceutical industry. This similarity is believed to enable easy recognition of benzimidazole by biological systems. As a result, benzimidazoles have been termed as ‘privileged structures’ for drug design. Moreover it is reported that benzimidazole exhibits high affinity for enzyme and protein receptors.

Schiff bases derived from aromatic amines and aromatic aldehydes are very important class of organic compounds because of their applications in many fields including biological², inorganic³, and analytical chemistry⁴. The hybrid molecules composed of the combination of part of a heterocyclic ring, like benzimidazole, and part of the Schiff base may exert potential biological activities⁵. Since benzimidazole derivatives are known to exhibit a wide variety of pharmacological activities, it was speculated for the fact that Schiff bases of benzimidazole analogues substituted with 4-methyl benzaldehyde moiety should also have interesting biological activity. Thus the aim of the present work is to synthesize the transition metal complexes of with a Schiff base derived from 2-Aminomethyl Benzimidazole and 4-methyl benzaldehyde(1-(1H-benzimidazol-2-yl)-N-[4-methyl benzylidene) methanamine-BMBM) to understand their structure and to evaluate their DNA binding and cleavage properties.

EXPERIMENTAL SECTION

Materials:- All the chemicals used were of analytical grade. 2-Aminomethylbenzimidazole.2HCl was of sigma Aldrich chemicals. 4-methyl 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 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.XBand spectrometer equipped with a 100KHZ field modulation unit, central university, Hyderabad.

PHYSICAL MEASUREMENTS AND ANALYSIS.

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 of 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 Shimadzu UV-1601 using DMSO as solvent. TGA studies were carried on Mettler Toledo Star system in the temperature range of 0-1000°C. The Mass spectra were recorded by ESI technique on VG AUTOSPEC mass spectrometer instrument. The ¹H-NMR spectra were 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 Guoy balance model 7550 at 23°C. The diamagnetic corrections was carried out using 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.

DNA BINDING STUDIES

The DNA binding experiments were carried out in Tris-HCl/ NaCl (pH 7.2) buffer using the copper and cobalt complexes in DMSO. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M⁻¹cm⁻¹) at 260 nm. Absorption titration experiments were performed by varying the concentration of the DNA with the complexes. All UV-Visible spectra were recorded after equilibration. The intrinsic binding constant K_b was determined from a plot of [DNA] / (a- f) versus [DNA] equation (1).

$$[DNA] / (a- f) = [DNA] / (a- f) + 1/ Kb (b- f) \text{ ----- (1)}$$

where a, f and b are the extinction coefficients of apparent, free and bound form of the complex to DNA respectively. By using the absorbance data, a plot of [DNA]/(a- f) vs [DNA] gives a straight line and K_b was determined from slope to intercept ratio⁶.

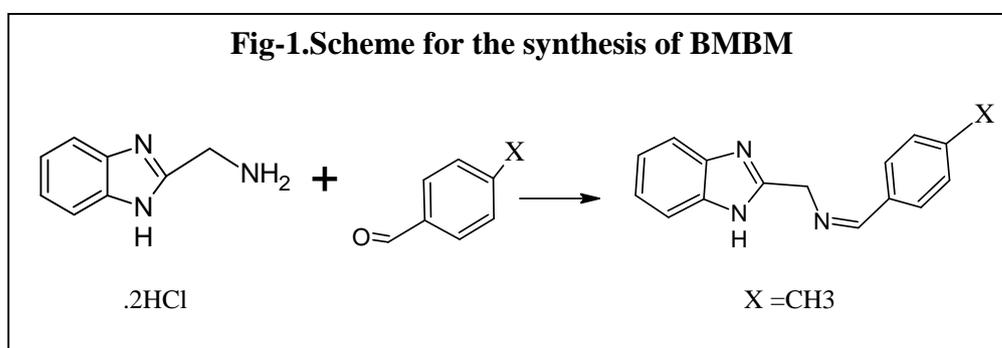
DNA CLEAVAGE&GEL ELECTROPHORESIS

The cleavage of pUC¹⁹ DNA was determined by agarose gel electrophoresis⁷. The gel electrophoresis experiments were performed by incubation of the samples containing 40 μM pUC¹⁹ DNA, 50

μM metal complexes and $50 \mu\text{M}$ H_2O_2 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 $1\mu\text{g cm}^{-3}$ ethidium bromide (EB) and photographed under ultraviolet light at 360nm. All the experiments were performed at room temperature unless otherwise mentioned.

