

SYNTHESIS, CHARACTERISATION AND DNA BINDING STUDIES OF SCHIFF BASE METAL COMPLEXES OF CEFIXIME

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ABSTRACT

Schiff base metal complexes of cefixime such as $[Cu(cefbenz)_2Cl_2] \cdot 2H_2O$, $[Zn(cefbenz)_2Cl_2] \cdot 2H_2O$, $[Ni(cefbenz)_2Cl_2] \cdot 6H_2O$, and $[Fe(cefbenz)_2(H_2O)Cl_3] \cdot 5H_2O$ have been synthesized and characterised by elemental analysis, IR, UV-Vis, ESR, NMR, XRD spectroscopic techniques. Thermal analysis was studied by TGA, DSC method. Morphological studies were carried out by scanning electron microscopic method. Further DNA binding studies were carried out for the complexes. Elemental analysis (Table 1) shows that the ligands and the metal salts are in the ratio of 2:1. The low molar conductance values of the complexes reveal their non-electrolytic nature. The IR spectrum of the Schiff base ligand shows a strong band in the region 1650-1661 cm^{-1} due to C=N bond. This band gets shifted to lower frequency in the complex, indicating the coordination through azomethine nitrogen. The complexes showed two bands in the region 550-532 nm and 626-612 nm which can be assigned to d-d transitions of the metal ions ($2B_{1g} \rightarrow 2A_{1g}$) and which strongly favour square-planar geometry around the central metal ion. In conclusion, prepared complexes showed enhanced biological activities than the parent drug that might be of interest for future research.

Keywords: *Metal Complexes, Cefixime, DNA Binding Studies, Ligand.*

I. INTRODUCTION

The Schiff bases are an important class of ligands in coordination chemistry. The study of structural and binding features of various Schiff base complexes can play an important role in better understanding of the complex in biological processes. Schiff bases derived from salicylaldehyde are well known for their interesting ligational properties and exclusive applications in different fields [1-3]. It is well known from the literature that Schiff bases derived from thiazide drugs have a strong ability to form metal complexes [4]. The interaction of these donor ligands and metal ions gives complexes of different geometries and literature survey reveals that these complexes are potentially more biologically active. Thus, in recent years Schiff bases and their metal complexes have attained much attraction because of their extensive biological activities [5,6]

The efforts are still underway to search or locate more potent and cheaper raw materials for fulfilling requirements of industry. The treatment of bacterial infections is carried out using cephalosporins, which have exhibited broad antibacterial activity with little toxicity and are employed extensively [7]. Most of the antimicrobial agents with improved spectra of activity and β -lactamase stability are obtained by structural modifications in cephem [8]. Cefixime belongs to cephalosporin i.e. third generation of antibiotics. The first and

second generations of cephalosporins have lesser activity against gram-negative bacteria as compared with the third generation.

II. SYNTHESIS

2.1 Synthesis of Schiff base ligands

Benzaldehyde (0.01 M, 1.321g), is dissolved in methanol (10 ml) and added to cefixime (0.01M, 3.61 g) dissolved in methanol. To this 10 ml KOH (0.1 % in methanol) was added to adjust the pH of the solution between 7-8 and then the mixture was refluxed for 3 hrs. The Schiff base separated out on removal of the solvent at room temperature. A light yellowish brown coloured crystalline solid was obtained and then dried over anhydrous CaCl_2 in vacuum.

2.2 Synthesis Schiff Base Metal Complexes

The Schiff base ligand (0.005M, 2.97g) and metal chlorides [M = Zn(II) (0.86 g), Ni(II)(1.187 g), Cu(II) (0.85 g), and Fe(III) (1.35g) were dissolved in methanol (10 ml) separately. To this KOH (0.1 % in methanol) was added to adjust the pH of the solution between 7-8 and the mixture was refluxed for 1 hr. A dark brown coloured product was isolated after reduction of solvent volume by evaporation, which was then filtered, washed, and dried over vacuum. The complexes are formulated as $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{cefbenz})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{cefbenz})_2\text{Cl}_2] \cdot 6\text{H}_2\text{O}$, and $[\text{Fe}(\text{cefbenz})_2(\text{H}_2\text{O})\text{Cl}_3] \cdot 5\text{H}_2\text{O}$,

III. RESULTS AND DISCUSSION

3.1 Elemental Analysis

Elemental analysis (Table 1) shows that the ligands and the metal salts are in the ratio of 2:1. The low molar conductance values of the complexes reveal their non-electrolytic nature.

