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Spectroscopic and electrochemical studies of the interaction of Cu (II) complex with DNA and its biological activity

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ABSTRACT

The complex $[CuL_2]Cl_2.H_2O$ (where L = 1-amidino-O-ethylurea) has been synthesized and characterized. X-ray powder diffraction study reveal that the complex have orthorhombic crystal lattice. The unit cell parameters of the complex are a = 13.6766A˚, b = 12.7978A˚, c = 5.2467A˚, and cell volume V = 918.35 A˚. The DNA binding property of this complex has been investigated under physiological conditions (pH 7.2, 25±0.2°C) by absorption spectroscopy, cyclic voltammetry and viscosity measurements. The results indicate that the complex bind with CT DNA by non-intercalative mode with intrinsic binding constant in the order of 10^4M^{-1}. Further, the complex has been screened for its antibacterial activity against Escherichia coli, Klebsiella pneumoniae sub sp. pneumoniae and Proteus mirabilis bacteria.

Key Words: orthorhombic, CT DNA, non-intercalative, antibacterial activity.

INTRODUCTION

Deoxyribonucleic acid (DNA) is the primary target molecule for most of anticancer and antiviral therapies according to the cell biology. The study of DNA interaction with transition metal complexes becomes increasingly important as it can elucidate how genetic information is expressed. Such study 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 anti-inflammatory, antifungal, antibacterial or anti-cancer agents [1-4]. 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 [5].

The ligand (1-amidino-O-alkylurea) is a nitrogen donor system having many hydrogen bonding sites, which may give the opportunity to form hydrogen bonding with the DNA bases. 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 [6-10]. In view of the above respect, in the present work, $[CuL_2]Cl_2.H_2O$ complex (where L = 1-amidino-O-ethylurea) have been studied for its DNA binding property by electronic absorption spectroscopy, cyclic voltammetry and viscosity measurements. Further, the antimicrobial activities of this complex have been screened using *Escherichia coli*, *Klebsiella pneumoniae* sub *sp. pneumoniae* and *Proteus mirabilis* bacteria.

EXPERIMENTAL SECTION

All the chemicals and reagents used were of analytical grade and used without further purification. $CuCl_2.2H_2O$, dicyandiamide and ethidium bromide (EB) (Merck), Calf thymus CT DNA fibrous type (Calbiochem) were used.

The C, H and N analyses were determined by using a Perkin–Elmer-2400 Series ll, CHNS/O elemental analyzer. Infrared spectra were obtained on a Shimadzu-8400S, FTIR spectrometer in wavelength region 4000-400 cm^{-1}.The spectra were recorded as KBr pellets. UV-Vis spectra were obtained on a Shimadzu 2450 UV–Vis spectrophotometer in wavelength region 800-200 nm. XRD data of the complex were collected on PANalytical powder diffractometer (X'Pert PRO) using radiation filled with Ni filter. Cyclic voltammetric measurements were performed on a CH602C Electrochemical analyzer.

All experiments involving CT DNA were performed in Tris buffer solution (50 mM NaCl/5mM Tris–HCl, pH 7.2) at 25±0.2^oC. 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}) [11]. 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 [12].

Absorption titration measurements were carried out by varying the concentration of CT DNA from 0 to 45x10^{-6}M, while keeping the metal complex concentration constant at 25x10^{-6}M. Samples were incubated at 25±0.2^oC 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 [13].

