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Research Article

SYNTHESIS, CHARACTERIZATION AND DNA INTERACTION OF METAL COMPLEX OF NITRILE GROUP LIGAND

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ABSTRACT

The copper complex of the type bis(1-amidino-O-ethylurea)copper(II)bromide, $[Cu(AEtUH)_2]Br_2$was synthesized and characterized.Based on the single crystal crystallographic data the synthesized complex crystallized in monoclinic structure with unit cell parameters a = 5.233A^o, b = 12.388 A^o, c = 12.752 A^o and β = 96.052^o respectively. Binding of this complex with calf thumus DNA (CT-DNA) was investigated by electronic absorption titration and cyclic voltammetric methods. The results indicate that the complex interact fairly with calf-thymus DNA by non-intercalative mode. The non-intercalative mode of interaction was further supportedby DNA model system.

Keywords: *Complex, Monoclinic, Calfthymus DNA, Non-Intercalative*

INTRODUCTION

Metals have a unique place within medical biochemistry, although until recently, this has been restricted predominantly to organic drugs. With their versatile structures, redox behaviour, physiochemical properties, transition metal complexes are often useful as chemical nucleases (Liu *et al.,* 2004; Macias *et al.,* 2007).

The interaction of these complexes with DNA becomes increasingly important as we elucidate how genetic information is expressed. A more complete understanding of how such complexes interact with DNA will lead not only to novel chemotherapeutics but also to highly sensitive diagnostic agents (Erkkila *et al.,* 1999). The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases (Raman and Joseph, 2010).

The metal complexes can interact with nucleic acids by noncovalent interactions, such as π-π stacking interactions, groove bindings, electrostatic interactions, hydrogen bonds and van der Waals interactions. Interest in the fashion of metal complex binding to DNA has been motivated not only by a desire to understand the basics of these interaction modes but also by the development of metal complexes into antimicrobial or anti-cancer agents (Sorenson, 1995).

This inspires the synthetic chemists to search for new metal complexes for bioactive compounds and copper in particular, has attracted the researchers.

Probably the most widely studied cation in this respect is Cu(ll), since a host of low molecular weight copper complexes have been proven beneficial against several diseases such as tuberculosis, rheumatoid, gastric ulcers and cancers (Sorenson, 1976).

Besides, copper is a bio-essential element in all living systems. Because of its biological activity and compatibility at normal concentrations, copper has been used in a number of medications throughout the history of present day man (Dollwet and Sorenson, 1985).

Numerous research groups have reported the biological importance of the hydrogen bonding interactions, their ability to interact with DNA bases and even showing the antimicrobial properties against several pathogenic microbes (Jeffrey, 1997, 1995; Jaffrey and Saenger, 1991; Suksangpanya *et al.,* 2004; Ibopishak *et al.,* 2004).

In view of the biological importance of the hydrogen bonding interactions, and their ability to interact with DNA bases here, we report the synthesis, characterizations and DNA binding activity of the copper complex $[Cu(AEtUH)_2]Br_2$where AEtUH = 1- amidino-*O*-methyl urea containing the CuN_4 chromophore.

MATERIALS AND METHODS

All the chemicals and reagents used were of analytical grade. Copper (II) chloride dihydrate, dicyandiamide, thymine (T), thymidine (TD) and thymidylic acid monophosphate disodium salt (TDM) were purchased from Merck, Germany. Calf thymus DNA (CT DNA) was purchased from Calbiochem, Germany.

The C, H and N analyses were determined by using a Perkin–Elmer-2400 Series ll, CHNS/O elemental analyzer.UV-Vis spectra were obtained on a Shimadzu 2450 UV–Vis spectrophotometer. Cyclic voltammetric measurements were performed on a CH602C Electrochemical analyzer.

The thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of the compound samples were performed using Perkin Elmer STA6000 Simultaneous Thermal Analyser Instruments under N_2 atmosphere in the temperature range of 30-700^{o}C at a heating rate of 5°C min^{-1}.

The powder X-ray diffraction studies were carried out using PAN analytical Philips diffractometer with Cu-kα radiation of wavelength 1.540 Å operating at a voltage of 40 kV and a current of 20 mA.X-ray crystallographic data were collected at 296 K with $Mo_{K\alpha}$ radiation (λ = 0.710 Å) using a Bruker Nonius SMART CCD diffractometer equipped with graphite monochromator at IIT, Guwahati.

All experiments involving interactions of complex with CT DNA, and DNA components (T, TD and TDM) were performed in Tris buffer solution (50 mM NaCl/5mM Tris–HCl, pH 7.2) at 25±0.2^{o}C. Double distilled water was used to prepare the buffer solution.

The concentration of CT DNA was determined from the intensity of absorbance at 260 nm with a known extinction coefficient value (ε_{260} = 6600 M^{-1} cm^{-1}) (Reichmann *et al.*, 1954). The ratio of the absorbance of CT DNA at 260 nm and 280 nm was found as 1.9. Therefore, no further purification was attempted (Marmur, 1961).

