

# SYNTHESIS, CHARACTERISATION AND DNA BINDING STUDY OF SCHIFF BASE METAL COMPLEXES OF ISONIAZID

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**Abstract:** Metal Complexes of isoniazid such as  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ ,  $[\text{Zn}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ ,  $[\text{Ni}(\text{inhbenz})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ , and  $[\text{Fe}(\text{inhbenz})_2(\text{H}_2\text{O})\text{Cl}]\text{Cl}_2 \cdot 5\text{H}_2\text{O}$ . have been synthesized and characterised by elemental analysis, IR, UV-Visibe, ESR, NMR, XRD spectrsopic techniques. Thermal analysis was studied by TGA, DSC method. Morphological studies were carried out by scanning electron microscopic method. DNA binding studies was carried out by cyclic voltammetric method. From the spectral studies a square planar geometry was proposed for the copper complex. In conclusion, prepared complexes showed enhanced DNA binding tendency than the parent drug that might be of interest for future research.

**Keywords:** Metal complexes. ligands, isoniazid, DNA binding.

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## I. INTRODUCTION

Isoniazid (INH) is a pro-drug that requires activation in isoniazid susceptible mycobacterial species. Based on in vitro experiments, it is reported that activation of isoniazid results in a number of reactive species that are capable of either oxidizing or acylating groups in proteins. However, the actual form of isoniazid that is active *in vivo* is still unknown. It was observed soon after isoniazid was introduced in the 1950s that isoniazid-resistant clinical isolates frequently lost catalase and peroxidase activity [1]. It was shown that superoxide stimulates INH activity in mycobacteria [2]. Activation of INH may also affect DNA, proteins, and other macromolecules through formation of ROS [3]. Thus oxidative activation of INH can both have a specific effect on mycolic acid synthesis and be generally toxic for proteins and nucleic acids. The limited number of effective antituberculosis drugs available and the problems associated with drug resistance and potential adverse reactions such as hepatotoxicity became a prerequisite for synthesis of more effective analogs of INH.

## II. SYNTHESIS OF SCHIFF BASE LIGAND

Benzaldehyde (0.01, 1.32g) is dissolved in methanol and added to the isoniazid (0.01 mol, 1.37g) 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 4hrs. The Schiff base was separated out on removal of the solvent at room temperature. A light yellowish brown coloured product was obtained and dried over anhydrous  $\text{CaCl}_2$  in vacuum.

## III. SYNTHESIS OF SCHIFF BASE METAL COMPLEXES

0.01M of Schiff base ligand and metal chlorides (0.01M) were dissolved in methanol separately. To this 10 ml KOH (0.1 % in methanol) was added to adjust the pH of the solution between 7.5-8 and the mixture was refluxed for 6hrs. A dark brown coloured product was isolated after reduction of solvent volume by evaporation, which was then filtered, and then dried over vacuum. The complexes were formulated as  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ ,  $[\text{Zn}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ ,  $[\text{Ni}(\text{inhbenz})_2]\text{Cl}_2 \cdot 6\text{H}_2\text{O}$ , and  $[\text{Fe}(\text{inhbenz})_2(\text{H}_2\text{O})\text{Cl}]\text{Cl}_2 \cdot 5\text{H}_2\text{O}$ .

#### IV. RESULTS AND DISCUSSION

##### A. IR spectrum:

In the IR spectrum of the Schiff bases ligand, a sharp band observed at  $1616\text{ cm}^{-1}$  is assigned to the  $\nu(\text{C}=\text{N})$  mode of the azomethine group. This shifts to lower wave numbers,  $1606\text{--}1609\text{ cm}^{-1}$  in all the complexes suggesting the co-ordination of the azomethine nitrogen to the metal centres. This is further substantiated by the presence of a new band around  $420\text{--}463\text{ cm}^{-1}$  assignable to  $\nu(\text{M-N})$ . The IR values  $\nu(\text{C-H})$   $720\text{ cm}^{-1}$  and  $633\text{ cm}^{-1}$  observed for isonizid drug are shifted to  $840\text{ cm}^{-1}$  and  $799\text{ cm}^{-1}$ . These shifts can be explained by the fact that each of the two nitrogen atoms of ligands donates a pair of electrons to the central copper metal forming a coordinate covalent bond. The bands around  $3206\text{ cm}^{-1}$  and  $2929\text{ cm}^{-1}$  can be assigned to the N-H stretches of the isonicotinamide. Metal-ligand vibrations are generally observed in the far-IR region and usually gives valuable information regarding the bonding of ligands to metal ions. However, non-ligand new bands appearing in the region  $530\text{--}518\text{ cm}^{-1}$  and  $460\text{--}411\text{ cm}^{-1}$  were assigned to  $\nu(\text{M-O})$  and  $\nu(\text{M-N})$  stretching vibrations [4-6]. The weak intensity bands appearing in the region  $390\text{--}362\text{ cm}^{-1}$  are assigned to  $\nu(\text{M-Cl})$  stretching vibrations (Fig 1).

