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SYNTHESIS, CHARACTERISATION AND DNA BINDING STUDY OF MIXED LIGAND METAL COMPLEXES OF CHLORAMPHENICOL AND OXYTETRACYCLINE

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Abstract: **Mixed ligand metal complexes of chloramphenicol of oxytetracycline such as have been [Cu(oxy)(chl)].2H_2O, [Zn(oxy)(chl)].2H_2O, [Ni(oxy)(chl)].6H_2O and [Fe(oxy)(chl)(H_2O)Cl].5H_2O synthesized and characterised by elemental analysis, IR ,UV-Visibe, ESR, NMR,XRD spectrscopic 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 byelectronic absorption studies. 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, thermal denaturation, DNA binding.**

1. INTRODUCTION

Oxytetracycline and chloramphenicol are antibiotics used in the prevention of wide range of infections. They can act against Gram-negative and Gram-positive micro-organisms. Because of the increased resistance of micro-organisms to these antibiotics a large doses of the drugs are required for treatment of infections. So, more effective drugs with enhanced activities and improved physical and bioavailability properties are urgently needed to replace these antibiotics. Chloramphenicol is a bacteriostatic antimicrobial that became available in 1949. Chemically it is 2,2-dichloro-N-[1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl] acetamide. It is considered as a broad-spectrum antibiotic and it is cheap and easy to manufacture. It is frequently used as an antibiotic of choice in the developing world. Oxytetracycline is a broad-spectrum antibiotic, active against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infections. Chemically it is (4S,4aR,5S,5aR,6S,12aS)-4-(dimethylamino)-3,5,6,10,11,12a-hexahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,5a,6,12,12a-octahydrotetracene -2-carboxamide.

2. SYNTHESIS OF METAL COMPLEXES

0.01 M (3.231 g) of chloramphenicol dissolved in 10 ml of distilled water was mixed with 0.01 M (4.604 g) of oxytetracycline hydrochloride in 10 ml of distilled water. The solution formed was mixed with the solution of each metal salts (0.01 M in 10 ml of distilled water) in a round bottom flask fitted with a condenser. The reaction mixture was refluxed for 2 hrs, cooled, and filtered. The metal complexes were formulated as [Cu(oxy)(chl)].2H_2O, [Zn(oxy)(chl)].2H_2O, [Ni(oxy)(chl)].6H_2O and [Fe(oxy)(chl)(H_2O)Cl].5H_2O

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3. RESULTS AND DISCUSSION

A. IR spectrum

The IR spectrum of the ligand (chloramphenicol) showed bands in the range of 3358.5 to 3562.3 cm^{-1} due to the stretching vibrations of OH group. This band shifted in the complex [Cu(oxy)(chl)].$2H_2O$. The IR spectrum of the ligand (oxytetracycline) showed bands in the range of 3328.5 to 3572.3 cm^{-1} due to the stretching vibrations of OH group[1,2]. This band shifted in the complex [Cu(oxy)(chl)].$2H_2O$ indicating the involvement of –OH group during complexation (Fig.1)

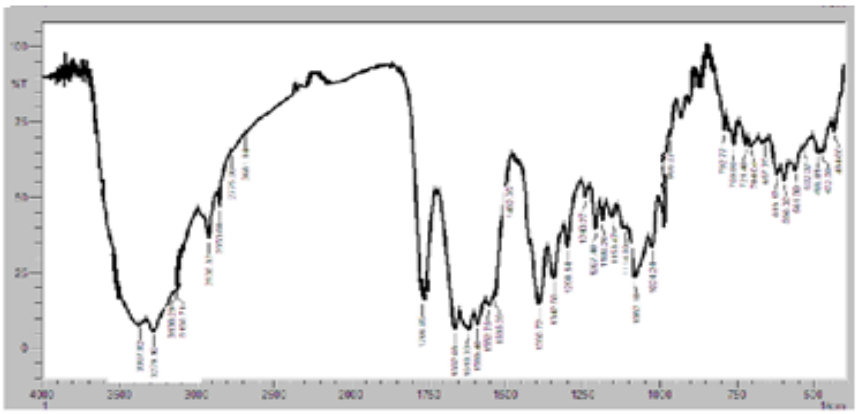

Figure 1: IR spectrum of [Cu(oxy)(chl)].$2H_2O$ Wave number(cm^{-1})