SYNTHESIS OF SCHIFF BASES

2-aminomethyl benzimidazole. 2HCl (1.10g, 5mmol) was dissolved in 15ml of water and neutralized by adding aqueous Na_2CO_3 solution⁸. A stirred solution of the p-methyl benzaldehyde (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 vacuum at room temperature.



SYNTHESIS OF METAL COMPLEXES

Methanolic solution of ligand (0.005mol) and methanolic solution of corresponding metal salts (0.005mol)($\text{MX}_2 = \text{Copper(II) Chloride, Nickel(II) chloride, Cobalt(II) chloride, Zinc(II) acetate, Manganese(II) Chloride}$ and Vanadyl Sulphate) were mixed together with constant stirring. The mixture was refluxed for 4 hours at 80°C . On cooling coloured solid metal complexes were precipitated out. The products were filtered, washed with petroleum ether and dried under vacuo over CaCl_2 .

Characterization of metal complexes of BMBM

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 .

Elemental analysis

It is clear from the data that the experimental values shown for each of the complexes, are in good agreement with the theoretical values calculated for 1:1 ratio. The composition assigned to the complexes may, therefore be formulated as presented in the table 3.17.

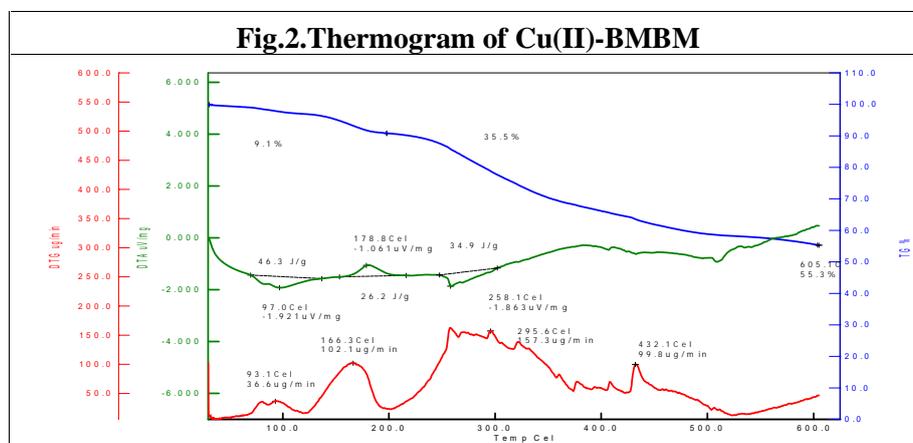
Conductivity Studies The molar conductance values of the metal complexes dissolved in DMSO were measured at 10^{-3}M concentrations. Conductance values show that Co(II) and Mn(II) complexes are 1:1 electrolytes and Cu(II) , VO(II) , Ni(II) and Zn(II) complexes are non electrolytes.

Thermal analysis (TGA & DTA)

Thermo gravimetric analysis of the metal complex is used: (i) to get information about the metal complexes, (ii) to decide whether the water molecule (if present) are inside or outside the coordination sphere of the metal complex⁹⁻¹¹. Thermal decomposition of Cu(II)-BMBM studied in Nitrogen atmosphere using TG, DTG and DTA techniques. The TGA curves of Cu(II)-BMBM show that the initial mass loss occurring at 93.1°C is interpreted as loss of one lattice water molecules and the second weight loss at 166.3°C is due to loss of coordinated water molecules.