Table 1. Elemental analysis of Schiff base copper complex of cefixime

Compound	C%(found)	H%(found)	N%(found)	Metal%(found)
$[\text{Cu}(\text{cefbenz})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$	45.22(45.92)	3.47(3.42)	11.47(11.38)	5.21(5.27)
$[\text{Zn}(\text{cefbenz})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$	45.15(45.34)	3.46(3.42)	11.45(11.43)	5.35(5.39)
$[\text{Fe}(\text{cefbenz})_2(\text{H}_2\text{O})\text{Cl}_3] \cdot 5\text{H}_2\text{O}$	41.81(42.78)	3.82(3.78)	10.60(10.59)	4.23(4.35)
$[\text{Ni}(\text{cefbenz})_2\text{Cl}_2] \cdot 6\text{H}_2\text{O}$	42.86(42.79)	3.91(3.93)	10.87(10.82)	4.56(4.49)

3.2 IR spectra

The IR spectrum of the Schiff base ligand shows a strong band in the region $1650\text{--}1661\text{ cm}^{-1}$ due to C=N bond. This band gets shifted to lower frequency in the complex, indicating the coordination through azomethine nitrogen (Fig 1). It is found from the IR spectra of the complexes that there are wide and strong band at $564\text{--}670\text{ cm}^{-1}$ due to (M-N) bonding. A strong band at 1626 cm^{-1} due to $>\text{C}=\text{O}$ stretching frequency of the carboxylate is not shifted in the copper complex indicating that the carboxylate group is not involved in complexation.

