

SYNTHESIS, CHARACTERISATION AND DNA BINDING STUDY OF MIXED LIGAND METAL COMPLEXES OF SULPHADOXINE & PYRIMETHAMINE

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Abstract: Mixed ligand metal complexes of Sulfadoxine and pyrimethamine such as $[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 6\text{H}_2\text{O}$ and $[\text{Fe}(\text{sul})(\text{pym})\text{Cl}_3]\cdot 6\text{H}_2\text{O}$ 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. DNA binding studies were 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, cyclic voltammetric method, DNA binding.

I. INTRODUCTION

Sulfadoxine/pyrimethamine (Fansidar) is a combination of antimalarials containing the sulphonamide antibacterial sulfadoxine and the antiparasitic drug pyrimethamine. Chemically sulphadoxine is 4-Amino-N-(5,6-dimethoxy-4-pyrimidinyl)benzene sulphonamide and pyrimethamine is 5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine. Both drugs are antifolates that inhibit the production of enzymes involved in the synthesis of folic acid within the parasites. The combination is considered to be more effective in treating malaria caused by *Plasmodium falciparum* than that caused by *Plasmodium vivax*, for which chloroquine is considered more effective [1,2]

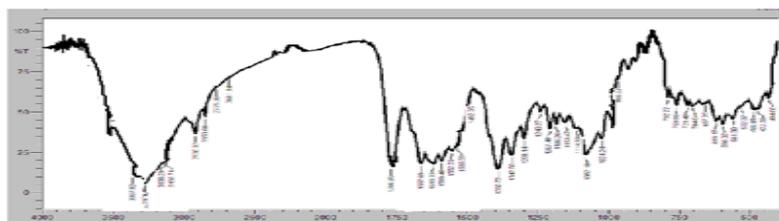
II. SYNTHESIS OF COMPLEXES

Each of the complexes was prepared by adding aqueous solution of metal salts to an ethanolic solution of the ligands in 1:1 mole ratios as preferred by the metal geometry. The solution was refluxed with constant stirring for 2 hrs until a colour change is observed or formation of precipitate. The complex formed was recovered from the solution by filtration followed by washing with ethanol and drying in vacuum. On the basis of elemental analysis the complexes were formulated as $[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 6\text{H}_2\text{O}$ and $[\text{Fe}(\text{sul})(\text{pym})\text{Cl}_3]\cdot 6\text{H}_2\text{O}$.

III. RESULTS AND DISCUSSION

A. IR spectrum:

Bands at 3466 cm^{-1} and 3557 cm^{-1} undergo a bathochromic shift in the spectra of Fe(III) complex (Fig.1). The spectrum of pyrimethamine and sulfadoxine also show absorption bands at 3472 cm^{-1} and 3454 cm^{-1} assigned to N-H stretching vibrations. This peak is found in the spectra of the metal complexes with a great shift in their intensity and position. This observation suggests that the (N-H) group is one of the co-ordination sites [3,4]. The free pyrimethamine spectrum showed a band attributed to (C=N) stretching vibration at 1630 cm^{-1} . This band is also found in the complexes with red shift attributed to complexation.

Figure 1: IR spectrum of $[\text{Fe}(\text{sul})(\text{pym})\text{Cl}_3].6\text{H}_2\text{O}$ wavenumber(cm^{-1})

B. Electronic spectrum:

The electronic spectrum of the copper complex (Fig.2.) is compared with those of the ligands. Two bands appeared at 256-251 nm and 348-328 nm, which can be assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, respectively, in all the ligands [5]. The complexes showed two bands in the region 552-530 nm and 626-612 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 [6]. In addition, the μ_{eff} values for this compounds, in range 1.74-1.84 BM, indicative of one unpaired electron per Cu(II) ion and suggesting a square-planar geometry [7]. Nickel(II) complex exhibits two bands at 624-610 nm and 494-472 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 Zn(II) complex expectedly shows only charge transfer transition from $\text{M} \rightarrow \text{L}$ and $\pi \rightarrow \pi^*$ transitions, as no d-d transition is expected. This complex is diamagnetic, confirming its tetrahedral geometry [9-11]. The Fe(III) complex exhibits bands around 234-253 nm, 324-365 nm, and 477-498 nm. The broad intense and poorly resolved bands around 324-365 nm may be assigned to LMCT. The high intensity band around 250 nm is due to ligand [12].