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

Compound	mass	MP °C	Molar conductance $\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$	Found (calculated)		
				C	H	N
BMBM	249	155	----	77.15 (77.18)	7.56 (7.58)	15.05 (15.04)
[Cu(BMBM)Cl ₂]	386	203	07	49.6 (49.7)	3.85 (3.88)	10.79 (10.88)
[Co(BMBM)Cl(H ₂ O) ₃]Cl	431	218	72	44.3 (44.5)	4.86 (4.87)	9.69 (9.74)
[Ni(BMBM)(H ₂ O) ₂ Cl ₂]	413	190	09	45.9 (46.1)	4.57 (4.6)	10.14 (10.16)
[Zn(BMBM)(OAc) ₂]	434	>300	18	49.8 (49.7)	3.86 (3.83)	9.68 (9.67)
[Mn(BMBM)Cl(H ₂ O) ₃]Cl	428	139	68	44.9 (44.85)	4.93 (4.9)	9.83 (9.81)
[VO(BMBM)SO ₄]	396	>300	11	47.9 (48.4)	3.76 (3.78)	10.45 (10.6)

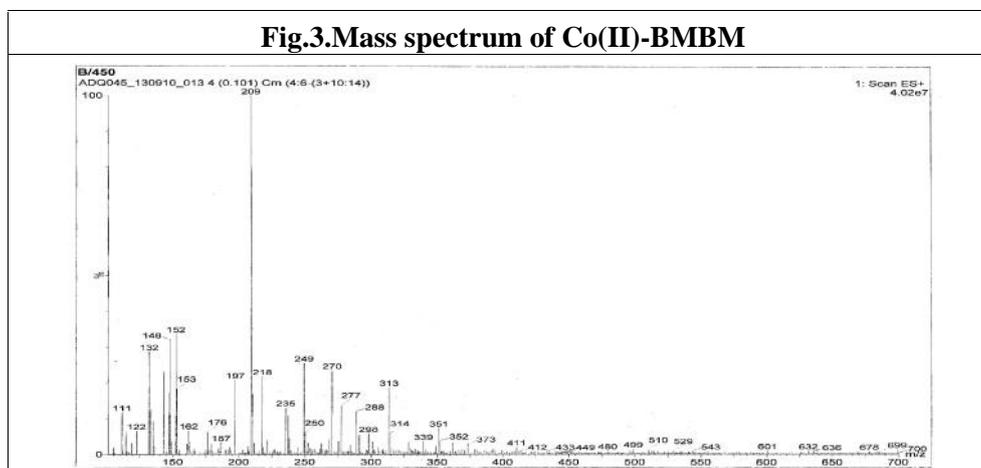


In this complex, rapid weight loss was observed at 295.6°C, indicative of decomposition of coordinated ligand. Finally, the horizontal nature of thermogram above 500°C observed, suggests the formation of final decomposition products corresponding to the metal oxide i.e. CuO.

Mass spectra

Electron spray ionization (ESI) mass spectra of the BMBM and its metal complexes were recorded at room temperature and were used to compare their stoichiometric composition. The molecular ion peaks for the metal complexes confirms the stoichiometry of metal chelates as ML type. It is also supported by the micro analytical data of the complexes. The spectra of [Cu(BMBM)Cl₂] showed M+1 peak at 387m/z, [Co(BMBM)(H₂O)₃]Cl showed M+2 peak at 433m/z, [Ni(BMBM)(H₂O)₂Cl₂] showed M+ peak at 413m/z, [Zn(BMBM)(OAc)₂] showed M⁺ peak at 434m/z, [Mn(BMBM)Cl(H₂O)₃]Cl showed M+1 peak at 429m/z and

[VO(BMBM)(SO₄)] showed M+1 peak at 397m/z which are in good agreement with theoretically calculated values.

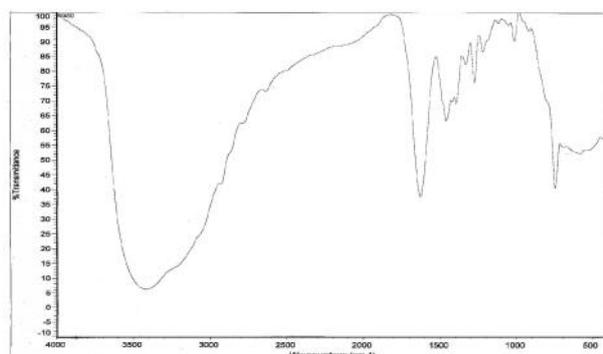
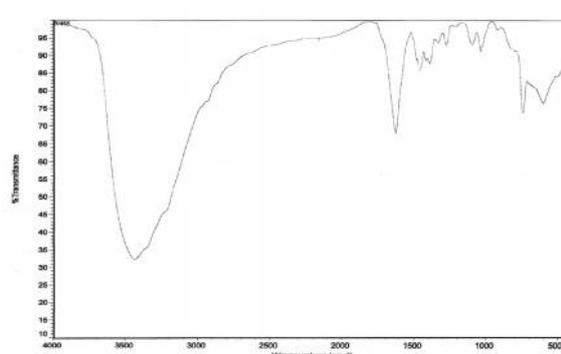