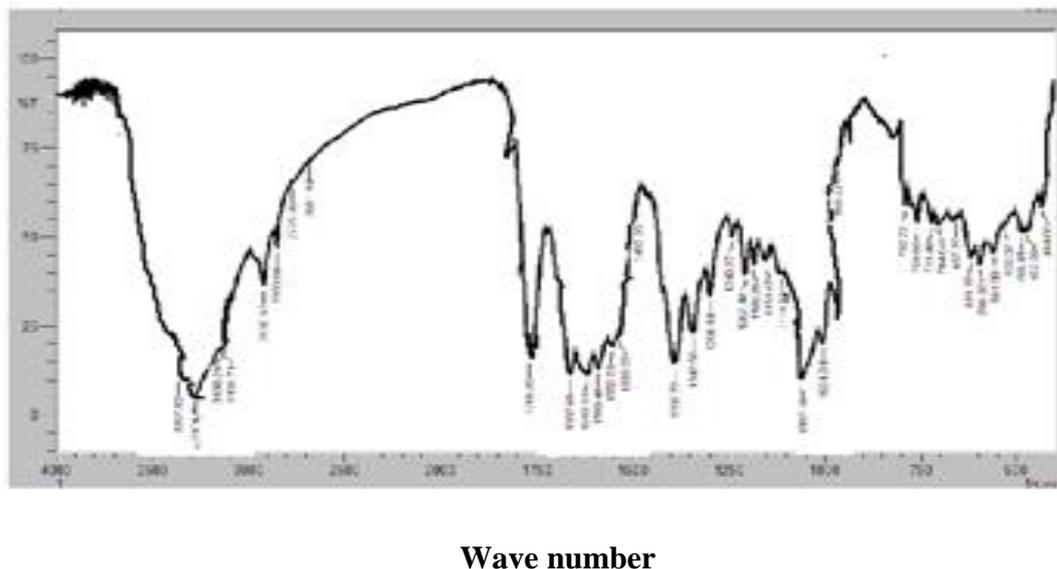


Fig 1. IR spectrum of $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$

3.3 Electronic Spectra

The electronic spectra of the Cu(II) complex (Fig 2) are compared with those of the ligands. Two bands appeared at $256\text{--}251\text{ nm}$ and $348\text{--}328\text{ nm}$, which are assigned to $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions respectively [9]. The complexes showed two bands in the region $550\text{--}532\text{ nm}$ and $626\text{--}612\text{ nm}$ which can be assigned to d-d transitions of the metal ions (${}^2\text{B}_{1g}\rightarrow{}^2\text{A}_{1g}$) and which strongly favour square-planar geometry around the central metal ion [10]. In addition, the μ_{eff} values for this compound in the range of $1.74\text{--}1.84\text{ BM}$, is indicative of one unpaired electron per Cu(II) ion suggesting the square-planar geometry [11]. Nickel(II) complex exhibit two bands at $624\text{--}600\text{ nm}$ and $494\text{--}474\text{ nm}$, assigned to the ${}^3\text{T}_1(\text{F})\rightarrow{}^3\text{T}_1(\text{P})$ and charge transfer (ct) transitions respectively in tetrahedral geometry [12]. The Zn(II) complex expectedly shows only charge transfer transition from $\text{M}\rightarrow\text{L}$ and $\pi\text{--}\pi^*$ transitions. This complex is diamagnetic, confirming its tetrahedral geometry [13-14]. The Fe(III) complex exhibits bands around $234\text{--}253\text{ nm}$, $324\text{--}365\text{ nm}$ and $477\text{--}498\text{ nm}$. The broad intense and poorly resolved bands around $324\text{--}365\text{ nm}$ may be assigned to LMCT. The high intensity band around 250 nm is of ligand origin and assignable to intraligand $n\text{--}\pi^*/\pi\text{--}\pi^*$ transition. The band around $477\text{--}498\text{ nm}$ is assigned to ${}^6\text{A}_{1g}\rightarrow{}^4\text{T}_{2g}(\text{G})$ [16] transition suggesting octahedral geometry which is confirmed by the magnetic moment value of $5.9\text{--}5.63\text{ BM}$ [17].

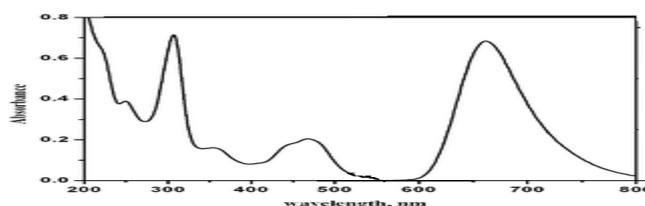


Fig 2 Electronic spectrum of [Cu(cefbenz)₂Cl₂].2H₂O

3.4 ESR Spectrum

In square-planar complexes the unpaired electrons lie in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with $g_{||} > g_{\perp}$ while the unpaired electron lies in the d_z^2 orbital giving $^2A_{1g}$ as the ground state with $g_{\perp} > g_{||}$. From the observed values, it is clear that $g_{||} > g_{\perp}$, which indicates that the structure of the complex (Fig 3) is square-planar and that the unpaired electron is predominantly in the $d_{x^2-y^2}$ orbital.