Absorption titration measurements were carried out by varying the concentration of CT DNA while keeping the metal complex concentration constant. Samples were incubated at 25±0.2^{o}C for 24 hr before recording each spectrum.

The intrinsic binding constant (K_b) for the interaction of the complex with CT DNA was determined using the following equation (Wolfe *et al.*, 1987).

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

where [DNA] is the concentration of CT DNA, the apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obsd}/[Cu], the extinction coefficient for the free copper (ll) complex and the extinction coefficient for the Cu (II) complex in the fully bound form, respectively.

A plot of [DNA]/ (ε_a-ε_f) versus [DNA] gave a slope of 1/(ε_b-ε_f) and a Y-intercept equal to 1/K_b (ε_b-ε_f), K_b is the ratio of the slope to the Y-intercept.

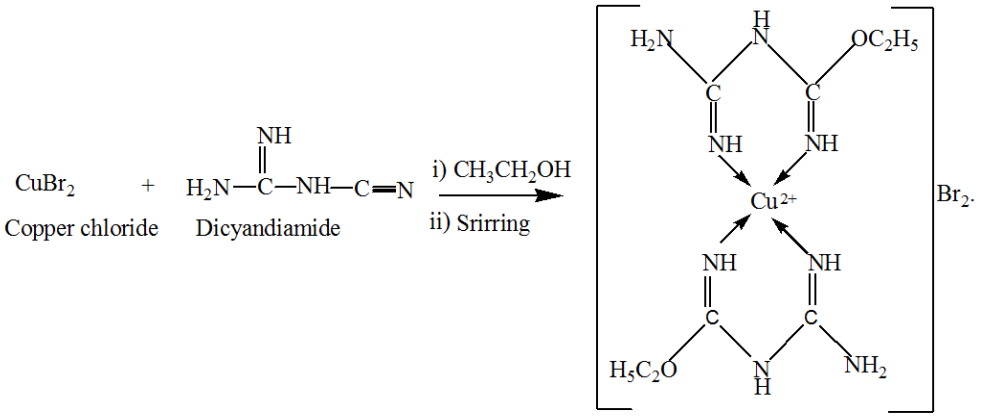
Cyclic voltammetric measurements were performed on a CH602C Electrochemical analyzer in Tris buffer at 25±0.2^{0}C, pH 7.2.

A standard three electrode system comprising platinum electrode working electrode, platinum wire auxiliary electrode and an Ag/AgCl reference electrode were used. Solutions were deoxygenated by purging with nitrogen gas for 20 minutes prior to the measurements.

Synthesis of the Complex $[Cu(AEtUH)_2]Br_2$

For the synthesis of this complex, 1.116 g of copper bromide ($CuBr_2$) and 0.420 g of dicyandiamide ($C_2N_4H_4$) were dissolved separately in ethanol. The solutions were mixed together in 1:1 molar ratio and heated at 45^{o}C on the water bath with constant stirring for about 12 h. The blackish colour solution mixture turns slightly dark green.

The reaction mixture was filtered and the filtrate was kept undisturbed with tight covering leaving only one hole at the center for slow evaporation. After few days pink crystals were separated out. The crystals were collected and washed by ethanol and then finally by acetone. Synthesis route of the complex is shown in (Scheme 1).



Scheme 1: Synthesis of the bis(1-amidino-O-ethylurea) copper(II)bromide complex $[Cu(AEtUH)_2]Br_2$.

RESULTS AND DISCUSSION

Analytical Data of the Complex

The complex was stable at room temperature and non-hygroscopic. The complex had shown good solubility in water and all the common organic solvents. The analytical data of the complex is shown in Table 1.

Table 1: Analytical data of the complex

Compound	Color	Yield	Analytical found (%) (Calculated) (%) Cu	C	H	N	μ_{eff} (B.M.)
$[Cu(AEtUH)_2]Br_2$	Pink	80	12.26 (13.10)	20.01 (19.86)	4.53 (4.10)	23.62 (23.17)	1.71

where AEtUH =1-amidino-O-ethylurea

Thermal Analysis (TGA-DTA)

The thermo gravimetric (TGA and DTA) analysis of the complex is shown in (Figure1). The complex was thermally decomposed in three decomposition steps within the range of temperature 35-700°C.