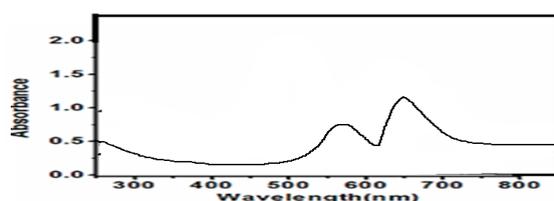
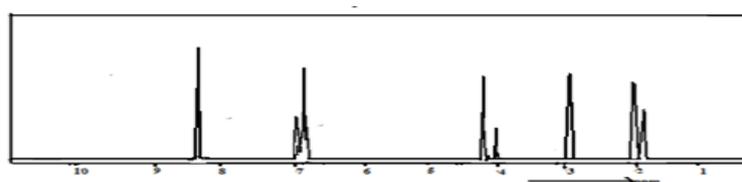
Figure 2: Electronic spectrum of $[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$

TABLE I: ELECTRONIC SPECTRAL DATA OF MIXED LIGAND COMPLEXES OF SULPHADOXINE AND PYRIMETHAMINE

Compound	Transition	Wavelength(nm)	Geometry	μ_{eff}
Ligand	$n - \pi / \pi - \pi^*$	below 250	-	-
$[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$	$({}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g})$	552-530	square planar	1.74-1.84
$[\text{Zn}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$	-	-	tetrahedral	diamagnetic
$[\text{Ni}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.6\text{H}_2\text{O}$	${}^3\text{T}_1(\text{F}) \rightarrow {}^3\text{T}_1(\text{P})$		tetrahedral	3.72
$[\text{Fe}(\text{sul})(\text{pym})\text{Cl}_3].6\text{H}_2\text{O}$	${}^3\text{T}_1(\text{F}) \rightarrow {}^3\text{T}_1(\text{P})$	477-498	Octahedral	5.9-5.63

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

In the NMR spectrum of the ligand (sulphadoxine), the aromatic NH proton peak appears at δ 4.0, NH_2 protons appear at δ 6.27, CH protons of 4-pyrimidine appear at δ 8.38, CH proton appear at δ 7.61 and CH_3 protons appear at δ 3.86 respectively. The NH_2 protons appear at δ 6.99, CH protons appear at δ 7.17, CH_2 protons appear at δ 3.1 and CH_3 protons appear at δ 1.25 respectively in pyrimethamine drug. In the complexes the shifts in position of peaks due to NH protons to downfield confirm the involvement of these groups in metal coordination (Fig.3).

Fig 3: ${}^1\text{H}$ NMR spectrum of $[\text{Zn}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$

D. ESR spectrum:

In square-planar complexes (Fig.4.) the unpaired electrons lie in the $d_{x^2-y^2}$ orbital giving $^2B_{1g}$ as the ground state with $g_{\parallel} > g_{\perp}$ while the unpaired electron lies in the d_z^2 orbital giving $^2A_{1g}$ as the ground state with $g_{\perp} > g_{\parallel}$. From the observed values, it is clear that $g_{\parallel} > g_{\perp}$, which indicates that the structure of the complex is square-planar and that the unpaired electron is predominantly in the $d_{x^2-y^2}$ orbital [13].