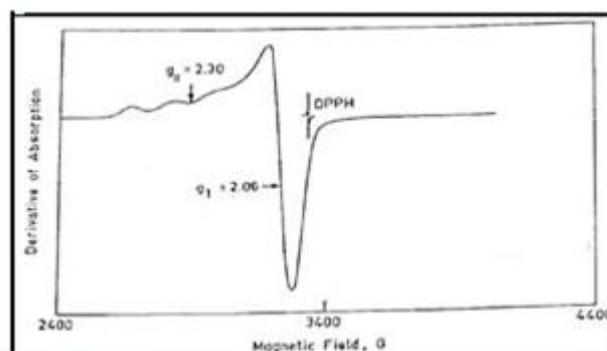


Fig 3. ESR spectrum of [Cu(cefbenz)₂Cl₂].2H₂O

Table 2 ; ESR spectral data of [Cu(cefbenz)₂Cl₂].2H₂O

Compound	$g_{ }$	g_{\perp}	g_{iso}	$K_{ }$	K_{\perp}	α^2	β^2	γ^2	G
[Cu(cefbenz) ₂ Cl ₂] 77K	2.234	2.226	-	0.62	0.514	1.712	0.712	0.719	6.4
[Cu(cefbenz) ₂ Cl ₂] 300K	-	-	2.10	-	-	-	-	-	-

3.5 ¹H NMR Spectrum

The ¹H NMR free ligand showed signals at 6.97 δ , 7.28-7.59 δ and 2.40-2.72 δ assigned to the azomethine group proton, aromatic protons and methyl protons respectively. These signals were slightly shifted upfield or downfield in the complexes. The Schiff base complex showed a signal at 10.97 δ due to the coordinated NH₂ group of the drug. The lattice and coordinated water molecules were observed at 3.03-3.10 δ and 3.39-3.48 δ , respectively .

3.6 TGA Study

From the results of TGA analysis of the Schiff base copper complex (Fig 4) of cefixime it is observed that lattice water molecules are lost in an endothermic step at relatively low temperature 80-120°C, while the coordinated water molecules are lost at relatively higher temperatures, >150°C in an endothermic step. The decomposition of the copper complex takes place at 450°C [18,19].

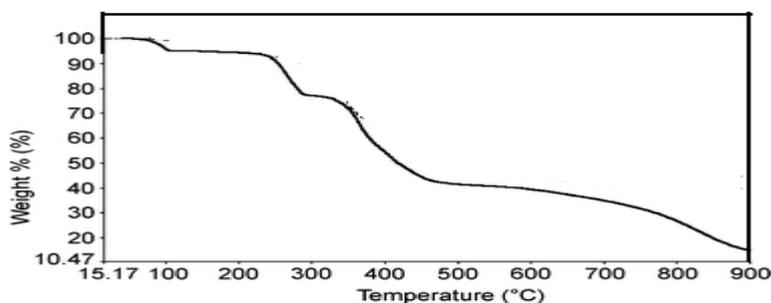


Fig 4; TGA pattern of $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$

3.7 DSC Study

DSC behaviour of the copper complex has been studied in the temperature range of 0-400°C. The metal complex (Fig 5) shows the single T_g at 191.4°C. The copper complex shows a sharp endothermic peak at 165.1°C indicating the melting of the complex. The endothermic peak noted at 236.7°C can be attributed to the decomposition of the complex. Broad exothermic peak obtained at 280 °C and 381.6°C can also be due to the decomposition of the complex [20,21].

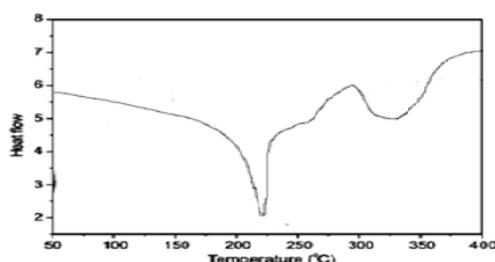


Fig 5. DSC pattern of $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$

3.8 XRD Study

The XRD pattern of the metallo drug are studied in the in the 2θ range of 5-35° and the crystalline size of the complex was calculated from Scherer's formula.(Fig 6). From the observed d_{XRD} pattern the average crystalline size for the metallo drug was found to be 39 nm indicating its nanocrystalline in nature.