TABLE I: IR SPECTRAL DATA OF OXYTETRACYCLINE AND CHLORAMPHENICOL

Compound	**υ(OH)**	**υ(M-Cl)**	**υ(M-O)**	**υ(H_2O)**
Ligand	3358	-	460	3600
[Cu(oxy)(chl)].$2H_2O$	-	-	456	3564
[Zn(oxy)(chl)].$2H_2O$	-	-	455	3554
[Fe(oxy)(chl)(H_2O)Cl]. $5H_2O$	-	425	450	3544
[Ni(oxy)(chl)].$6H_2O$	-	-	450	3543

B. Electronic spectrum

The electronic spectra of [Cu(oxy)(chl)].$2H_2O$ is compared with those of the ligand. Two bands appeared at 259-251 nm and 348-328nm, assigned to π→π* and n→π* transitions in all the ligands. The complex(Fig.2) showed two bands in the region 556-530 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 [3]. In addition, the μ_{eff} values in the range of 1.74-1.84 BM, is indicative of one unpaired electron per Cu(II) ion suggesting the square-planar geometry [4]. Nickel(II) complexes exhibit two bandss at 624-600 nm and 494-472 nm assigned to the $^3T_1(F) \rightarrow {}^3T_1(P)$ and charge transfer(ct) transitions respectively in tetrahedral geometry [5]. The Zn(II) complex shows only charge transfer transition from M→L and π-π* transitions, as there is no d-d transition. This complex is diamagnetic, confirming its tetrahedral geometry [6,7]. 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 of ligand origin assignable to intraligand n-π*/π-π* transition. The band around 477-498 nm is assigned to $^6A_1g \rightarrow {}^4T_2g(G)$[8] transition suggesting octahedral geometry which is confirmed by the magnetic moment value of 5.9 – 5.63 BM [9].

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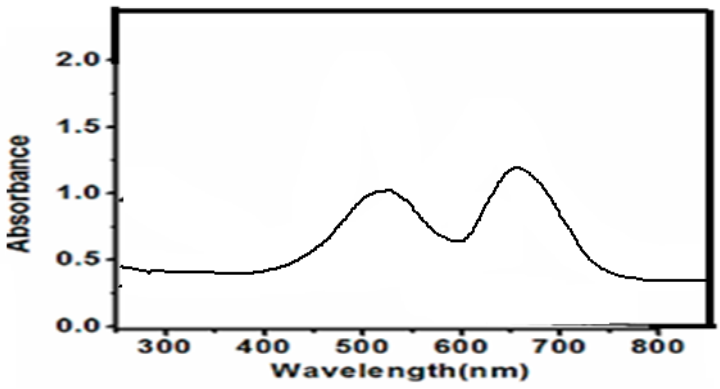

Figure 2: Electronic spectrum of [Cu(oxy)(chl)].2H_2O

TABLE II: ELECTRONIC SPECTRAL DATA OFOXYTETRACYCLINE AND CHLORAMPHENICOL

Compound	Transition	Wavelength(nm)	Geometry	μ_{eff}
Ligand	Ligand	below250		
[Cu(oxy)(chl)].2H_2O	($^2B_1g \rightarrow {}^2A_1g$)	556	square planar	1.74-1.84
[Zn(oxy)(chl)].2H_2O	-	-	tetrahedral	diamagnetic
[Fe(oxy)(chl)(H_2O)Cl]. 5H_2O	$^6A_1g \rightarrow {}^4T_2g(G)$	528	octahedral	5.31
[Ni(oxy)(chl)].6H_2O	$^3T_1(F) \rightarrow {}^3T_1(P)$	494	tetrahedral	3.26

C. 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

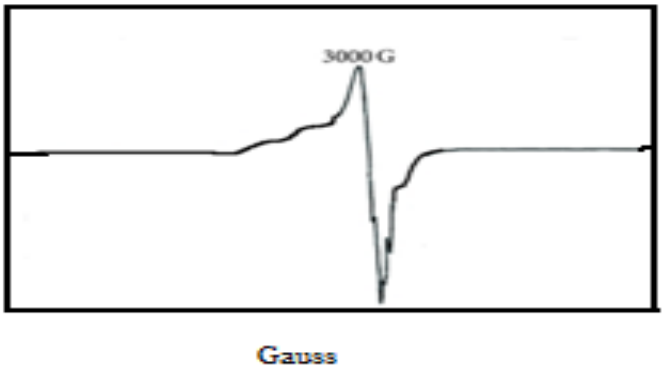

Figure 3: ESR spectrum of [Cu(oxy)(chl)].2H_2O at 300K

TABLE III: ESR SPECTRAL DATA OF[Cu(oxy)(chl)].2H_2O

Complex	g‖	g⊥	g_{iso}	K‖	K⊥	α^2	β^2	γ^2	G
[Cu(oxy)(chl)]. 2H_2O 77K	2.214	2.206	-	0.92	0.524	1.226	0.7251	0.755	6.4
[Cu(oxy)(chl)]. 2H_2O 300K	-	-	2.16	-	-	-	-	-	-