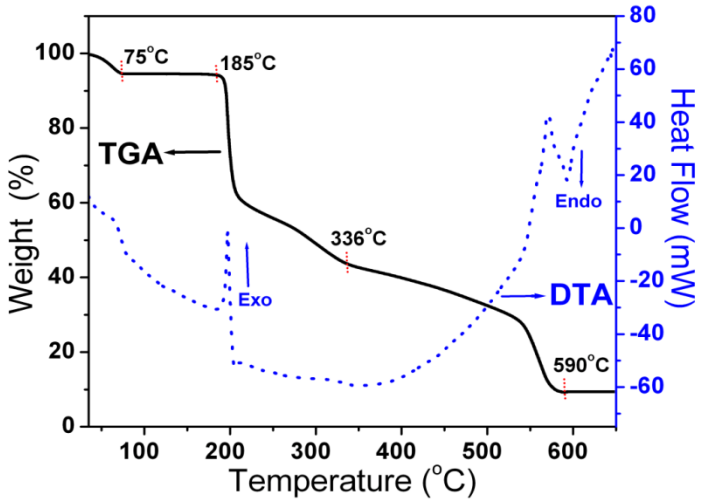


Figure 1: TGA and DTA curves of the complex $[Cu(AEtUH)_2]Br_2$

The first step of decomposition within the temperature range of 35-75°C with a mass loss of (4.3%) may be attributed to the liberation of surface water molecules. The second decomposition step with an estimated mass loss of 52.43% (calcd. 53.80%) within the temperature range of 185– 336°C corresponds to the loss of 2 molecules of ligand $C_4H_{10}N_4O$. The subsequent steps with a mass loss of 32.17% (calcd. 33.03%) within the temperature range of 336 – 590°C correspond to the removal of Br_2 molecule leaving copper oxide as a residue. The DTA curve of the complex further shows that the decomposition of the ligand and bromide molecule is accompanied by respective exothermic and endothermic processes within the temperature ranges.

UV-Vis Spectra

The UV-Vis spectra of the complex exhibited a broad band at 546nmcorresponding to the $^2E_g \rightarrow {}^2T_{2g}$transition (Figure 2A). In the spectra the $^2B_{1g} \rightarrow {}^2B_{2g}$, $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions are not resolved, suggesting the presence of square planar geometry [Ibopishak *et al.*, 2005; Unchulee *et al.*, 2010]. The $^2E_g \rightarrow {}^2T_{2g}$ transition band observed at 546nm was slightly shifted to 542 nm while recorded in Methanol. The complex also exhibit n→π* or π→π* charge transfer band at 225 nm (Figure 2B).

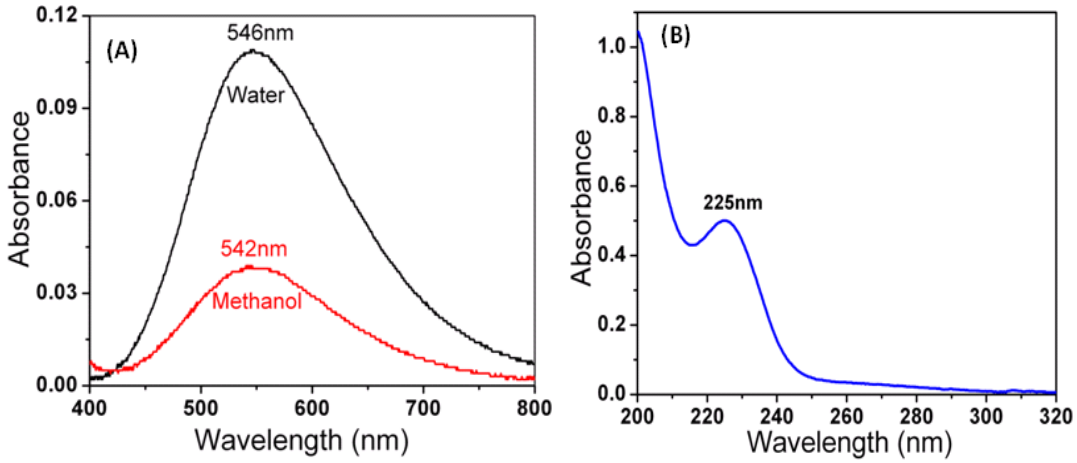


Figure 2 (A and B): UV-Vis spectra of the complex $[Cu(AEtUH)_2]Br_2$ (A) $^2E_g \rightarrow {}^2T_{2g}$ transition bands recorded in water and methanol solvents, (B) n→π* or π→π* charge transfer band recorded in water

X-Ray Diffractions (XRD)

X-ray powder diffractions (XRD) of the free ligand dicyandiamide and complex are depicted in (Figure 3A and 3B), and the observed diffraction data of the complex is given in (Table 2).

