

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

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Abstract: Schiff base metal complexes of amoxillin such as $[\text{Cu}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{amoxbenz})_2] \cdot 6\text{H}_2\text{O}$ and $[\text{Fe}(\text{amoxbenz})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$ 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 viscosity 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, amoxillin, DNA binding.

I. INTRODUCTION

The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species [1]. Coordination compounds can act as enzyme inhibitor due to their pharmacological application. In view of the importance of such imines, we describe here the synthesis and characterization of Schiff base metal complexes derived from amoxillin drug.

II. SYNTHESIS OF SCHIFF BASE LIGAND

Benzaldehyde (0.01M, 1.32g) was dissolved in methanol and added to the amoxicillin trihydrate (0.01 M, 4.19 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 was separated out on removal of the solvent at room temperature. A light yellowish brown coloured crystalline solid was obtained which was dried over anhydrous CaCl_2 in vacuum.

III. SYNTHESIS OF SCHIFF BASE METAL COMPLEXES

The Schiff base ligand and metal chlorides $[\text{M} = \text{Zn}(\text{II}) (0.199 \text{ g}), \text{Ni}(\text{II}) (0.136 \text{ g}), \text{Cu}(\text{II}) (0.197 \text{ g}) \text{ and } \text{Fe}(\text{III}) (0.237 \text{ g})]$, 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 6 hrs. A dark colour product was isolated after reduction of solvent volume by evaporation which was then filtered, washed with methanol and dried over vacuum. On the basis of elemental analysis the complexes are formulated as $[\text{Cu}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Zn}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$, $[\text{Ni}(\text{amoxbenz})_2] \cdot 6\text{H}_2\text{O}$ and $[\text{Fe}(\text{amoxbenz})_2(\text{H}_2\text{O})\text{Cl}] \cdot 5\text{H}_2\text{O}$.

IV. RESULTS AND DISCUSSION

A. IR spectrum:

In the IR spectrum, the (Ar-OH) stretching frequency observed at 3428, 3389 cm⁻¹ in the ligands got disappeared in the iron complex (Fig.1) showing the participation of the M-O group in coordination. The Schiff base ligand shows strong band in the region 1650-1661 cm⁻¹ due to the azomethine group. This band gets shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen. The wide and strong bands at 564–670 cm⁻¹ (M-N) and 460-490 cm⁻¹ (M-O) are assigned to metal-ligand stretching vibration

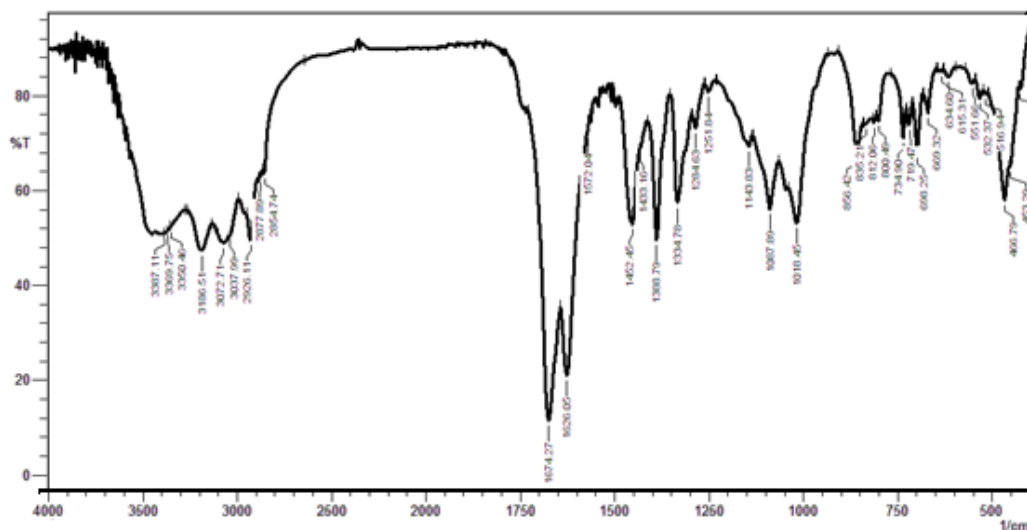


Figure 1: IR spectrum of [Cu(amoxbenz)₂].2H₂O

TABLE 1: IR SPECTRAL DATA OF SCHIFF BASEMETAL COMPLEXES OF AMOXILLIN (cm⁻¹)