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D. ^1H NMR spectrum

The NMR spectrum of oxytetracycline shows the signal at 5.35δ for -OH protons 10.77δ for phenolic -OH protons, 7.68δ for $–NH_2$ protons, 3.29δ for CH protons and 2.26 δ for CH_3 protons respectively. In the second ligand, chloramphenicol, the –OH protons appears at 3.65δ, NH proton appears at 8.3δ, CH proton appears at 8.19δ and CH_2 protons appear at 3.5δ, respectively. Upon complexation (Fig.4) it is found that the -OH signal that appeared in the spectrum of the ligand at 5.35δ completely disappeared in the spectrum of its Zn(II) complex, indicating that the -OH proton is removed by chelation with the Zn(II) ion.

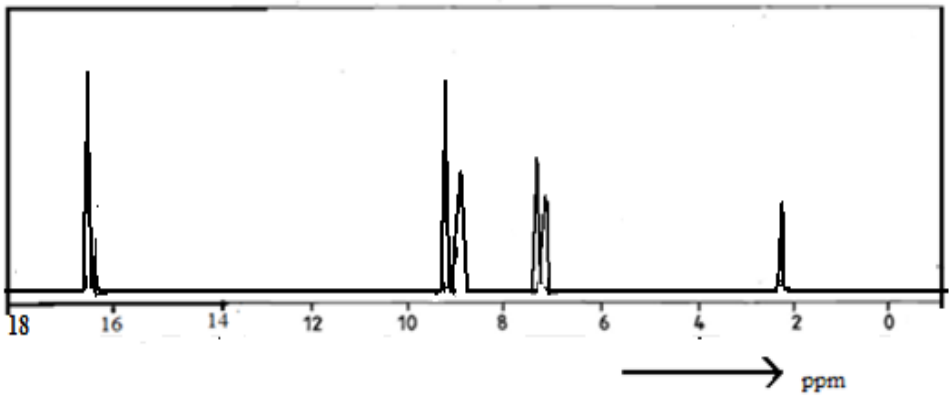

Figure 4: ^1H NMR spectrum of [Zn(oxy)(chl)].$2H_2O$

E. XRD studies

The X-ray diffractogram of the complex (Fig.5) was recorded in the range 0-80°. Each diffractor was well resolved into sharp reflux suggesting highly crystalline nature for the mixed ligand complexes. The d-spacing of the crystalline part of the compounds was calculated using the Bragg's equation. The width 'β' at half the maximum of crystalline peak was measured and the crystallite size was calculated using the Debye-Scherer's relation. The result showed that the crystalline size of the mixed ligand Cu(II) complexes ranges from 5.38-36.44 A°. The X-ray diffractogram of all mixed ligand complexes also showed that, the nature of peaks are well resolved, sharp with very good intensity. Hence the mixed ligand Cu(II) complex synthesized in the present investigation is crystalline in nature.

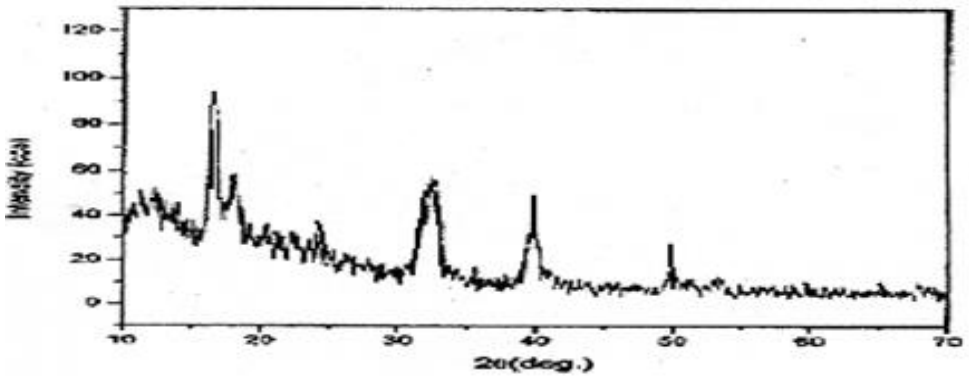

Figure 5: Powder XRD pattern of [Cu(oxy)(chl)].$2H_2O$

E. SEM studies

The scanning electron micrograph of the drugs showed that the surface was spongy and soft with large macroscopic phase separation. This phase separation was minimized after formation of mixed ligand complexes (Fig.6). Also the spongy and softness were reduced due to introduction of metal ion. From SEM images it was clear that there was a strong change in morphology of ligands on complexation