IR spectral studies

The infrared spectral bands that provide conclusive structural evidence for the Coordination of the ligands to the central metal ions are given in the Table2. The ligand shows a strong band at 1611cm⁻¹ characteristic of (C=N) stretching vibration, this band undergoes a negative shift in the complexes indicating the participation of the azomethine nitrogen in coordination¹². In addition, the ligand exhibits a band at 1499 cm⁻¹ due to cyclic(C=N) of the imidazole nitrogen (N3). This band is shifted in all the complexes, indicating the involvement of the imidazole (N3) atom in complex formation. The IR spectrum of the BMBM shows a broad band around 3379cm⁻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 group¹³. Appearance of a broad band around 3400cm⁻¹ region was observed in the spectra of metal complexes indicating the presence of coordinated or lattice water molecules. Water molecules are coordinated, confirmed by the occurrence of additional band in the range of 800-850cm⁻¹ arising due to OH rocking vibrations¹⁴. Further conclusive evidence of the coordination of these Schiff base with metals was shown by the appearance of weak low-frequency new bands at 430–450 cm⁻¹ corresponding to (M–N) and (M–O) stretching modes¹⁵. In addition, vanadyl complex shows a band at 981cm⁻¹ attributed to V=O frequency¹⁶⁻²⁰.

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

Compound	CH=N	C=N	M-N	M-OH	V=O
BMBM	1611	1499	---	---	----
Cu(II)BMBM	1626	1480	446	3437,809	---
Co(II)BMBM	1627	1480	430	3417,815	---
Ni(II)BMBM	1626	1459	430	3390,843	---
Mn(II)BMBM	1623	1454	435	3385,812	---
VO(II)BMBM	1608	1491	434	3413,840	981
Zn(II)BMBM	1572	1436	444	3422,853	---

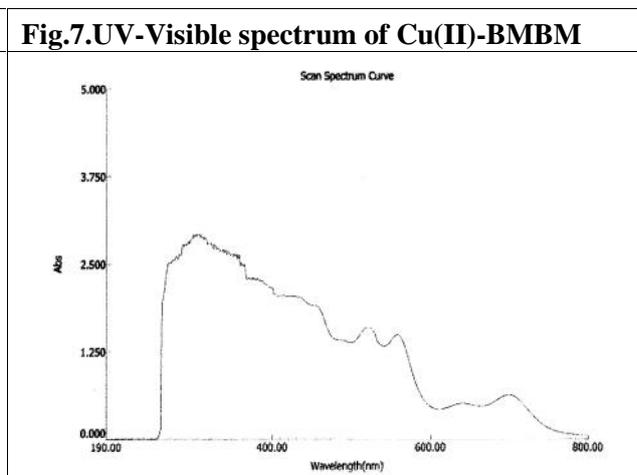
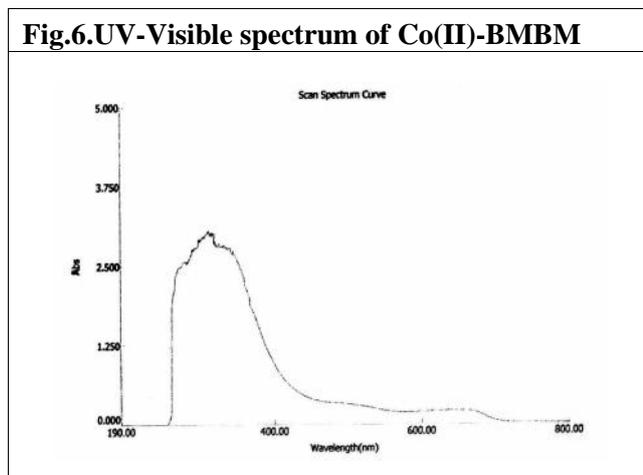
Fig.4. IR spectrum of Co(II)-BMBM