Compound	$\nu(\text{ar-OH})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$	$\nu(\text{CH=N})$
Ligand	3428	-	-	-
[Cu(amoxbenz) ₂].2H ₂ O	-	460	560	1650
[Zn(amoxbenz) ₂].2H ₂ O	-	470	570	1630
[Fe(amoxbenz) ₂ (H ₂ O)Cl].5H ₂ O	-	450	540	1620
[Ni(amoxbenz) ₂].6H ₂ O	-	465	550	1630

B. Electronic spectra:

The electronic spectra of the Cu(II) complex (Fig;2) are compared with those of the ligands. Two bands appeared at 260-253 nm and 345-328 nm, which can be assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions respectively for the ligand [2]. The complexes showed two bands in the region 550-530 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 addition, the μ_{eff} value for this compound, in the range of 1.74-1.84 BM, is indicative of one unpaired electron per Cu(II) ion and suggesting a square-planar geometry [3]. Nickel(II) complex exhibits two bands at 620-600 nm and 490-476 nm, assigned to the ${}^3T_1(F) \rightarrow {}^3T_1(P)$ and charge transfer (ct) transitions respectively in tetrahedral geometry [4]. The Zn(II) complex expectedly shows only charge transfer transition from M \rightarrow L and $\pi \rightarrow \pi^*$ transitions, as no d-d transition is expected. This complex is diamagnetic, confirming its tetrahedral geometry. The Fe(III) complexes exhibit 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 of ligand origin assignable to intraligand $n \rightarrow \pi^*/\pi \rightarrow \pi^*$ transition. The band around 477-498 nm is assigned to ${}^6A_{1g} \rightarrow {}^4T_{2g}(G)$ [5] transition suggesting octahedral geometry which is confirmed by the magnetic moment value of 5.9 – 5.63 BM [6].

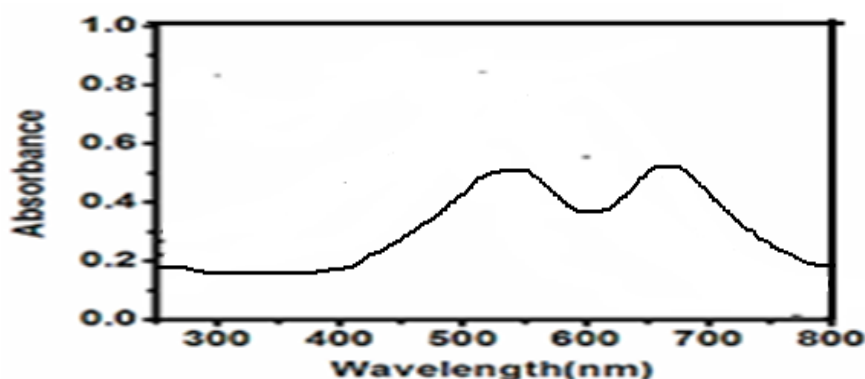


Figure 2: Electronic spectrum of [Cu(amoxbenz)₂].2H₂O

TABLE 2: ELECTRONIC SPECTRAL DATA OF SCHIFF BASE METAL COMPLEXES OF AMOXILLIN (cm⁻¹)

Compound	Transition	Wavelength(nm)	Geometry	μ _{eff}
Ligand	n - π / π - π*	Below 250	-	-
[Cu(amoxbenz) ₂].2H ₂ O	(² B _{1g} → ² A _{1g})		Square planar	1.74-1.84
[Zn(amoxbenz) ₂].2H ₂ O	-	-	tetrahedral	diamagnetic
[Fe(amoxbenz) ₂ (H ₂ O)Cl].5H ₂ O	⁶ A _{1g} → ⁴ T _{2g} (G)	470-498	octahedral	5.9-5.63
[Ni(amoxbenz) ₂].6H ₂ O	³ T ₁ (F) → ³ T ₁ (P)	623-605	Tetrahedral	3.26

C. ESR spectrum:

In square-planar complexes the unpaired electrons lie in the d_{x²-y²} orbital giving ²B_{1g} as the ground state with g_{||} > g_⊥ while the unpaired electron lies in the dz² orbital giving ²A_{1g} as the ground state with g_⊥ > g_{||}. From the observed values, it is clear that g_{||} > g_⊥, which indicates that the structure of the complex (Fig.3) is square-planar and that the unpaired electron is predominantly in the d_{x²-y²} orbital [7].