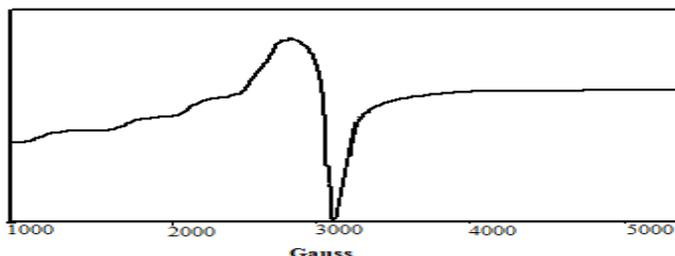


Fig 4: ESR spectrum of [Cu(sul)(pym)Cl]Cl.2H₂O at 300K

TABLE II: ESR SPECTRAL DATA OF [Cu(sul)(pym)Cl]Cl.2H₂O

Complex	g_{\parallel}	g_{\perp}	g_{iso}	K_{\parallel}	K_{\perp}	α^2	β^2	γ^2	G
[Cu(sul)(pym)Cl] Cl.2H ₂ O 77K	2.224	2.213	-	0.752	0.524	1.236	0.725	0.735	6.3
[Cu(sul)(pym)Cl] Cl.2H ₂ O 77K			2.10						

E. XRD study:

The crystalline size of the copper complex (Fig.5.) calculated from Scherer's formula was found to be 46 nm indicating its nanocrystalline nature. The line broadening of the crystalline peak in the copper complex shows higher crystalline nature. The average crystalline size of the Zn(II) complex is found to be 38nm showing that it is nanocrystalline in nature.

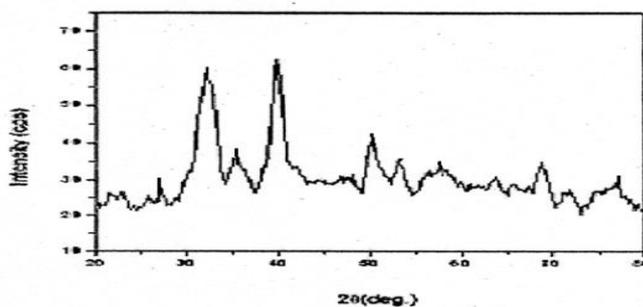


Fig 5: Powder XRD pattern of Zn (sul)(pym)Cl]Cl.2H₂O

F. TGA study:

The zinc complex undergoes three stages of decomposition. The complex (Fig.6.) starts to decompose above 100°C. The first stage of decomposition occurs at 210°C corresponding to 20% weight loss. The decomposition take place in the second step at 285.7°C bringing a weight loss of 37.5%. At 300°C the Zn(II)complex undergoes 32% decomposition. The residual weight at 79°C for the Zn(II)complex is 41.50% corresponding to the amount of respective metal oxide formed at that temperature [14,15]

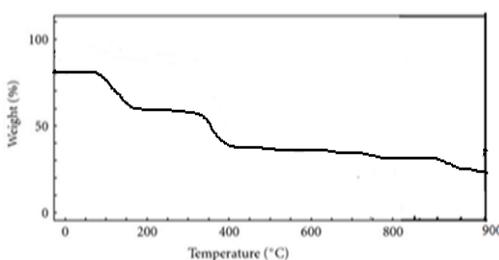


Fig 6: TGA pattern of [Zn(sul)(pym)Cl]Cl.2H₂O

G. DSC studies:

DSC behaviour of the copper complex (Fig.7.) have been studied in the temperature range of 0-400°C. The glass transition temperature of the Cu(II) complex is found to be 208.8°C. The narrow endothermic peak observed at 308°C may be due to the melting of the complex. The exothermic peaks observed at 149.9°C and 200.7°C may be due to the decomposition of the complex as shown from the figure [16,17].

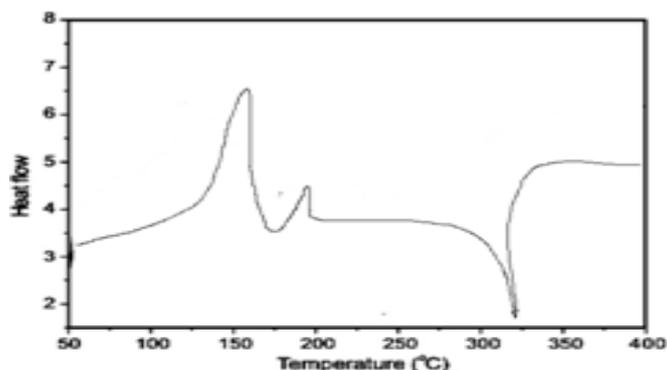


Fig 7: DSC pattern of $[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$

H. SEM studies:

In the Ni(II) complex (Fig.8.) and (Fig.9.), the particles have platelet like structures with uniform thickness. This can be due to the precipitated powder and are made of very fine particles. In the Cu(II) complex and Ni(II) complex, the particles have sponge like structures with irregular shapes and the average size of the particle is 35µm.