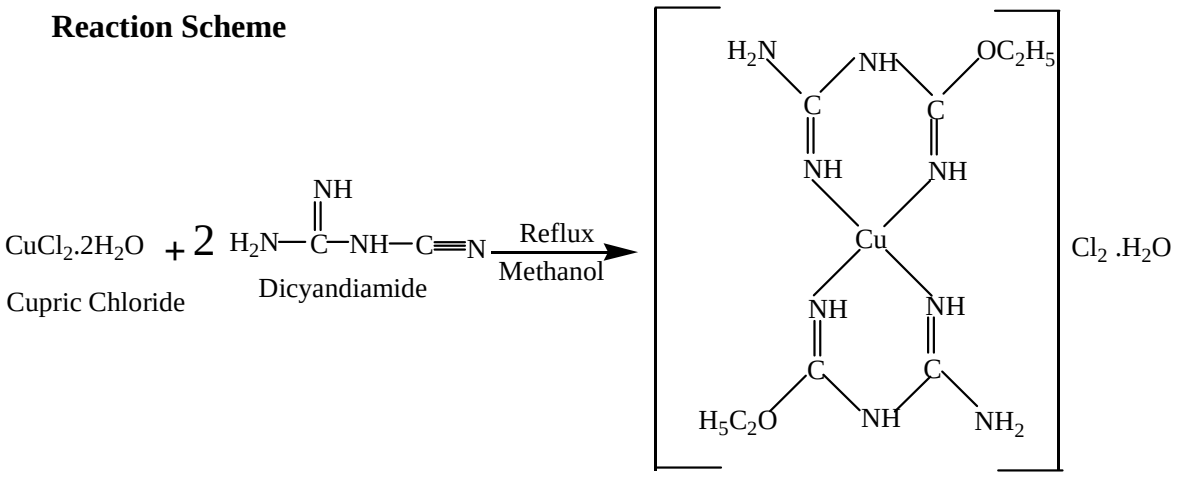
$$[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA]/ (\varepsilon_b - \varepsilon_f) + 1/ K_b (\varepsilon_a - \varepsilon_f) \quad (1)$$

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 $[DNA]/ (\varepsilon_a-\varepsilon_f)$ versus [DNA] gave a slope of $1/(\varepsilon_b-\varepsilon_f)$ and a Y-intercept equal to $1/K_b (\varepsilon_b-\varepsilon_f)$, 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.

Viscosity measurements were carried out by using Ubbelodhe viscometer maintained at a constant temperature of 25±0.2^{0}C in a thermostatic bath. The CT DNA concentration was kept constant (0.5x10^{-3}M) and the concentration of the Cu(II) complex was varied to give [complex]/[DNA] (r) ratios in the range of 0.00-0.157. Flow time was measured with a stopwatch. The experiment was carried out in triplicates and an average flow time was calculated. Viscosity values were calculated from the observed flow time of DNA-containing solutions corrected for the flow time of the buffer alone (to): $\eta=(t-t_0)/t_0$ [14]. Data were presented as relative viscosity $(\eta/\eta_0)^{1/3}$ versus binding ratio (r) [DNA]/[complex], where η is the viscosity of CT DNA in the presence of complex and η_0 is the viscosity of CT DNA alone.

Reaction Scheme



Cupric Chloride Dicyandiamide

Bis (1-amidino-O-ethylurea)copper(II)chloride

$[CuL_2]Cl_2.H_2O$

The biological activities of the complex and parent salt $CuCl_2.2H_2O$ were carried out by standard filter paper disc diffusion method. Overnight grown bacteria (1 O.D.) were spread on nutrient agar and kept for about half an hour to allow the bacterial cells to rest. Filter discs (about 6 mm in diameter) were placed on the inoculated plates into which 0.01 cm^3 each of the test solutions (0.1 mg/ml, 1 mg/ml and 2 mg/ml) were loaded and sterile, kept for about 1hr to enable diffusion of the test solutions into the medium. They were incubated at 37±0.2^{0}C. Inhibition of microbial growth was determined by measuring the diameter of the inhibition zone after 24-hr incubation. The antibacterial activities of the synthesized complex and parent metal salt were compared with the reference antibiotic, gentamycin sulfate.

Synthesis of the Complex $[CuL_2]Cl_2.H_2O$

The complex was synthesized by refluxing cupric chloride dihydrate and dicyandiamide in a 1:2 stoichiometric ratio in ethanol for 1-3hr, as previously reported [15]. The amount of copper was determined by decomposing the complex with a mixture of HNO_3 and H_2SO_4; and finally by

performing an iodometric titration. Yeild: 85%. Anal. Calc. for $CuC_8H_{22}N_8O_3Cl_2$: Cu, 15.06; C, 22.63; H, 5.24; N, 26.02. Found: Cu, 15.89; C, 22.51; H, 5.67; N, 26.20.