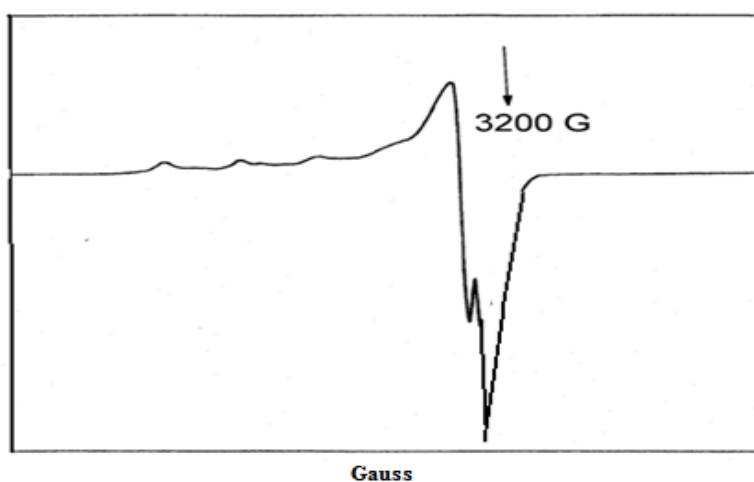


Figure 3: ESR spectrum of [Cu(amoxbenz)₂].2H₂O at 300K

TABLE 3: ESR SPECTRAL DATA OF [Cu(amoxbenz)₂].2H₂O

Complex	g	g _⊥	g _{iso}	K	K _⊥	α ²	β ²	γ ²	G
[Cu(amoxbenz) ₂].2H ₂ O 77 K	2.314	2.236	-	0.72	0.524	1.246	0.7151	0.765	5.3
[Cu(amoxbenz) ₂].2H ₂ O 300 K	-	-	2.10	-	-	-	-	-	-

D. ^1H NMR spectrum:

The ^1H NMR spectral data of ligand (amoxillin and bezaldehyde) shows signals at δ 7.45-7.60 and δ 7.48-7.57 respectively due to aromatic ring remain unchanged in their metal complexes. The ^1H NMR spectrum of Zn(II) complex (Fig.4) showed a down-field shift in the frequency of azomethine protons confirming coordination of the metal ion. In all the complexes, no signal was recorded for phenolic hydrogen in the 12.5 to 14.00 ppm region, as in the case of the Schiff base, indicating deprotonation of the ortho hydroxyl group and confirmed coordination through phenolic oxygen.

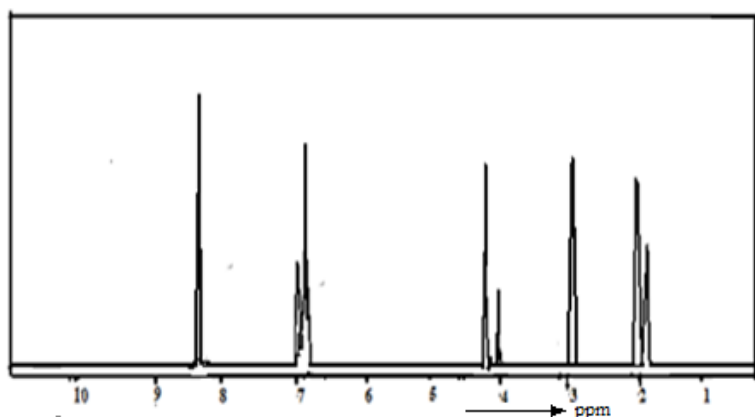


Figure 4: ^1H NMR spectrum of $[\text{Zn}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$

E. XRD studies:

The XRD pattern of the metallo drug was recorded in the 2θ range of $5-35^\circ$ and the crystalline size of the complex was calculated from Scherer's formula. From the observed d_{XRD} pattern the average crystalline size for the metallo drug was found to be 49 nm indicating that it is in nano crystalline in nature (Fig 5).