Table 2: Powder XRD data of the complex $[Cu(AEtUH)_2]Br_2$

Sl.No.	d-spacing (A°)		2 θ values		Δ2θ	(h k l)
	Observed	Calculated	Observed	Calculated		
1.	8.44320	8.418	10.469	10.501	-0.032	(001)
2.	7.33517	7.33428	12.056	12.057	-0.001	(200)
3.	6.87514	6.90547	12.866	12.809	0.057	(101)
4.	6.37233	6.36856	13.886	13.894	-0.008	(010)
5.	4.02203	4.02323	22.083	22.076	0.007	(211)
6.	3.45671	3.45274	26.278	25.782	-0.030	(202)
7.	3.38870	3.38585	26.278	26.300	-0.022	(311)
8.	3.20484	3.20555	27.815	27.809	0.006	(401)
9.	2.99327	2.99418	29.825	29.816	0.009	(302)
10.	2.52353	2.5239	35.546	35.541	0.005	(21-3)
11.	2.4677	2.46827	36.378	36.369	0.009	(122)

Using a set of program called P-INDEX, which is based on least-squares approach, the unit cell parameters of the complex were determined and found to be (a = 14.807, b = 6.369, c = 8.497)A˚, α = γ =90º, β = 97.847º and cell volume V = 793.82 A˚^{3}. This data of the complex supports monoclinic system. The crystallite size of this complex was determined using Debye–Scherer formula (Warren, 1990) and found to be 59 nm.

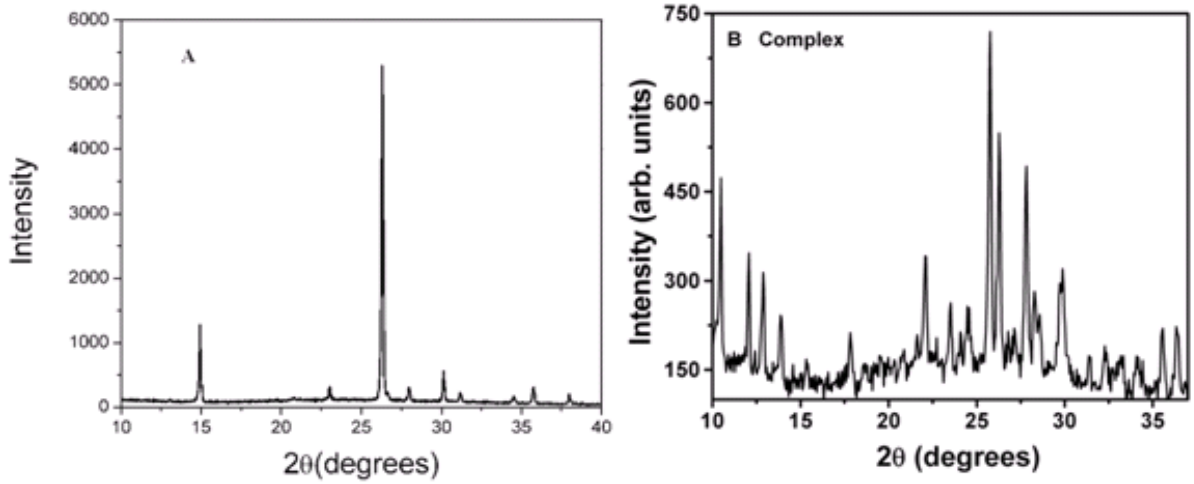


Figure 3(A and B): XRD spectrum of (A) free ligand (dicyandiamide); (B) complex $[Cu(AEtUH)_2]Br_2$

X-Ray Crystallographic Studies:

Single crystals suitable for X-ray diffraction were obtained by the slow evaporation of an ethanolic solution of the complex. The molecular structure of the complex with the atom numbering is shown in (Figure 4), while a view of its packing in the unit cell is shown in (Figure 5). The crystallographic data of the complex is given in (Table 3). The structure consists of $[Cu(AEtUH)_2]^{2+}$ cation and $2Br^-$ anions. The complex shows monoclinic structure where the copper(II) cation is located on inversion centre coordinated by two N, N-bidentate ligand in a trans-configuration symmetrically to give square planar $[Cu(AEtUH)_2]^{2+}$ cation (AEtUH = 1-amidino-O-ethylurea). The charge of the Cu^{2+} is neutralized by two bromide ions. The complex maintained a distorted square planar geometry with the bond angles N(1)-Cu(1)-N(1), 180º; N(5)-Cu(1)-N(5), 180º; N(5)-Cu(1)-N(1), 91.16º; N(5)-Cu(1)-N(1), 88.84º.

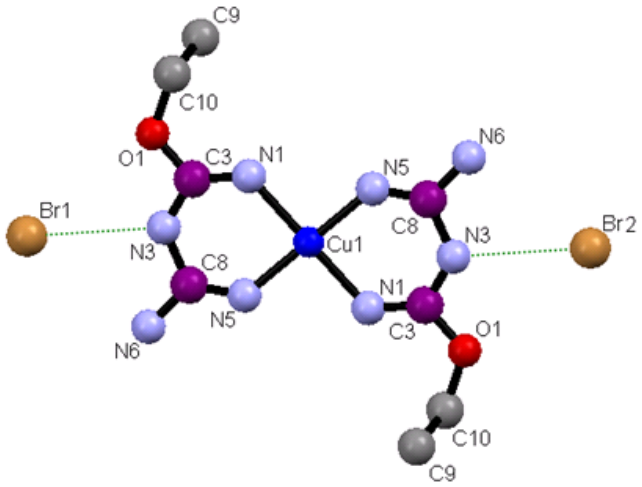


Figure 4: Ball and stick structure of the complex $[Cu(AEtUH)_2]Br_2$; (Hydrogen atoms are omitted for clarity)