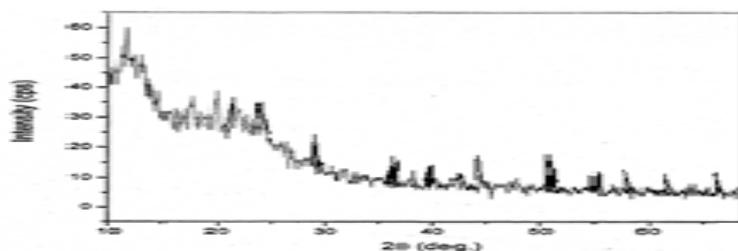


Fig 6 .Powder XRD pattern of $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$

3.9 SEM Study

The SEM micrograph of Schiff base ligand and its copper complex [(Fig 7) and (Fig8)] was recorded at an energy of 20 KV with magnification X150. The particles are agglomerated that these complexes have polycrystalline with nanosized grains in the complex.

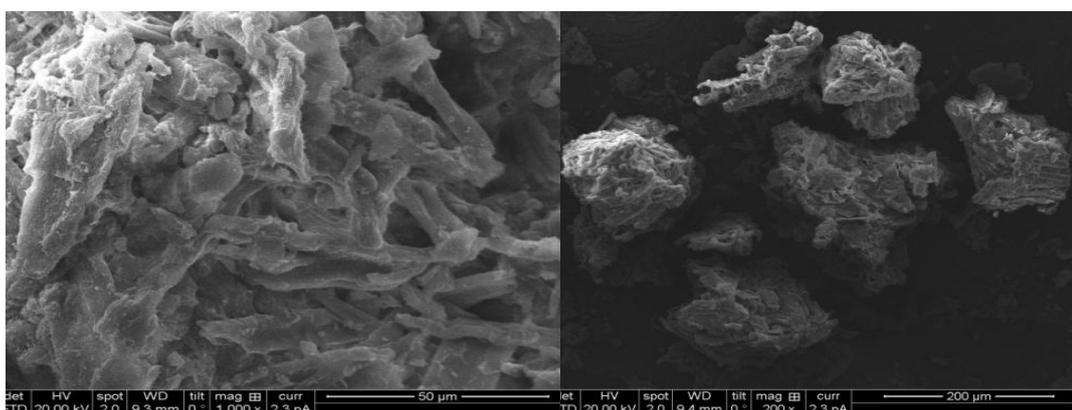


Fig 7. SEM image of Schiff base ligand Fig 8. $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$

IV. DNA BINDING STUDIES

4.1 Electronic Absorption Spectroscopic Method

Absorption spectroscopy is one of the most useful techniques to study the binding of any drug to DNA. The extent of hypochromism generally indicates the intercalative binding strength [22]. “Hyperchromic” and “hypochromic” effects are the spectral features of DNA concerning its double helical structure[23]. Hyperchromism has been observed for the interaction of many drugs with DNA [24]. The hyperchromic effect might be ascribed to external contact (electrostatic binding [25] or to partial uncoiling of the helical structure of DNA, exposing more bases of the DNA [26]. Copper (II) complexes do not give any intense d-d or charge transfer band to monitor the changes upon the addition of DNA. The electronic absorption titration of the complexes has been carried out at the fixed concentration of the complexes (100μm) in DMSO at 25 °C, with varying the concentration of DNA and is illustrated in figures (6.12-6.15). Copper complexes in DMSO buffer mixtures shows bands in the region 320-390 nm and are assigned to ligand to metal charge transfer (LMCT) transitions. When the amount of DNA is increased, the intensity of charge transfer band is also changed, due to either hypochromism or hyperchromism. The complexes such as $[\text{Cu}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{cefbenz})_2\text{Cl}_2]\cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{cefbenz})_2\text{Cl}_2]\cdot 6\text{H}_2\text{O}$, and $[\text{Fe}(\text{cefbenz})_2(\text{H}_2\text{O})\text{Cl}_3]\cdot 5\text{H}_2\text{O}$

show hyperchromism with slight red shift. A similar hyperchromic effect has been observed for certain metal complexes when interacted with DNA

For comparing the binding strength of metal complexes, the intrinsic binding constant K_b was determined using the equation .

$$[DNA]/(\epsilon_a - \epsilon_f) = [DNA/(\epsilon_b - \epsilon_f)] + 1/K_b(\epsilon_b - \epsilon_f)$$

The ' K_b ' values show that the interaction is very strong between the complexes and CT-DNA. The binding constant for values of the above complexes is in the range of 4.5×10^6 greater than those observed for other known DNA intercalative agents. All these results show that the complexes have high DNA binding affinity.