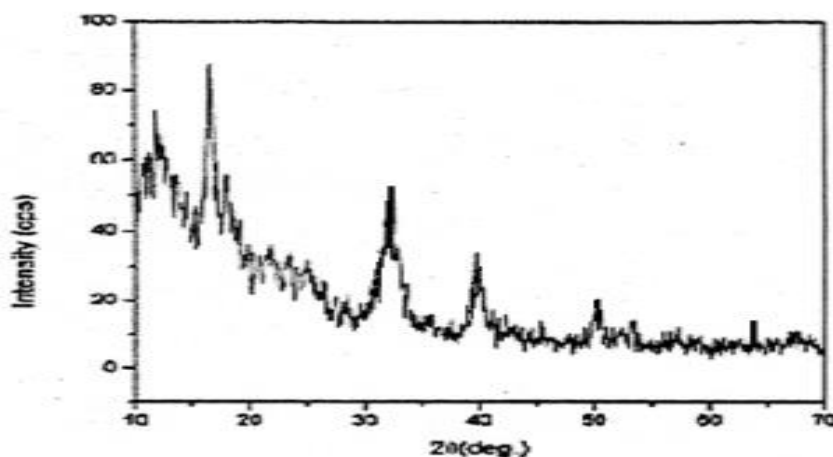


Figure 5: Powder XRD pattern of $[\text{Cu}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$

F. TGA studies:

The TGA experiment was carried out to explore the thermal stability of the complexes. The thermal behaviours of Fe(III) complex (Fig.6) was studied in the temperature range of $25-800^\circ\text{C}$. The TGA study reveal that the decomposition proceeds in three steps. In the first stage weight loss at 98°C corresponds to the presence of the lattice water in the complexes. The weight losses in the temperature range $110-200^\circ\text{C}$ are due to the presence of the coordinated water in both the complexes. A plateau was obtained after heating above 600°C , which corresponds to the formation of stable metal oxide [8,9].

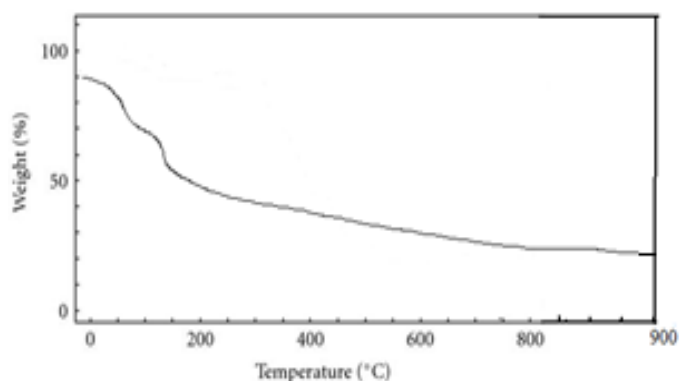


Figure 6: TGA pattern of $[\text{Fe}(\text{amoxbenz})_2(\text{H}_2\text{O})\text{Cl}]\cdot 5\text{H}_2\text{O}$

G. DSC studies:

DSC behaviour of the metal complex has been studied in the temperature range of 0-400°C. The copper complex shows a sharp endothermic peak at 165.1°C indicating the melting of the complex (Fig. 7). The endothermic peak noted at 236.7°C can be attributed to the decomposition of the complex. Broad exothermic peak obtained at 290°C and 391.6°C can also be due to the decomposition of the complex [10,11].

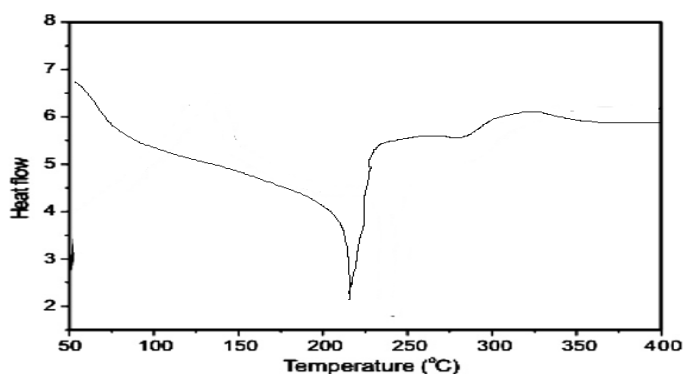


Figure 7: DSC pattern of $[\text{Cu}(\text{amoxbenz})_2]\cdot 2\text{H}_2\text{O}$