Fig.5. IR spectrum of Co(II)-BMBM


Electronic spectra and magnetic moment

The magnetic susceptibility and electronic spectral data of the metal complexes are given in Table3. The magnetic susceptibility data showed that all the metal complexes, except zinc complex, are paramagnetic. The values are consistent with monomeric nature of the metal ion. The Cu(II) complex showed three broad bands at 14306, 17921 and 19193 cm^{-1} assignable to the ${}^2B_{1g}$, ${}^2A_{1g}$, ${}^2B_{1g}$, ${}^2B_{2g}$ and ${}^2B_{1g}$, ${}^2E_{1g}$ transitions respectively, that are characteristic of a Square planar geometry²¹. The electronic spectrum of Co(II) complex showed bands at 14970, 19230, 24103 cm^{-1} , which are assignable to ${}^4T_{1g}(F)$, ${}^4T_{2g}(F)$, ${}^4T_{1g}(F)$, ${}^4A_{2g}(F)$ and ${}^4T_{1g}(P)$, ${}^4T_{1g}(F)$ transitions respectively, that are characteristic of octahedral configuration. The Ni(II) complex exhibited three transitions at 16890, 21340, 25250 cm^{-1} , which are assignable to ${}^3A_{2g}$, ${}^3T_{2g}(F)$, ${}^3A_{2g}$, ${}^3T_{1g}(F)$ and ${}^3A_{2g}$, ${}^3T_{1g}(P)$ transitions respectively. Electronic spectra of Mn(II) complex display absorption bands at 21263, 24890, 25203 and 27419 cm^{-1} , characteristic of octahedral geometry corresponding to ${}^6A_{1g}$, ${}^4T_{1g}({}^4G)$, ${}^6A_{1g}$, ${}^4E_{2g}({}^4D)$, ${}^6A_{1g}$, ${}^4T_{1g}({}^4P)$ and ${}^6A_{1g}$, ${}^4E_{g}({}^4G)$ transitions respectively. The VO (II) complex exhibited two transitions at 22779, 24937 cm^{-1} , which are assignable to 2B_2 , 2E , 2B_2 , 2B_1 respectively, that are characteristic of square pyramidal geometry²¹. The diamagnetic Zn(II) complex did not show any d-d bands and their spectra are dominated by charge transfer bands assigned due to transition possibly in a tetrahedral environment²⁰.

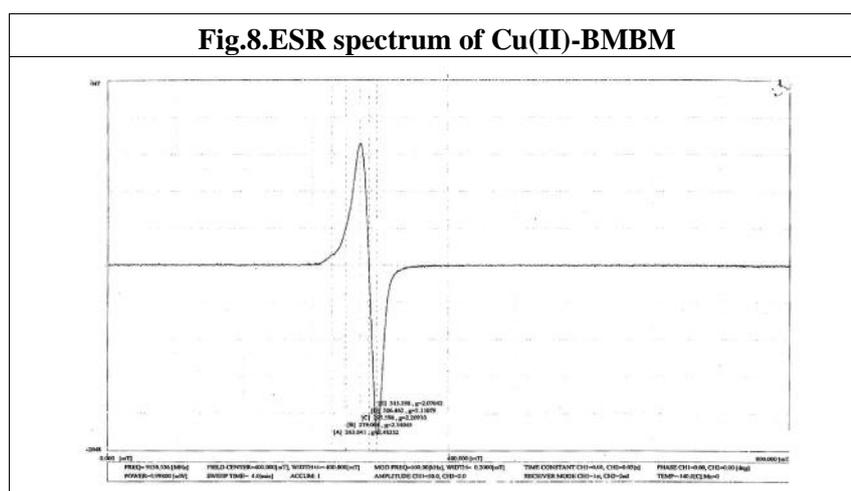
Table3. Electronic spectral data and magnetic moment data