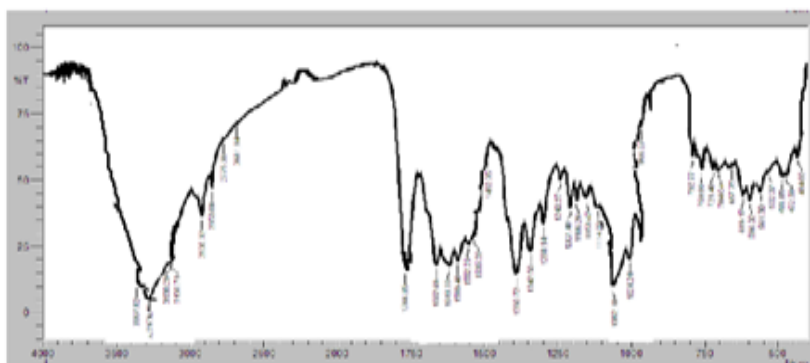


Figure 1: IR spectrum of  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  wavenumber( $\text{cm}^{-1}$ )

##### B. Electronic spectrum:

The copper complex exhibits absorption band in the region of  $345\text{ nm}$  indicating a four coordinate square planar geometry of  $\text{Cu}(\text{II})$  [7]. Nickel(II) complex (Fig2) exhibits two bands at  $623\text{--}605$ , and  $494\text{--}472\text{ nm}$  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 (8). The  $\text{Zn}(\text{II})$  complex does not show any d-d transitions. In general four coordinate  $\text{Zn}(\text{II})$  complexes would have tetrahedral geometry. The  $\text{Fe}(\text{III})$  complex exhibits bands around  $238\text{--}253\text{ nm}$ ,  $324\text{--}368\text{ nm}$  and  $487\text{--}448\text{ nm}$ . The broad intense and poorly resolved bands around  $325\text{--}367\text{ nm}$  may be assigned to LMCT. The band around  $477\text{--}498\text{ nm}$  is assigned to  ${}^6\text{A}_1\text{g} \rightarrow {}^4\text{T}_2\text{g}(\text{G})$  [9] transition suggesting octahedral geometry which is confirmed by the magnetic moment value of  $5.9\text{--}5.63\text{ BM}$  [10].

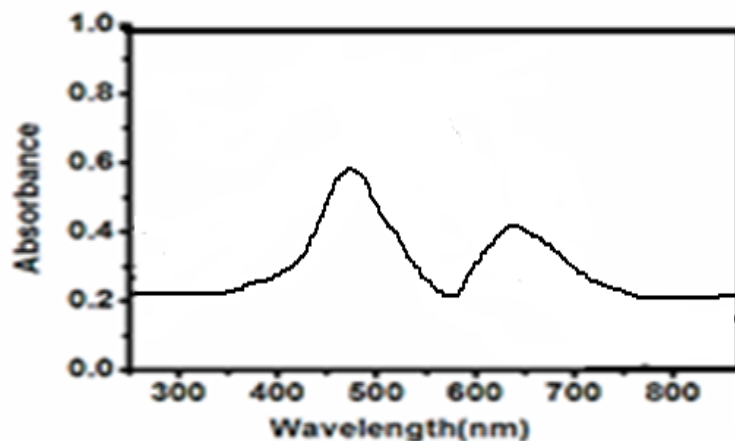


Figure 2: Electronic spectrum of  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$

**C.  $^1\text{H}$  NMR spectrum:**

The signal at  $\delta$  1.5 (s, 1H) is assigned to amide proton (-CONH-) and the signal at  $\delta$  8.44 (s, 1H) is assigned to azomethine proton (-N=CH-) in the Schiff base ligand. The signals due to (-CONH-) are shifted to downfield in the zinc complex, indicating coordination of oxygen of -CONH with metal ions. The azomethine protons shifts downfield in the spectra  $\delta$  8.65,  $\delta$  8.71 (s, 1H) supporting coordination of 'N' of the -N=CH- group with the metal ions. The aromatic protons at  $\delta$  6.94 – 7.83 shift downfield in the complexes. Thus  $^1\text{H}$  NMR observations are supplement with the assigned geometry (Fig3).