RESULTS AND DISCUSSION

Infrared Spectra

The IR spectra of ligand dicyandiamide show a strong nitrile $\nu_{C\equiv N}$ band at 2165cm^{-1}. In the IR spectra of the complex there is the absence of a band around 2165 cm^{-1} and also there is no C=O stretching band around 1740 cm^{-1} indicating that the complexing ligand is not substituted guanylurea. Instead the complex has a very strong ν_a (C–O–C) stretch at ca. 1220 cm^{-1} and vs(C–O–C) at ca. 981cm^{-1} to support 1-amidino-O-ethyllurea [13]. Further the IR spectra of dicyandiamide show a band at around 1558−1570 cm^{-1} for azomethine C=N stretching, this had a downward shift (20-35 cm^{-1}) in the complex showing that the azomethine nitrogen is coordinated to the metal ion. The increase in value of $\nu_{(C=N)}$ to 1668 cm^{-1} range in the complex is presumably due to change in the C=N bond order, or coordination through the nitrogen atom, facilitated by the transfer of electron density from the C–O–C moiety. The electron density on the N=C–O–C fragment of 1-amidino-O-ethylurea is delocalized in the complexes and the =C–O– bond order is raised [16-18]. The presence of new band in the 480cm^{-1} range is due to (Cu-N) in the complex.

Electronic Spectrum of the complex

The UV- Vis spectra of the complex exhibited n→π*or π→π* charge transfer band at 44,444 cm^{-1}. Another broad band, observed at 18,519) cm^{-1} was attributed to d - d transition ($^2B_{1g} \rightarrow {}^2A_{1g}$), typical for Cu (II) in square planar CuN_4 chromosphere.

Surface morphological studies

X-ray powder diffraction (XRD) of the free ligand and complex are depicted in Fig 1. The observed diffraction data is given in Table 1. Using a set of program called P-INDEX, which are based on least-squares approach, the unit cell parameters of the complex are found to be a = 13.6766A˚, b = 12.7978A˚, c = 5.2467A˚, and cell volume V = 918.35 A˚. This data of the complex supports orthorhombic system. To evaluate the crystallite size of the synthesized complex, D is determined using Debye–Scherer formula [19,20] given by

$$D = 0.94\lambda/\beta\cos\theta$$

where β is the full width at half maximum of the predominant peak and θ is the diffraction angle and λ is the wavelength of light. The size of the crystallite of the complex is found to be 67nm.

Electrochemical Studies

Typical cyclic voltammetric behavior of the complex ($0.5x10^{-3}$ M) scan between 0.4 to - 0.8 is shown in Fig 2. In the cathodic scan two reduction peaks were observed at -0.058 and -0.302 respectively, which are associated with the reduction of copper ions according to reaction:

$$Cu^{2+} + e^- \rightarrow Cu^+$$
$$Cu^+ + e^- \rightarrow Cu$$

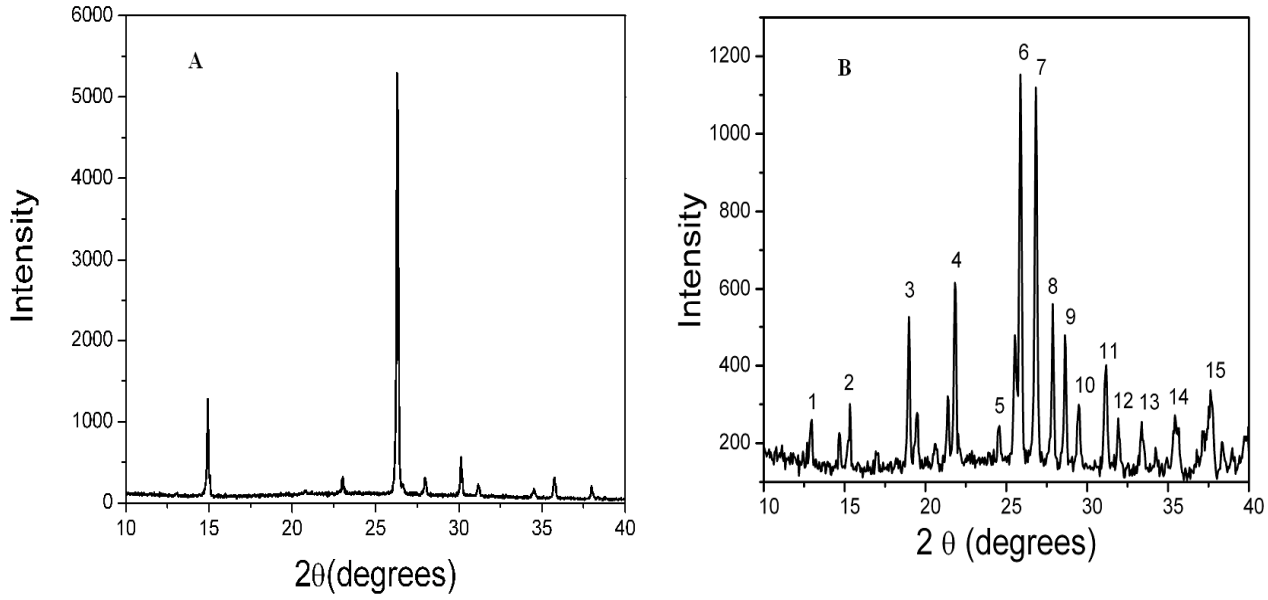


Fig.1. XRD spectrum of (A) free ligand (dicyandiamide); (B) complex

Table 1: Powder XRD data of Cu (II) complex

	d-spacing (A^0)		2 θ values		Δ2θ	(h k l)
	Observed	Calculated	Observed	Calculated		
1	6.84441	6.83833	12.924	12.936	-0.012	(200)
2	6.02696	6.03131	14.686	14.675	0.011	(210)
3	4.55691	4.55889	19.464	19.455	0.009	(300)
4	4.15094	4.16267	21.389	21.328	0.061	(201)
5	3.62345	3.61942	24.548	24.576	-0.028	(230)
6	3.44042	3.4413	25.876	25.869	0.007	(301)
7	3.32046	3.32325	26.828	26.805	0.023	(311)
8	3.1983	3.19946	27.873	27.863	0.01	(040)
9	3.11394	3.11535	28.644	28.631	0.013	(140)
10	3.02831	3.03081	29.472	29.447	0.025	(321)
11	2.86935	2.86458	31.145	31.198	-0.053	(401)
12	2.79461	2.79541	32.000	31.991	0.009	(411)
13	2.68099	2.67844	33.395	33.428	-0.033	(331)
14	2.52752	2.52573	35.488	35.514	-0.026	(112)
15	2.39014	2.38996	37.602	37.605	-0.003	(122)

In the reverse scans two prominent anodic peaks were observed at around 0.038 V and 0.178 V corresponding to oxidation of copper according to reaction:

$Cu \rightarrow Cu^{+} + e^{-}$

$Cu^{+} \rightarrow Cu^{2+} + e^{-}$

The non-equivalent current intensities of the anodic and cathodic peaks as well as large separation between the anodic and cathodic peak potentials indicates the redox processes are irreversible in nature [21].

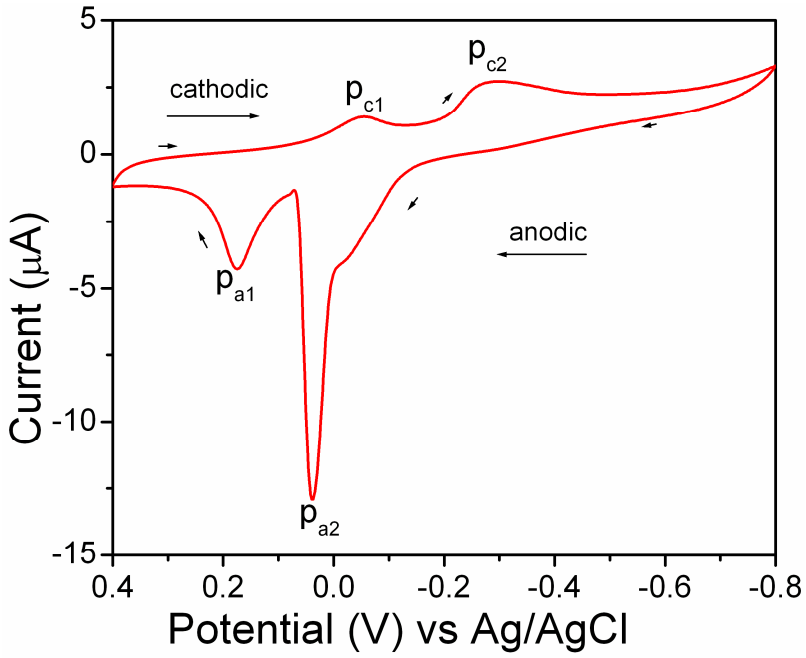


Fig. 2. Cyclic voltammograms of $0.5x10^{-3}$ M of the complex; Supporting electrolyte 50 mM NaCl/5 mM Tris–HCl (pH 7.2); Scane rate -0.01V s^{-1}.