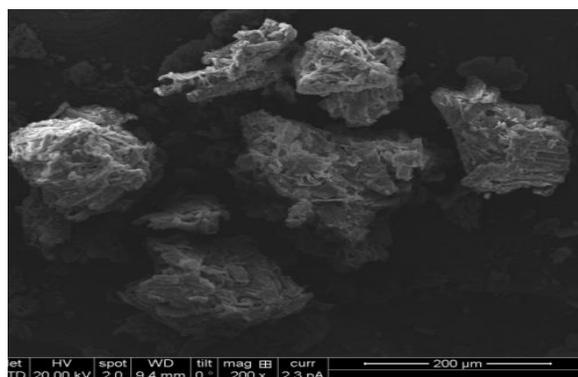


Fig 8: SEM image of $[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$

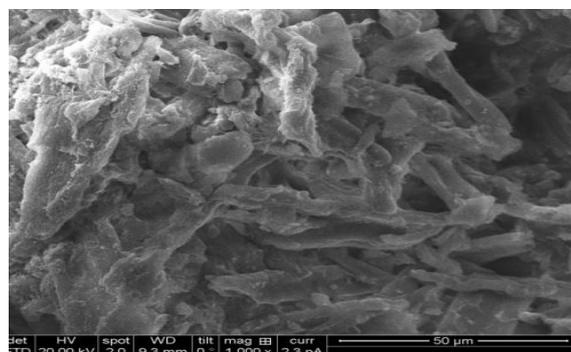


Fig 9: SEM image of $[\text{Ni}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}.2\text{H}_2\text{O}$

I. FAB mass spectra:

The FAB mass spectrum of copper complex of the mixed ligands (Fig.10.). of sulphadoxine–pyrimethamine drugs shows the molecular ion peak for the ligands at m/z 317 and at 248 m/z , where as its complex show the molecular peaks at m/z 620, which confirms the stoichiometry of the copper complex.

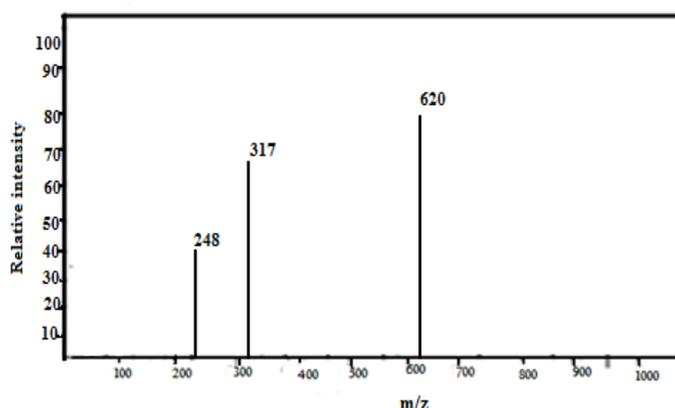


Fig 10: FAB mass spectrum of $[\text{Cu}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$

IV. PROPOSED GEOMETRY OF THE COMPLEX

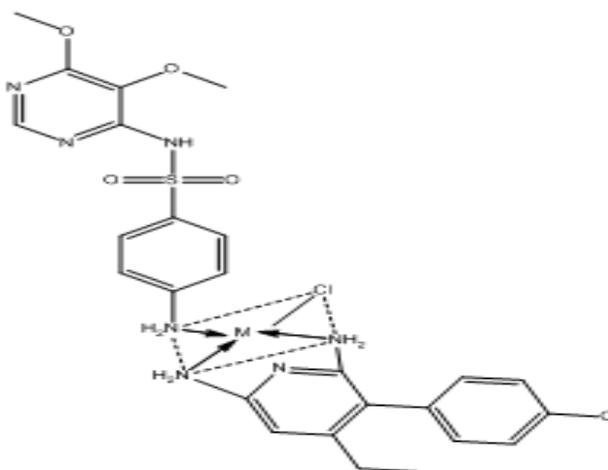


Fig 11: Geometry of mixed ligand metal complexes of sulphadoxine and pyrimethamine, $M = \text{Cu}(\text{II})$ ion