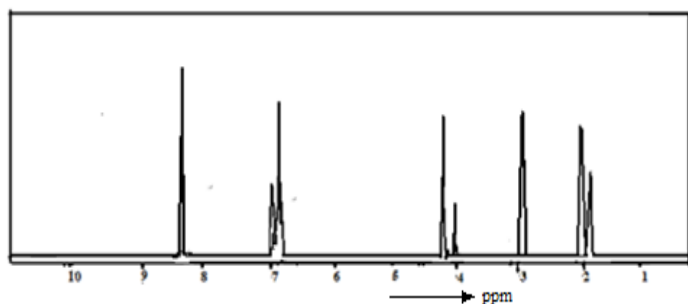


Figure 3:  $^1\text{H}$  NMR spectrum of  $[\text{Zn}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$

**D. ESR spectrum:**

ESR spectrum of the copper complex(fig,4) revealed the trend  $g_{\parallel} > g_{\perp}$  showing that the unpaired electron is delocalized in  $d_{x^2-y^2}$  orbital in the ground state of metal and the spectrum is characteristic of axial symmetry. According to Hathaway, if the value of G is greater than four ( $G > 4$ ) the exchange interaction is negligible whereas when the value of G is less than four ( $G < 4$ ), a considerable exchange interaction is indicated in the complex. It is observed that G value for the complex is greater than four ( $G > 4$ ) indicating the exchange interaction is negligible [11,12]. When the complex is frozen to liquid nitrogen temperature well resolved peaks are obtained. This shows the presence of square planar geometry of Cu(II) complex

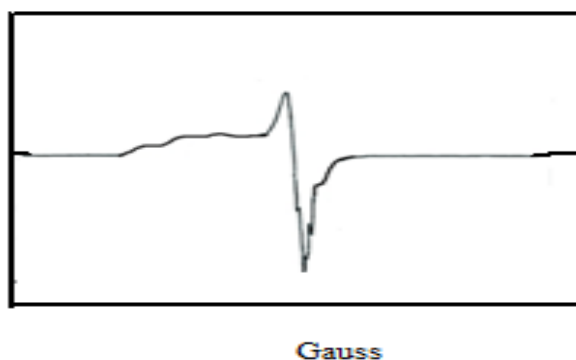


Figure 4: ESR spectrum of  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  at 300K

**E. XRD studies:**

The crystalline size of the complex (Figure 5) was calculated from Scherer's formula. From the observed  $d_{\text{XRD}}$  pattern the average crystalline size for the metallo drug was found to be 78 nm indicating that it is nano crystalline in nature.

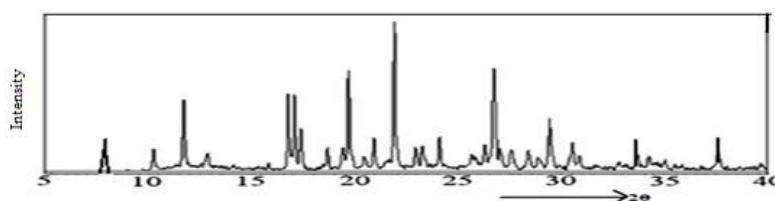
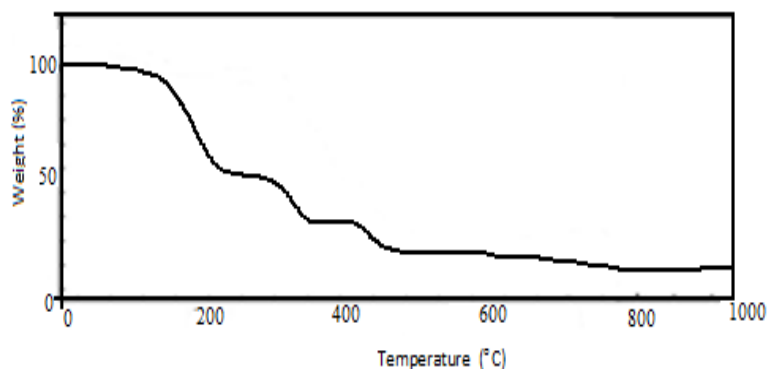


Figure 5: Powder XRD pattern of  $[\text{Zn}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$