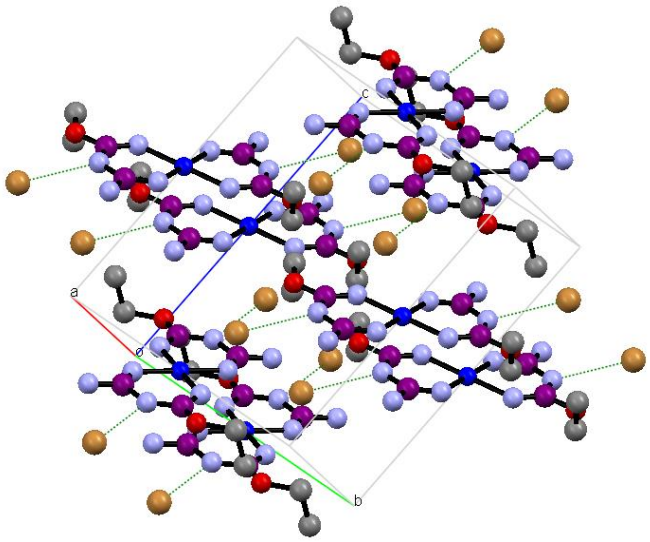


Figure 5: Packing structure of the complex $[Cu(AEtUH)_2]Br_2$; (Hydrogen atoms are omitted for clarity)

Table 3: X-ray crystallographic data and structure refinement of the complex $[Cu(AEtUH)_2]Br_2$

Empirical formula	**$CuC_8H_{20}N_8O_2Br_2$**
Formula weight (gM^{-1})	483.67
Temperature (K)	293 (2) K
Crystal system	Monoclinic
Space group	P $2_1/c$
a (A^o)	5.233 (9)
b(A^o)	12.388 (2)
c(A^o)	12.752 (2)
α	90.00
β	96.052
γ	90.00
Volume (A^{o3})	818.85
μ	1.49 mm^{-1}
Mo- K_α radiation, λ / Å	0.71073
$\Delta\rho$	-1.96 to 3.51 eA^{o-3}
Θ –range for data collection	1.5 to 28.3°
$R[F^2 > 2\sigma(F^2)]$	0.141
$wR(F^2)$	0.421

DNA Interaction Studies

Electronic Absorption Titration Method

The spectral changes of the complex in the absence and presence of CT-DNAis shown in (Figure 6). On addition of CT-DNA, the n→π* or π→π* absorption band of the complex centered at 225nm showed hyperchromism accompanied by a slight blue shift. Based on the hyperchromism exhibited and shifts in absorbance upon addition of CT DNA, non-intercalative interaction probably by an electrostatic interaction between complex ions and negatively charge phosphate groups of the CT DNA can be predicted (Long and Barton 1990; Senthil Kumar and Arunachalam 2006). In order to illustrate the DNA binding strength of this complex, the intrinsic binding constant K_b was determined by employing the equation 1 and was found to be $8.5x10^3\ M^{-1}$. (Figure 7) shows the plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$*vs.* [DNA] for the titration of complex with CT-DNA.

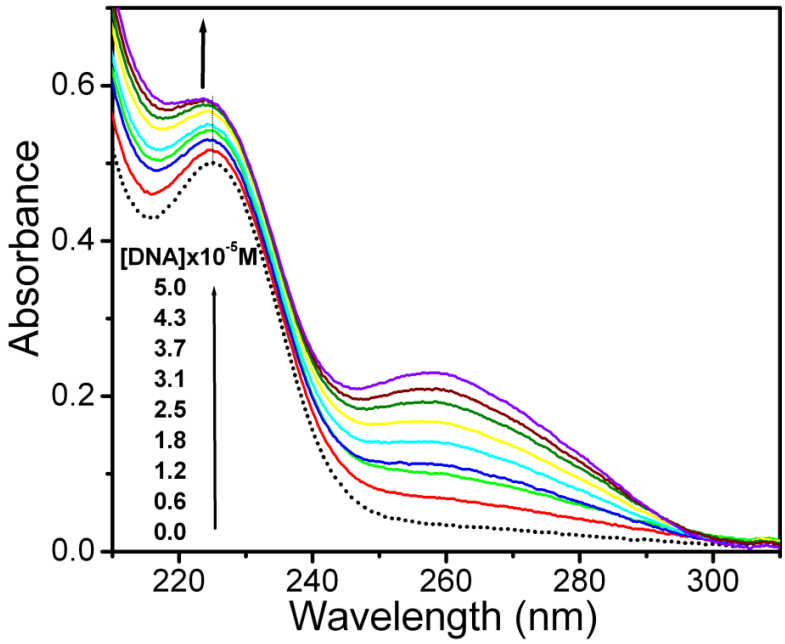


Figure 6: Absorption spectral traces of complex $[Cu(AEtUH)_2]Br_2$ ($2.5x10^{-5}$M) in the absence (dotted line) and presence (solid lines) of increasing amounts of CT-DNA. Arrows shows the absorbance changes upon increasing CT-DNA concentration