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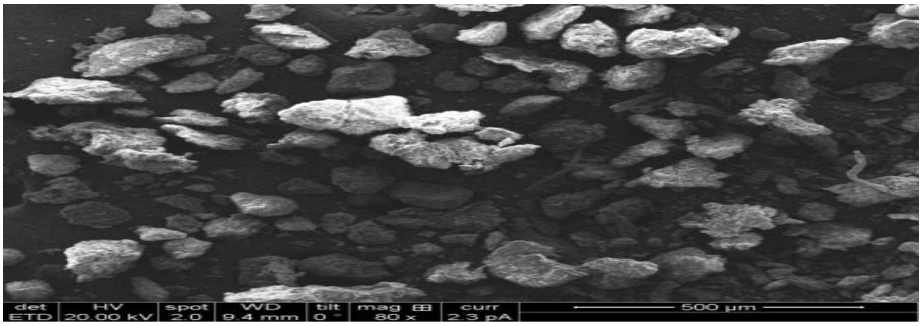

Figure 6: SEM image of [Zn(oxy)(chl)].2H_2O

F. TGA studies

The [Cu(oxy(chl))].2H_2O complex undergoes two step decomposition process (Fig.7). The first step of decomposition process started in the range 30-45°C and completed in the range 615-640°C which was accompanied by a weight loss in the range 29.618-32.022%. This may be attributed to the loss of two molecules of water of hydration. The second step started in the range 615-640°C and completed in the temperature range 900°C which was accompanied by a weight loss of 48.040 -50.432%. This may correspond to the combined weight loss due to remaining part of the complex and benzene moiety [10,11].

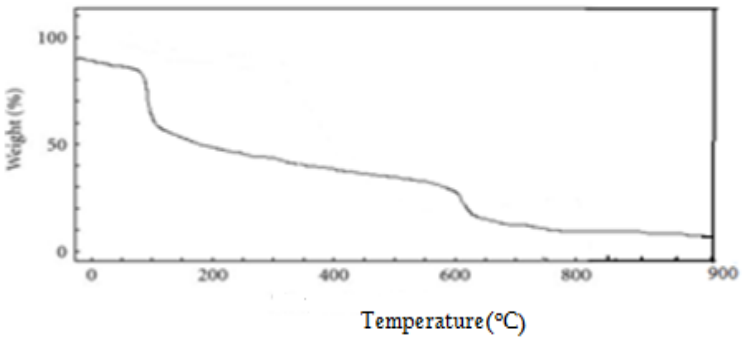

Figure 7: TGA pattern of [Cu(oxy)(chl)].2H_2O

G. DSC studies

The glass transition temperature of [Cu(oxy)(chl)].2H_2O complex (Fig.8) is found to be 155.2^oC. The sharp endothermic peak noted at 250.3^oC is due to the melting of the complex. The smaller dip in the endothermic curve at 354^oC may be attributed to the decomposition of the complex. No other remarkable feature is noticed in the DSC curve [12,13].

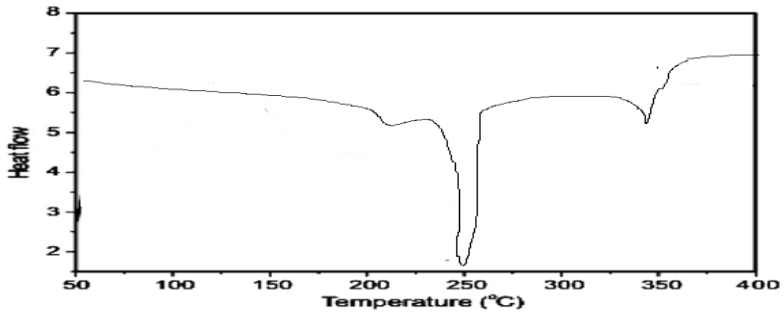

Figure 8: DSC pattern of [Cu(oxy)(chl)].2H_2O

H. FAB mass spectra

The FAB mass spectrum shows molecular ion peak for the complex [Cu(oxy)(chl)].2H_2O at m/z 954 which confirms the stoichiometry of the metal complex (Fig .9). The other fragments obtained are $(C_{22}H_{22}O_7)^+$, $(C_{10}H_{11}NOCl_2)^+$ and CuO and their molecular ion peaks are observed at m/z 398, 231 and 78.5, respectively