**F. TGA studies:**

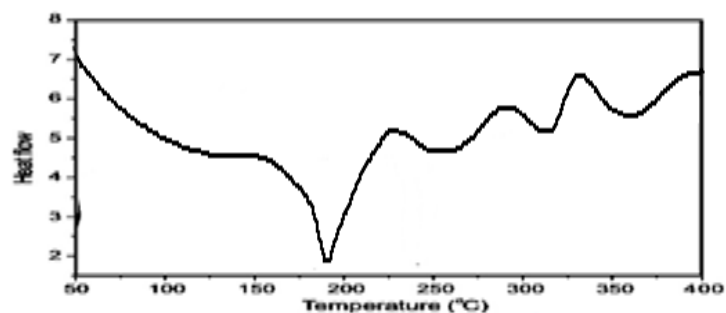
Thermogram of Fe(III) complex (Figure 6) indicated that the total weight loss of 79.9% up to 900°C, was observed in three steps, (i) a small weight loss at 101.7 °C was 4.2% (calculated value 4.4%) is assigned to the loss of lattice water, (ii) weight loss in the range of 234.6-484.4 °C was 8.5% (cal.8.4%) corresponding to the loss of coordinated water (iii) maximum weight loss 71.8%.



**Figure 6: TGA pattern of [Fe(inhbenz)<sub>2</sub>(H<sub>2</sub>O)Cl]Cl<sub>2</sub>.5H<sub>2</sub>O**

**G. DSC studies:**

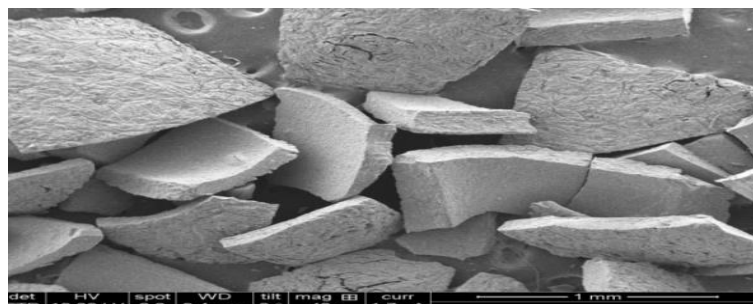
DSC behaviour of the Fe(III) complex (Figure7) has been studied in the temperature range of 0-400°C. The metal complex shows the single T<sub>g</sub> at 191.4 °C. The copper complex shows a sharp endothermic peak at 175.10 °C indicating the melting of the complex. The endothermic peak noted at 256.7 °C can be attributed to the decomposition of the complex. Broad exothermic peak obtained at 320 °C and 371.6 °C can also be due to the decomposition of the complex [13,14].



**Figure 7: DSC pattern of [Fe(inhbenz)<sub>2</sub>(H<sub>2</sub>O)Cl]Cl<sub>2</sub>.5H<sub>2</sub>O**

**H. SEM studies:**

From the SEM study it is clearly seen that the surfaces of the metal complexes containing copper exhibit(fig,8) heterogeneous network and formation of distinct domains.



**Figure 8: SEM image of [Cu(inhbenz)<sub>2</sub>]Cl<sub>2</sub>.2H<sub>2</sub>O**

### I. FAB-mass spectrum:

The FAB-mass spectrum (Fig. 9) has showed a molecular ion peak at  $m/z$  428 corresponding to the molecular weight of the metal complex  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ . Peaks at  $m/z$  90 and 78 corresponds to the fragments  $(\text{C}_7\text{H}_6)^+$  and  $(\text{C}_5\text{H}_4\text{N})^+$ . Thus mass studies support the proposed composition.

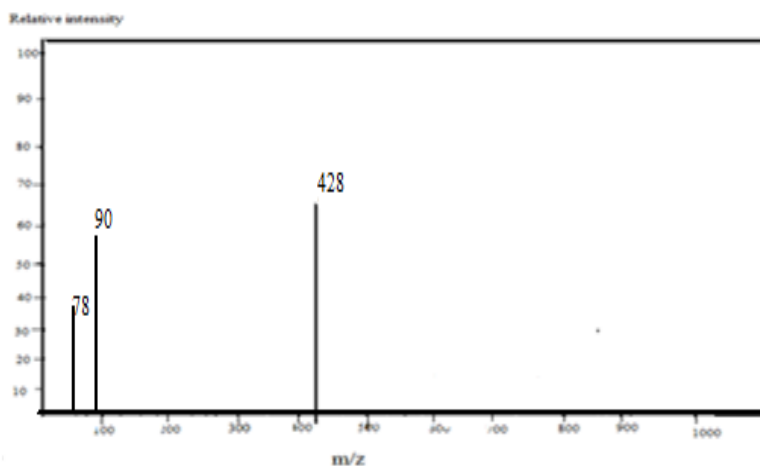


Figure 9: FAB mass spectrum of  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$