DNA Binding Studies

Absorption spectral measurement

The absorption spectra of the complex in the absence and presence of increasing amounts of CT DNA concentration is shown in Fig 3. With increasing concentrations of DNA, the complex exhibited hyperchromism with slight blue shifts of the absorption bands at 225 nm. Since the complex do not contain any fused aromatic ring to facilitate intercalation, classical intercalative interaction is excluded. 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 [22-23]. However, since DNA possesses several hydrogen bonding sites which are accessible both in the minor and major grooves, a favorable hydrogen bonding may be formed between the coordinated and non-coordinated amine –NH– groups of the complex with the base pairs in CT DNA. Further, the intrinsic binding constant (K_b) of the complex calculated by using Eq. (1) is found to be $1.4x10^4$ M^{-1}

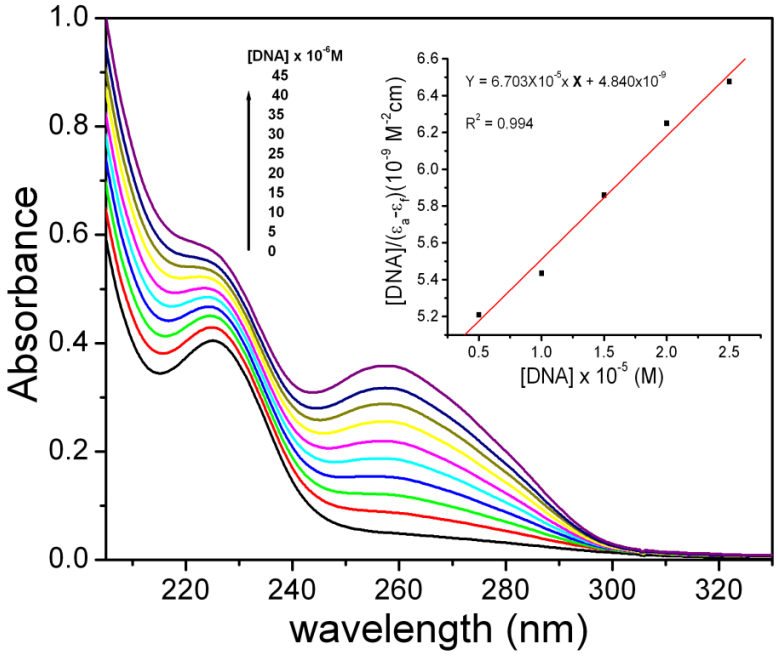


Fig. 3. Absorption spectral trace of the complex in Tris–HCl buffer upon addition of CT DNA. Insets: plots of [DNA]/(ε_a - ε_f) *vs* [DNA] for the titration of complex with CT DNA.

Cyclic voltammetric measurements

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. Pure CT DNA is electrochemically inactive under our experimental conditions in the potential range of +0.4 to -0.8V which is not shown in the figure. Cyclic voltammograms of the complex in the absence and presence of CT DNA are shown in Fig 4. A prominent reduction peak were not appeared in the cathodic scan where as cathodic current increases from -0.27V onward due to reduction of Cu^{2+}. So, only the oxidation peaks were used to investigate the CT DNA binding nature of the complex. The addition of CT DNA caused the peak currents of the anodic waves to diminish considerably as compared to the solutions without CT DNA. This decrease in peak currents is due to the decrease in the concentration of unbound Cu (II) complex. This is again due to the formation of CT DNA-complex system. Similar observation was reported by Shah *et al*. [24]. Further, with increasing concentration of CT DNA, the anodic peak potentials of the complex shifted to more negative values indicating the non-intercalative binding nature of the complex with CT DNA [25].

Viscosity measurements

Viscosity measurements were carried out for further clarifying the CT DNA binding nature of the complex. According to the classical intercalation concept put forward by Lerman [26], the presence of the intercalation bond between a drug and the base pairs of DNA forces these base-pairs away from each other and therefore, unwinding the double helix and lengthening a given amount of DNA which in turn, increases the viscosity of the DNA solution. In contrast, groove-face or electrostatic interactions typically cause less pronounced (positive or negative) or no change in the DNA solution viscosity [27]. Figure 5 shows the changes in viscosity of the CT DNA upon addition of complex as well as EB (a well known intercalating agent). With

increasing concentration of complex, relative viscosity of the CT DNA solution deviates from the nature of the EB. This result supports the non-intercalative binding nature of the complex with CT DNA.