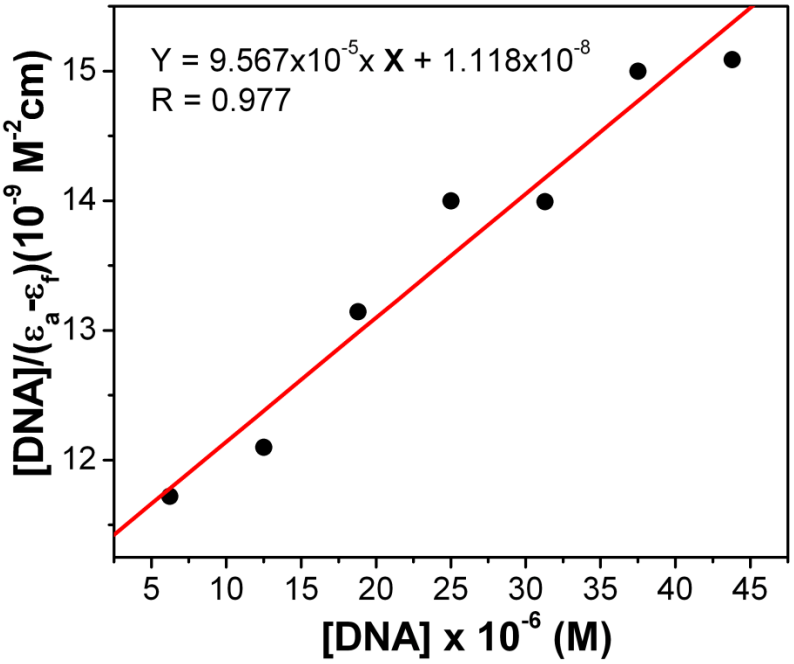


Figure 7: Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs. [DNA] for the titration of complex $[Cu(AEtUH)_2]Br_2$ with CT-DNA

Cyclic Voltammetric Method

The application of electrochemical methods in the study of complex-DNA interactions provides a useful complement to the previously used UV-Vis method of investigation. Typical cyclic voltammetric behaviors of complex in the absence and presence of CT-DNA are shown in (Figure 8). After the addition

of DNA, no new redox peak appears in voltammogram. The anodic peak current decreases extensively. So, only the anodic redox waves were selected for the study of the interaction of complex with CT-DNA. The decreased in anodic peak currents with increasing concentration of CT-DNA was due to the formation of CT-DNA-complex system. Similar observation was reported by (Shah *et al.*, 2008). Additionally, the oxidation peak potentials (a_{p1} and a_{p2}) of the complex were also shifted to more negative values indicating the non-intercalative binding nature of the complex with CT-DNA (Carter and Bard, 1987).