## V. DNA BINDING STUDIES BY CYCLIC VOLTAMMETRIC METHOD

Cyclic voltammetric studies have been carried out to probe the binding of the copper complexes  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  to DNA in buffer solution and the cyclic voltammogram shows both cathodic and anodic peak potentials. On increasing the concentration of DNA, the  $E_{pc}$  values undergo a positive shift of potential (shifts to less negative values). This shift might be due to interaction of the reduced form of the complexes with DNA. The obvious shift of peak potential indicates the strong association of the complexes with DNA. If the peak potential is shifted positively that interaction will be intercalation or groove binding [15].

It is clear from the figure.10 the peak potential shift occurs positively for  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  so the mode of binding is intercalative or groove. The formal potential  $E_{1/2}$  of this complex undergoes a positive shift from -0.699 to -0.630 V (nearly 70 mV). This high shift after the addition of CT-DNA suggest high binding affinity of  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$ . In addition to the changes in the formal potential, the voltammetric current decrease upon the addition of DNA to the complexes. This decrease in current is due to the diffusion of an equilibrium mixture of free and DNA bound complex to the electrode surface. All the observation point towards the fact that the complex  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$  interacts with CT-DNA either in intercalating way or through grooves.

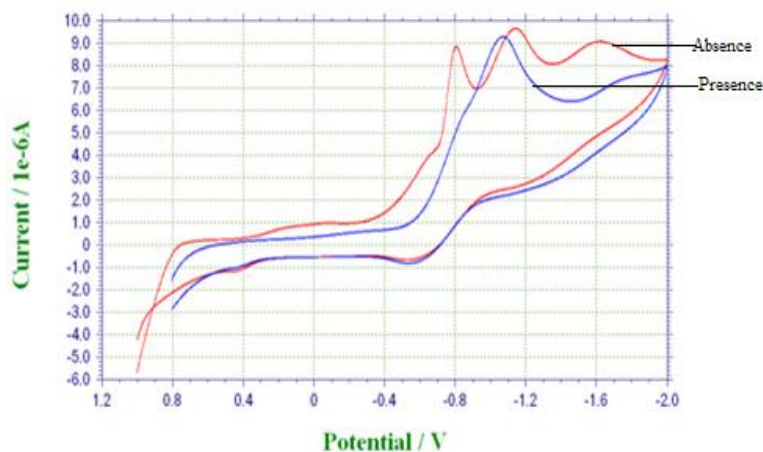


Figure 10: Cyclic voltammogram of  $[\text{Cu}(\text{inhbenz})_2]\text{Cl}_2 \cdot 2\text{H}_2\text{O}$

## VI. PROPOSED GEOMETRY OF THE COMPLEX

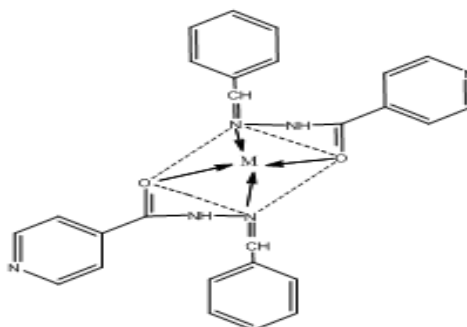


Figure 11: Geometry of Schiff base metal complex of isoniazid, M = Cu(II)ion

## VII. CONCLUSION

The interactions of metal complexes with DNA are a recent focus of research interest in bioinorganic chemistry. The binding and cleavage of DNA by metal complexes is related to the utility of such metal complexes in the design and the development of synthetic restriction enzymes, new drugs, DNA foot printing agents etc. Metal complexes have been found to be particularly useful for the above mentioned purposes because of their potential to bind DNA via multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities. The ligands or the metal ion in these complexes can be varied in an easily controlled manner to facilitate an individual application. All the studies reveal that modification of the metal or ligands would lead to substantial changes in the binding modes, location and affinity giving rise to changes to explore various valuable conformation or site-specific DNA probes and potential chemotherapeutical agents In conclusion, prepared complexes showed enhanced DNA binding tendency than the parent drug that might be of interest for future research.

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