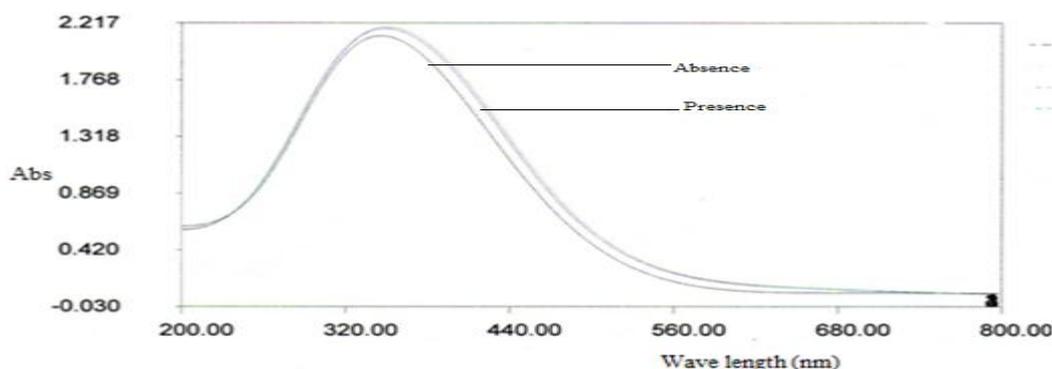


Fig 9. Electronic Absorption Spectra of [Cu(cefbenz)₂Cl₂].2H₂O with Increasing Addition of DNA

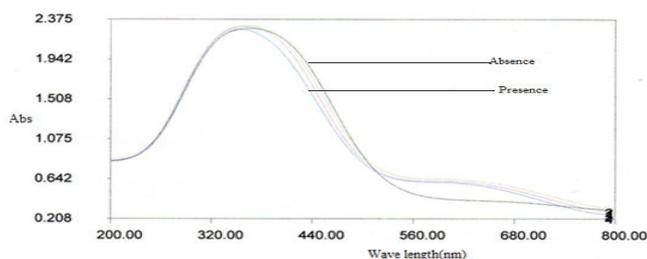


Fig 10 Electronic Absorption Spectra of [Zn(cefbenz)₂Cl₂].2H₂O with Increasing Addition of DNA

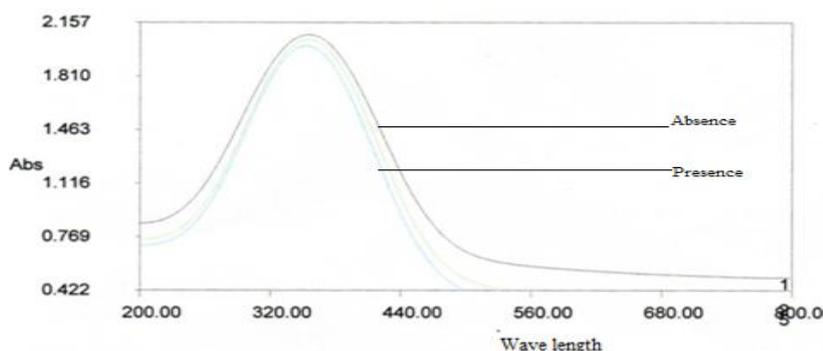


Fig 11. Electronic Absorption Spectra of [Ni(cefbenz)₂Cl₂].6H₂O, Complex with Increasing Addition of DNA