Complex	μ_{eff}	Frequency(cm^{-1})	Assignments	Geometry
Cu(II)-BMBM	2.01	14306 17921 19193	${}^2B_{1g}$, ${}^2A_{1g}$ ${}^2B_{1g}$, ${}^2B_{2g}$ ${}^2B_{1g}$, ${}^2E_{1g}$	Square planar
Co(II)-BMBM	4.79	14970 19230 24103	${}^4T_{1g}(F)$, ${}^4T_{2g}(F)$ ${}^4T_{1g}(F)$, ${}^4A_{2g}(F)$ ${}^4T_{1g}(P)$, ${}^4T_{1g}(F)$	Octahedral
Ni(II)-BMBM	3.1	16890 21340 25250	${}^3A_{2g}$, ${}^3T_{2g}(F)$ ${}^3A_{2g}$, ${}^3T_{1g}(F)$ ${}^3A_{2g}$, ${}^3T_{1g}(P)$	Octahedral
VO(II)BMBM	1.76	22779 24937	2B_2 , 2E 2B_2 , 2B_1	Square pyramidal
Mn(II)BMBM	4.89	21263 24890 25203 27419	${}^6A_{1g}$, ${}^4T_{1g}({}^4G)$ ${}^6A_{1g}$, ${}^4E_{2g}({}^4D)$ ${}^6A_{1g}$, ${}^4T_{1g}({}^4P)$ ${}^6A_{1g}$, ${}^4E_{g}({}^4G)$	Octahedral
Zn(II)-BMBM	Diamagnetic	-	-	Tetrahedral



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 of BMBM was recorded in DMSO at liquid nitrogen Temperature (LNT)

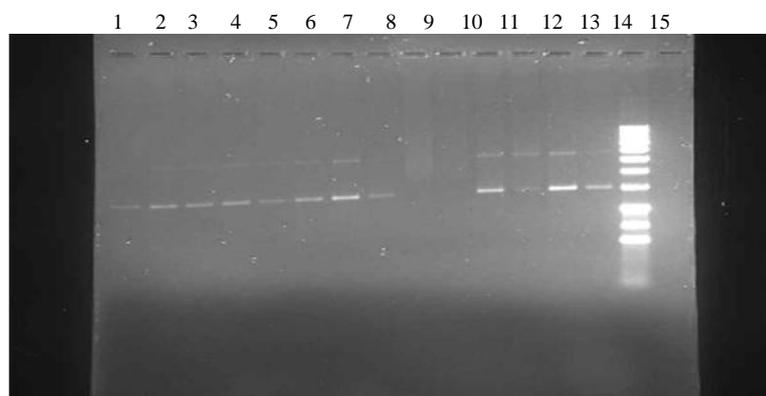


In distorted octahedral and square planar complexes, the unpaired electron lies in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with $g_{II} > g$ ($2.2 > 2.07$) and g_{avg} is calculated as 2.116. ESR spectrum of Cu(II)-BMBM is given in the Figure 6. The spin-orbit coupling constant, λ value (-416 cm^{-1}) calculated using the relations, $g_{avg} = 1/3[g_{II} + 2g]$ and $g_{avg} = 2(1 - \lambda/10Dq)$, is less than the free Cu(II) ion (-832 cm^{-1}) which also supports covalent character of M-L bond in the complex.

DNA CLEAVAGE STUDIES

DNA cleavage is measured by relaxation of super coiled pUC19 DNA to nicked circular conformation and linear conformation. During electrophoresis process super coiled DNA will migrate faster when compared with DNA in nicked and linear confirmations. Figure.9 illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC¹⁹ DNA induced by BMBM metal complexes.