H. SEM studies:

The surface morphology of the ligand and the copper complex (Fig.8&9) was recorded with energy of 20 KV with magnification X150. The SEM images of the complex showed that the surfaces of the crystals formed by the complexes are different from the ligand. This indicates that the ligand changed the surface property of the metal complex. Although the complex appeared with the same colour and stereochemistry with the copper salt but the surface of the compound showed a different morphology compared to the starting drug molecule.

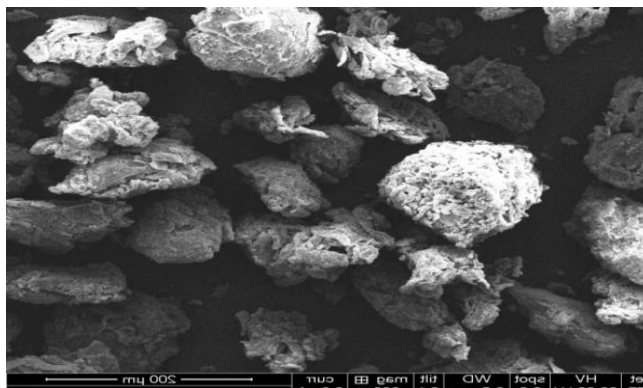


Figure 8: SEM image of ligand

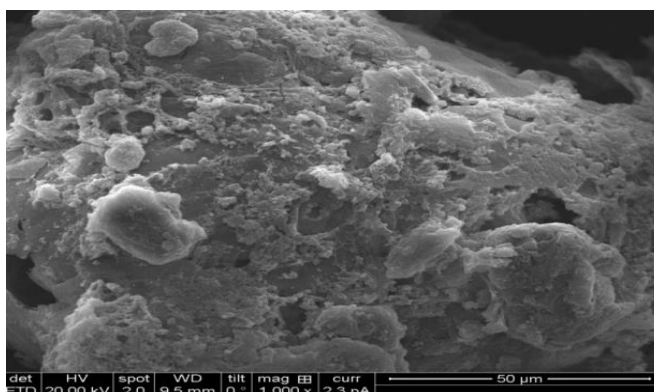


Figure 9: SEM image of $[\text{Cu}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$

I. FAB mass spectra

The FAB spectrum of the ligand and its Zn(II) complex (Fig.10) shows a molecular ion peak M^+ at m/z 365, which corresponds to the molecular mass of the ligand. The observed molecular ion peaks M^+ at m/z 643 in Zn(II) complex accounts for the molecular mass of $[\text{Zn}(\text{amoxbenz})_2(\text{H}_2\text{O})]^+$ and the loss of two coordinated water molecules with the molecular mass of $[\text{Zn}(\text{C}_{23}\text{H}_{29}\text{N}_3\text{O}_8\text{S})_2]^+$ respectively.

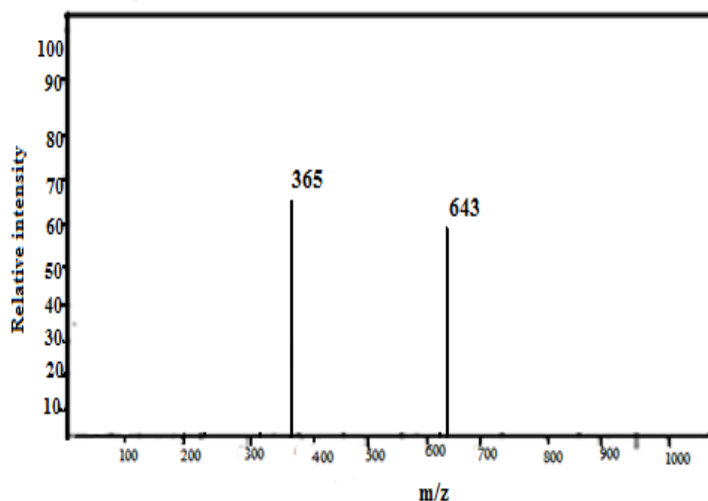


Figure 10: FAB mass spectrum of $[\text{Zn}(\text{amoxbenz})_2] \cdot 2\text{H}_2\text{O}$