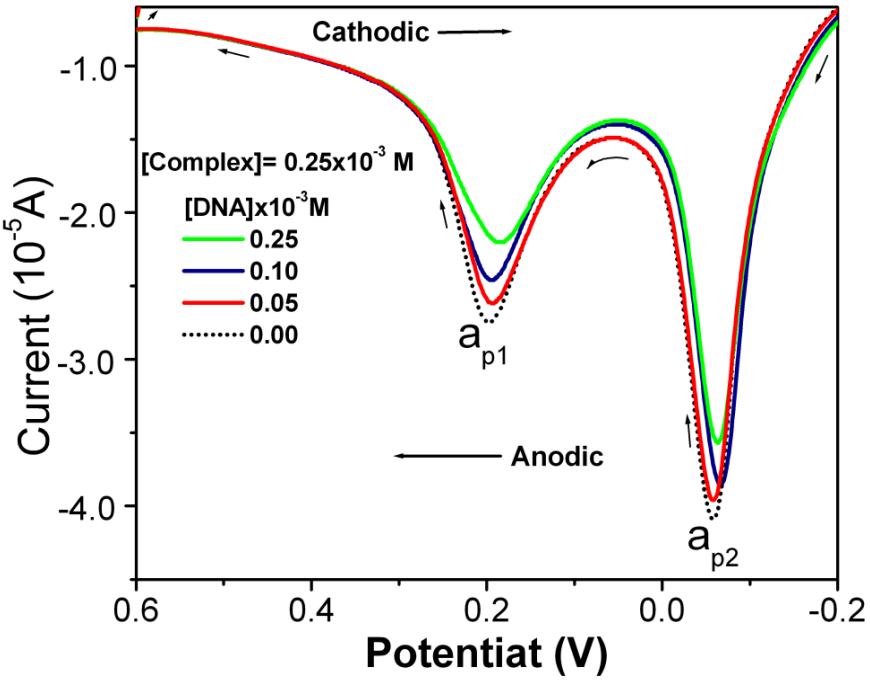


Figure 8: Cyclic voltammograms of complex $[Cu(AEtUH)_2]Br_2$ in the absence and presence of CT-DNA; where [complex] = $2.5x10^{-3}$ M. Supporting electrolyte- 50 mM NaCl/5 mM Tris–HCl (pH 7.2 ± 0.2); Scan rate- 10 mVs^{-1}

DNA Model System

DNA model system is one of the methods for investigating the most preferable mode of DNA interaction of the studied complex. Figure 9 shows the spectral changes of the synthesized complex $[Cu(AEtUH)_2]Br_2$ both in the absence and presence of thymine and thymidilic acid monophosphate disodium salt respectively. In all the three cases, the absorption band of the complex centered at 225 nm showed hyperchromism with slight blue shift upon addition of increasing amounts of above three chemicals.

The bands observed at around 266 nm were due to the present of excess of thymine, thymidine and thymidilic acid monophosphate disodium salt.

The intrinsic binding constant value K_b of the complex with thymine, thymidine and thymidilic acid monophosphate were further determined by monitoring the changes in absorbance centered at 225 nm, by employing the equation 1and were found to be $1.48x10^4$ M^{-1}, $1.50x10^4$ M^{-1} and $2.64x10^4$ M^{-1} respectively (Figure 10).

The determined binding constant K_b value of the complex with thymidilic acid monophosphate was found to be greater than that with thymine and thymidine. From this, it can be concluded that the possible mode of interaction of the synthesized complex with CT-DNA will be the electrostatic interaction between the negatively charged phosphate group of the DNA and positively charged central copper(II) ion of the complex.

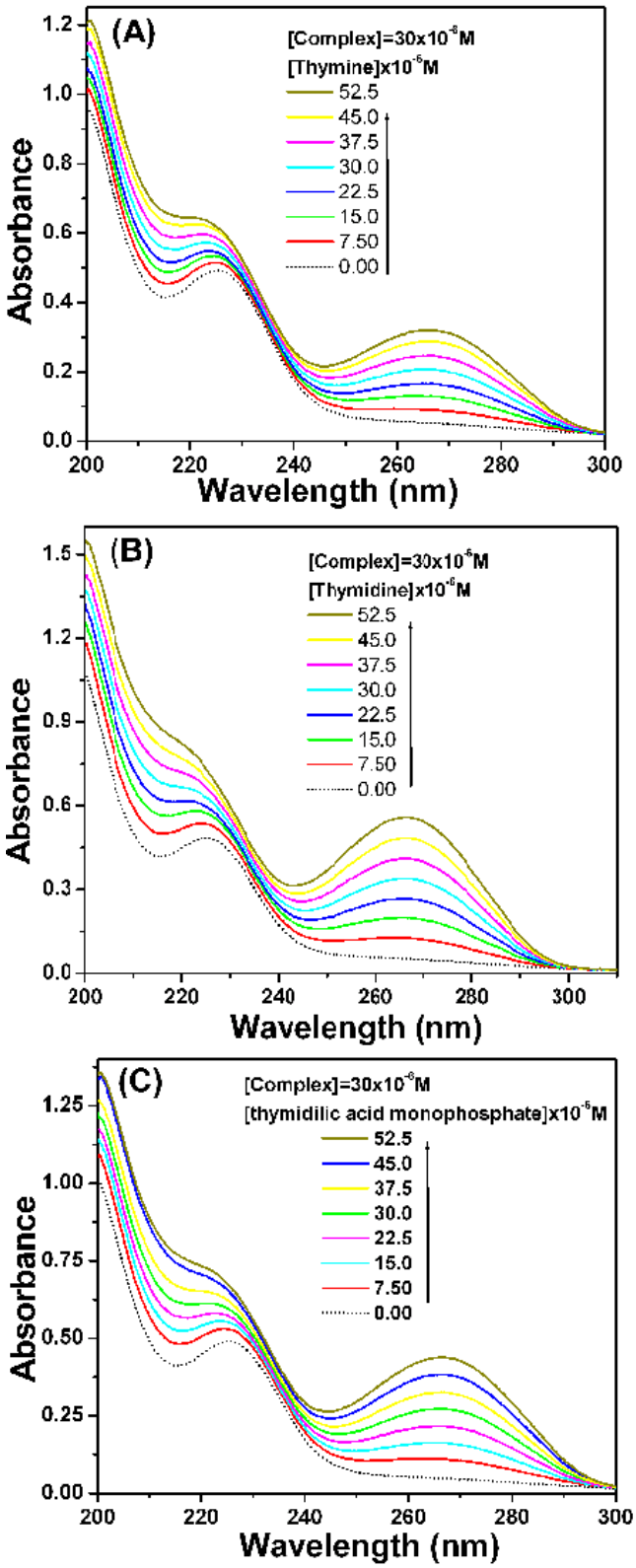


Figure 9: Absorption spectral traces of complex ($2.5x10^{-5}$M) in the absence (dotted line) and presence (solid lines) of increasing amounts of (A) thymine, (B) thymidine and (C) thymidilic acid monophosphate disodium salt respectively