V. BIOLOGICAL STUDIES

A. DNA binding studies:

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 [18-21]. 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 [22,23] by virtue of their intrinsic chemical, electrochemical and photochemical reactivities [24]. Indeed, there is already a considerable literature involving the practical use of transition metal complexes as chemical nucleases [25,26].

The cyclic voltammogram of $[\text{Zn}(\text{sul})(\text{pym})\text{Cl}]\text{Cl}\cdot 2\text{H}_2\text{O}$ complex (0.01 M) in the absence of molecular oxygen at room temperature in 1.0 to -1.0 V potential range at scan rate 50 mVs^{-1} indicates a quasi-reversible two electron process. During the forward scan it shows cathodic reduction peak, at -0.61V which is attributed to reduction of $\text{Zn}(\text{II}) \rightarrow \text{Zn}(0)$. During the reverse scan it shows oxidation peaks, one at -0.26V which is attributed to oxidation of $\text{Zn}(\text{II}) \rightarrow \text{Zn}(0)$. The presence of DNA in the solution at the same concentration of complexes caused a negative shift in the potential ΔE_p by 0.160V and a decrease in $E_{1/2}$ by 0.35V . The value of I_{pa}/I_{pc} also decreased with increasing DNA concentration. The decrease of the anodic and cathodic peak currents of the complex in the presence of DNA is due to a decrease in the apparent diffusion coefficient of the $\text{Zn}(\text{II})$ complex upon complexation with the DNA macromolecules. These results show that complexes stabilize the duplex (GC pairs) by intercalation. The cyclic voltammogram of zinc complexes in the presence and absence of CT-DNA in Tris-HCl (5mm) buffer pH=7.1 is shown in figure 12.

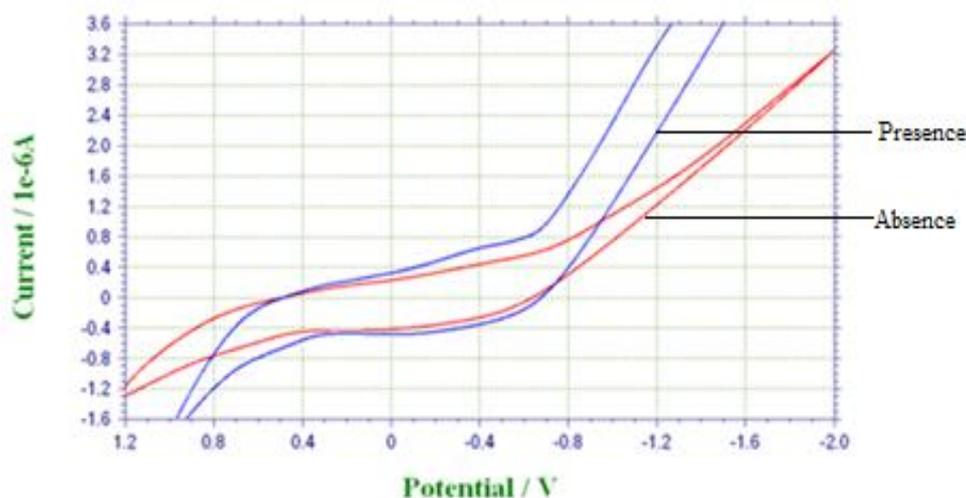


Fig 12: Cyclic voltammogram of $[Zn(sul)(pym)Cl]Cl.2H_2O$

VI. CONCLUSION

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. Indeed, there is already a considerable literature involving the practical use of transition metal complexes as chemical nucleases. However, most of these complexes contain only planar aromatic ligands and investigations of such complexes with ligands containing substituents as DNA-binding reagents have been relatively few. 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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