V. PROPOSED GEOMETRY OF THE COMPLEX

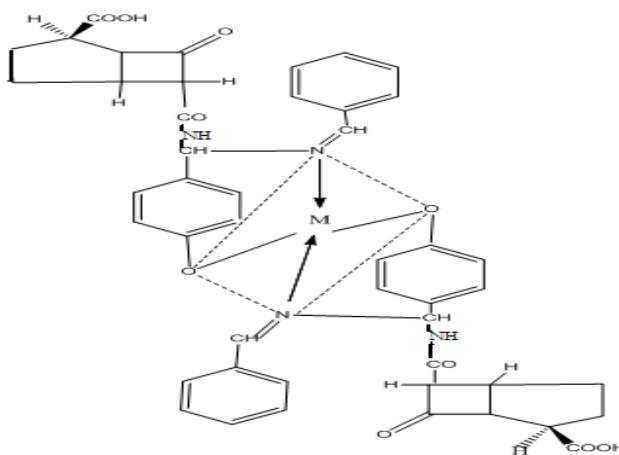


Figure 11: Geometry of Schiff base metal complex of amoxicillin, $\text{M} = \text{Cu}(\text{II})$

VI. BIOLOGICAL STUDIES

A. Viscosity method:

Optical photophysical probes generally provide necessary, but insufficient clues to further clarify the interactions between the study complex and DNA. Hydrodynamic measurements that are sensitive to length change (i.e. viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data. A classical intercalation model demands that the DNA helix lengthens as base pairs are separated in order to accommodate the binding ligand, leading to an increase in DNA viscosity. In contrast, a partial, non-classical intercalation of compound could bend (or kink) the DNA helix, reducing its effective length and, concomitantly, its viscosity [12]. Viscosity experiment results clearly show that both the compounds can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increase the viscosity of DNA. The effects of increasing concentration of metal complexes of cefixime on the relative viscosity $(\eta/\eta_0)^{1/3}$ of DNA is shown in Fig 12.

Series-1 [Cu(amoxbenz)₂].2H₂O Series-2 [Zn(amoxbenz)₂].2H₂O,

Series-3 [Ni(amoxbenz)₂].6H₂O Series -4 [Fe(amoxbenz)₂(H₂O)Cl].5H₂O

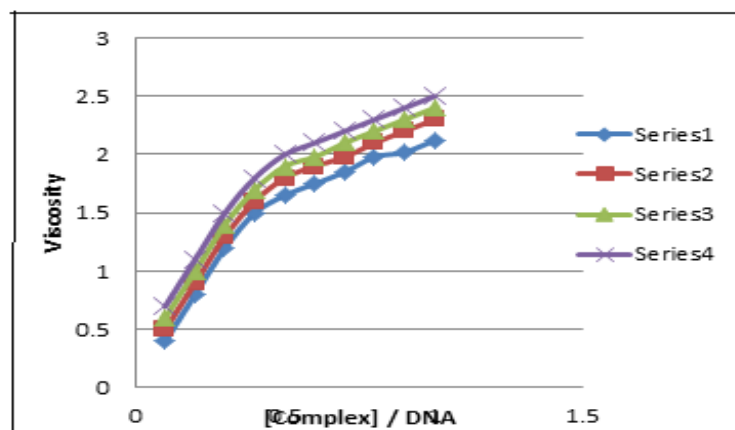


Figure 12: Effects of increasing concentration of Schiff base metal complexes of amoxilin with the relative viscosity $(\eta/\eta_0)^{1/3}$ of DNA

VII. CONCLUSION

The binding of metals to nucleotides or polynucleotide influence the sugar conformation. Interaction of DNA with transition metal complexes has gained considerable Metal-mediated hydrolysis of phosphate esters by metallo nuclease enzymes is therefore a common catalytic pathway in nucleic acid biochemistry. The conformation of nucleic acids depends on the kind of metal ion that binds to DNA. Metal binding to the bases will usually disrupt base pair hydrogen bonding and destabilize the double helix. On the other hand current interest due to its various applications in cancer research and nucleic acid chemistry. 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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