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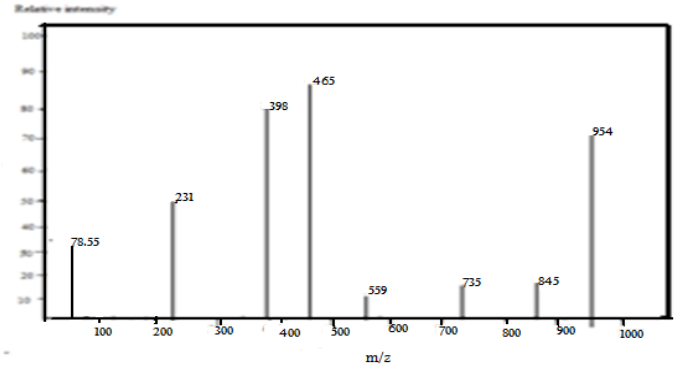

Figure 9: FAB mass spectrum of [Cu(oxy)(chl)].2H_2O

4. PROPOSED GEOMETRY OF THE COMPLEX

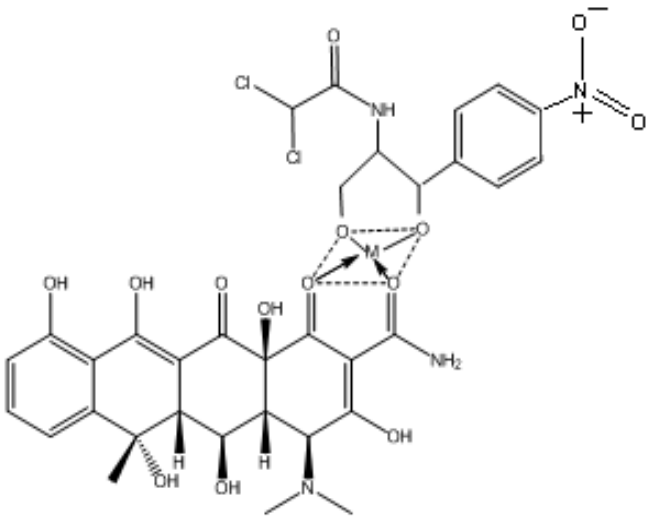

Figure10: Geometry of mixed ligand complex of oxytetracycline and chloramphenicol,M= Cu(II) ion

5. DNA BINDING STUDY

A. Electronic absorption studies

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 [14]. "Hyperchromic" and "hypochromic" effects are the spectral features of DNA concerning its double helical structure[15]. Hyperchromism has been observed for the interaction of many drugs with DNA [16]. The hyperchromic effect might be ascribed to external contact (electrostatic binding [17] or to partial uncoiling of the helical structure of DNA, exposing more bases of the DNA [18]. 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 ^oC, 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 [Cu(oxy)(chl)].2H_2O, [Zn(oxy)(chl)].2H_2O, [Ni(oxy)(chl)].6H_2O and [Fe(oxy)(chl)(H_2O)Cl].5H_2O 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.

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$$[DNA]/ (\varepsilon_a-\varepsilon_f) = [DNA/(\varepsilon_b-\varepsilon_f)] \quad +1/K_b (\varepsilon_b-\varepsilon_f)$$

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.

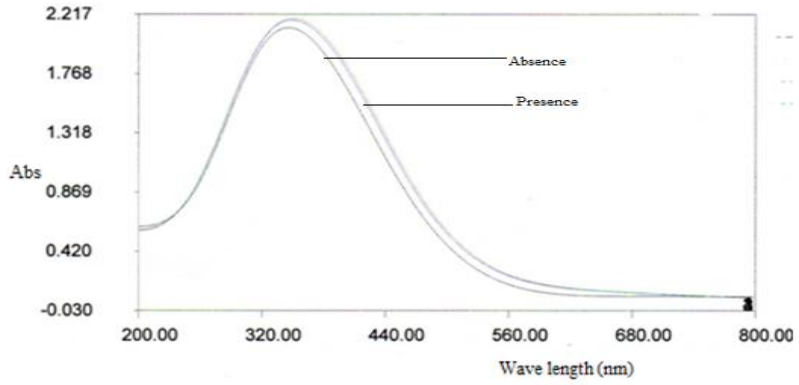

Figure 11: Electronic absorption spectra of[Cu(oxy)(chl)].2H_2O with increasing addition of DNA

6. CONCLUSION

Intercalation binding results when small molecules or the drug intercalate in to the nonpolar interior of the DNA helix. Aromatic group is stacked between the base pairs in this type of bonding and this happens when ligands of an appropriate size and chemical nature fit themselves in between base pairs of DNA. The ligand is suitable for intercalation are mostly polycyclic, aromatic, planar and therefore often make good nucleic acid strains. There is a current interest in designing and synthesizing DNA strand, as these molecules might function as chemotherapeutic 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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