Fig.9. DNA Cleavage Activity of Metal complexes of BMBM



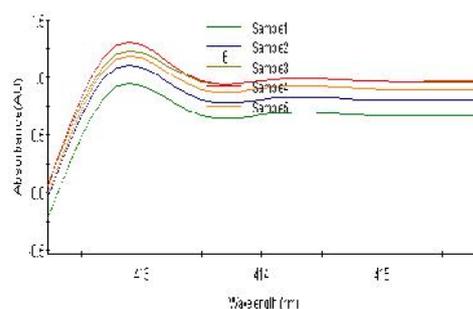
1 – DNA alone, 2 – DNA + H₂O₂, 3 – DNA + DMSO, 4 – DNA + DMSO + H₂O₂, 5 – DNA + Compound VO(II), 6 – DNA + H₂O₂ + BMBM-VO(II), 7 – DNA + BMBM- Ni(II), 8 – DNA + H₂O₂ + BMBM-Ni(II) 9 – DNA + BMBM-Co(II), 10 – DNA + H₂O₂ + BMBM-Co(II), 11 – DNA + BMBM-Cu(II), 12 – DNA + H₂O₂ + BMBM-Cu(II), 13 – DNA + BMBM-Zn(II), 14 – DNA + H₂O₂ + BMBM-Zn(II), 15 – Marker

The control experiments did not show any apparent cleavage of DNA (lane 1, 2, 3 and 4). Cu(II) and Zn(II) complexes in the presence of H₂O₂ (lane 12 and 14) at 50 μM concentration shows more cleavage activity when compared in absence of H₂O₂. Co(II) 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 (lane 9 and 10). The super coiled plasmid DNA was completely degraded resulting in the disappearance of bands on agarose gel²². Further the presence of a smear and decreased intensity of bands in the gel diagram indicates the presence of radical cleavage²³.

DNA BINDING STUDIES

There has been considerable interest in studying the affinity and selectivity in binding of metal complexes to DNA. The results of such studies may find applications in chemotherapy and in the development of tools for biotechnology. Much work has been carried out to understand the non covalent interactions of DNA by metal complexes. We have studied the interaction of Cu (II) and Co(II) complexes with DNA using absorption spectral studies.

Fig.10. Absorption spectra of Co(II)-BMBM in 5mM Tris-Hcl buffer at pH 7.2 in the presence of increasing amounts of CT-DNA. (The arrow indicates the change in absorbance up on increasing the DNA concentration)



Absorption spectral studies

Electron absorption spectroscopy is one of the powerful techniques for probing metal ion DNA interactions²⁴⁻²⁶. The 'Hyperchromic' effect and 'Hypochromic' effect are spectral features of DNA concerning its double helix structure. Hypochromism results from the concentration of DNA in the helix axis as well as from the change in the conformation of DNA, while 'hyperchromism' results from the structural damage of DNA^{27,28}. The extent of hypochromism suggests the strength of intercalative binding; on the other hand hyperchromism may result with metal complexes which bind non-intercalatively or electrostatically with DNA.

The electronic absorption titration of Cu(II) and Co(II) complexes has been carried out at a fixed concentration of the complexes (100 μM) in DMSO at RT by varying the concentration of DNA. The absorption spectra of Co(II)-BCTM (Fig.10) and Cu(II)-BMBM complexes with increasing concentration of DNA showed that there is a minor bathochromic shift along with significant hypochromicity. These results indicate that Cu(II) and Co(II) complexes bind to DNA through intercalation due to strong stacking interaction between the planar aromatic chromophore and base pairs of DNA^{29,30}. The intrinsic binding constant (K_b) of the copper complex is 1.92×10^5 and that of Cobalt complex is 2.26×10^5 and these values suggest that these complexes reasonably bind with CT-DNA.

Conclusion

Cu(II), Co(II), Ni(II), Mn(II), VO(II) and Zn(II) complexes of the Schiff base derived from 2-(amino methyl) benzimidazole and p-methyl benzaldehyde were prepared and characterized.

The study reveals that

- (i) Co(II) and Mn(II) complexes are 1:1 electrolytes and Cu(II), VO(II), Ni(II) and Zn(II) complexes are non electrolytes.
- (ii) The Schiff base behaves as a neutral bidentate ligand and is coordinated through the azomethine nitrogen and pyridyl nitrogen of benzimidazole moiety.
- (iii) Mn(II), Co(II), Ni(II) complexes have octahedral geometry and Square planar geometry for Cu(II), Tetrahedral geometry for Zn(II) and Square pyramidal geometry for VO(II) was proposed.

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