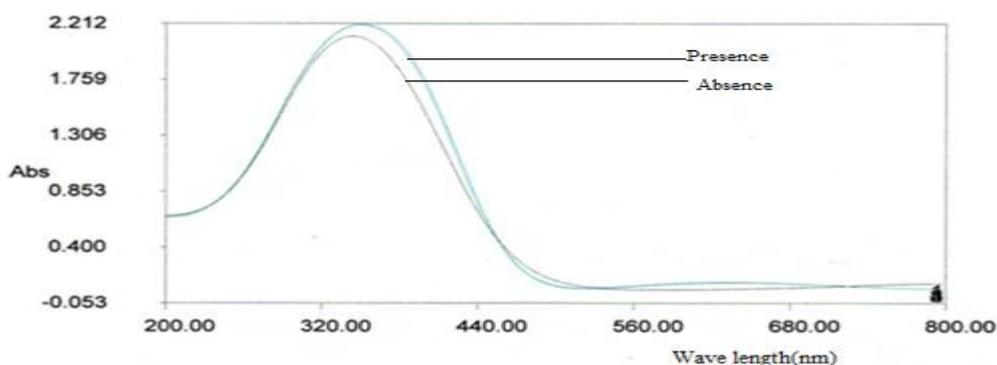


Fig 12. Electronic Absorption Spectra of $[\text{Fe}(\text{cefbenz})_2(\text{H}_2\text{O})\text{Cl}_3]\cdot 5\text{H}_2\text{O}$ Complex with Increasing Addition of DNA

V. CONCLUSION

The study of the reaction between the Schiff base metal complexes and the drug indicates its high stability. This encourages the synthesis and careful investigation of the nature of bonding between the drug and the transition metal cation of important biological role, using physicochemical method of analysis. It is clear from above discussion that the fragmentation pattern and spectral studies of the complex confirm and illustrate the proposed geometry obtained by elemental analysis, IR, ^1H NMR, and mass spectra.

Hence, the proposed tentative structure of the metal complex is given as in Figure 13 .

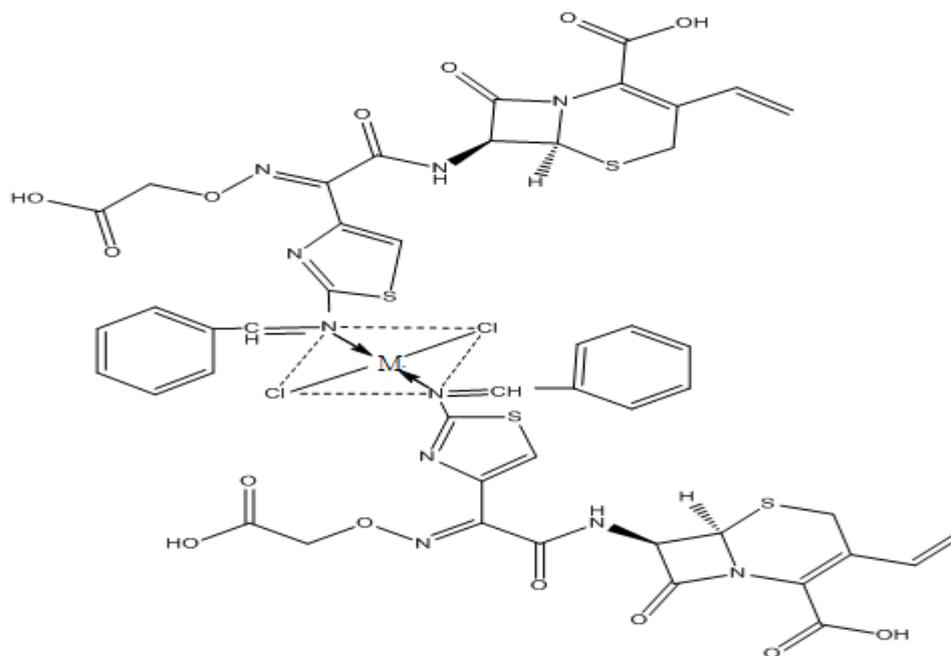


Fig 13. Geometry of Schiff Base Complex of Cefixime, M = Cu(II) ion

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