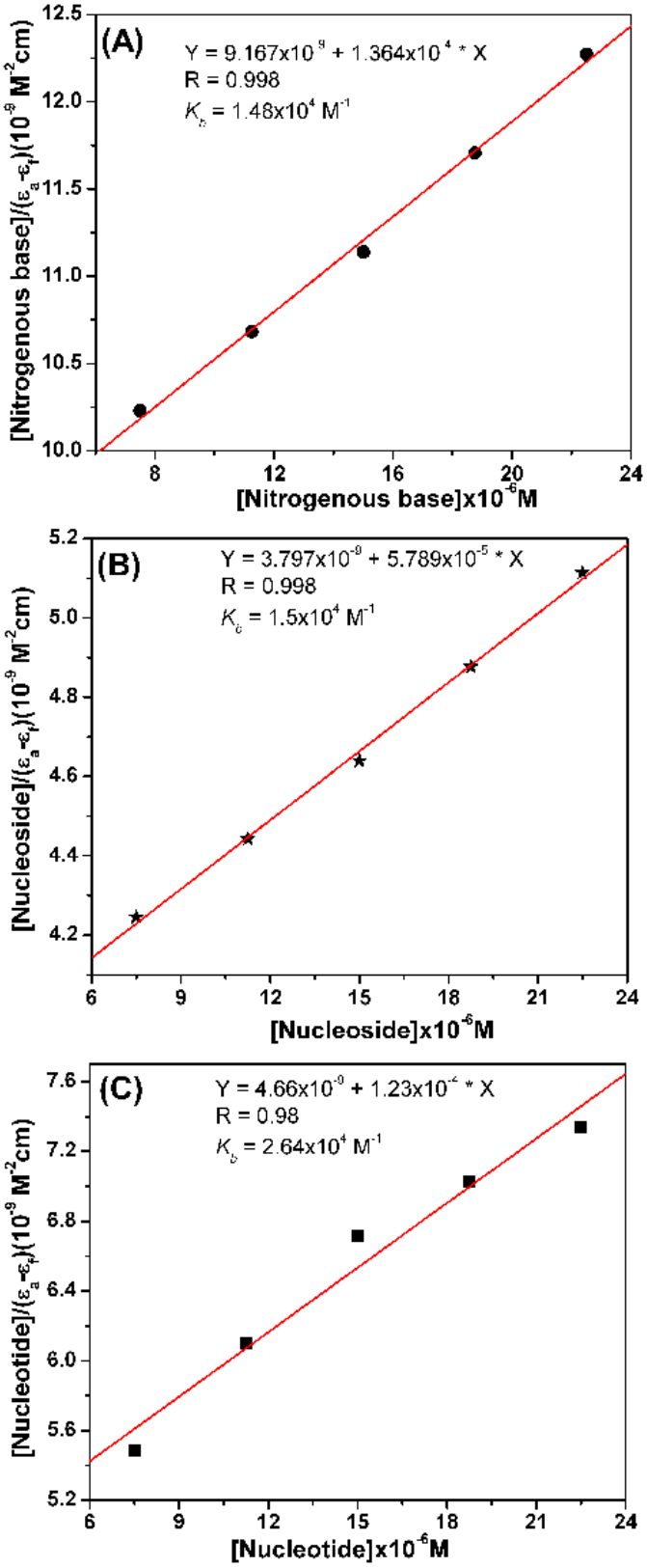


Figure 10: **Plots for determining intrinsic binding constant K_b values of the complex with (A) thymine, (B) thymidine and (C) thymidilic acid monophosphate disodium salt respectively**

Conclusion

The X-ray powder diffraction (XRD) data suggested that the synthesized complex crystallizes in the monoclinic system with the unit cell parameters (a = 14.807, b = 6.369, c = 8.497)A˚, α = γ =90º, β = 97.847º and cell volume V = 793.82 $A^{\circ 3}$.The X-ray crystallographic data further supported the monoclinic system with distorted square planar geometry. The DNA interaction study indicated that the synthesized complex can interact with CT DNA in the non-intercalative mode of fashion with the binding constant $K_b$value $8.5x10^3$ M^{-1}. The DNA model system further provides the most probable route of binding of the synthesized complex with DNA.

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