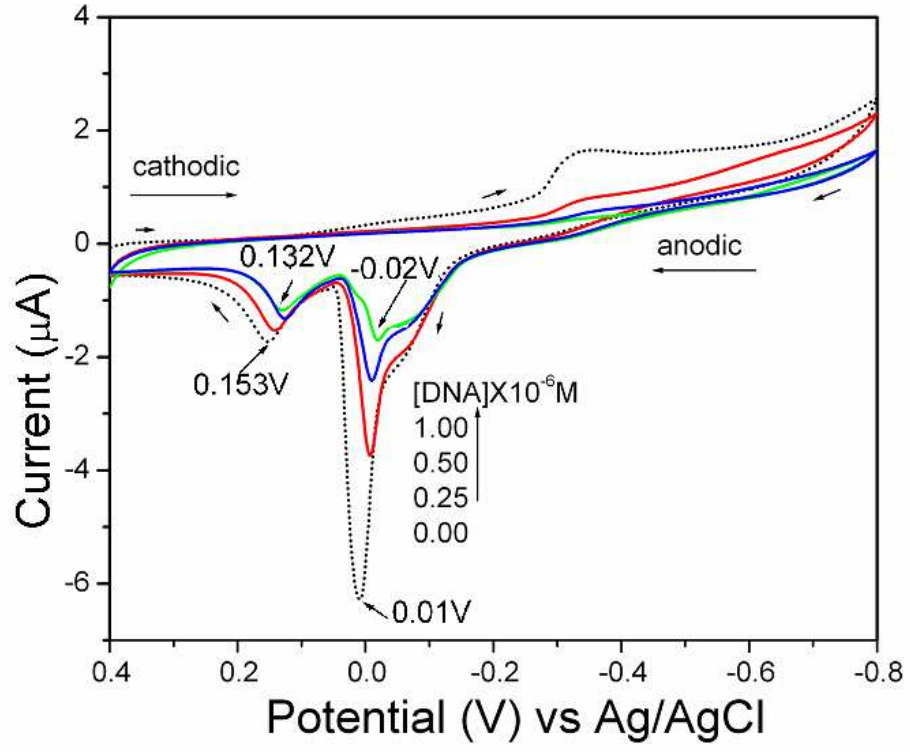


Fig. 4. Cyclic voltammograms of complex in the absence and presence of CT DNA; where [complex] = 2x10^{-3} M. Supporting electrolyte, 50 mM NaCl/5 mM Tris–HCl (pH 7.2); Scan rate-0.01V s^{-1}.

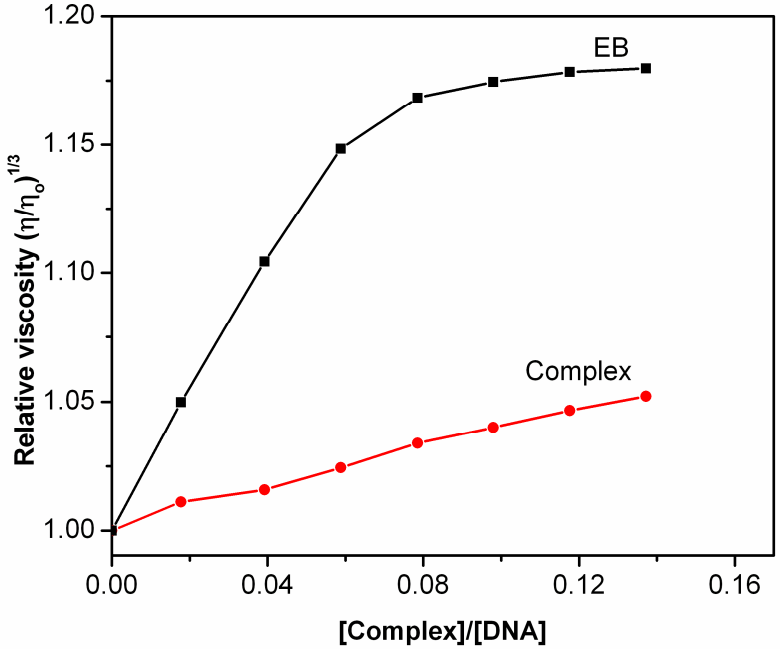


Fig. 5. Effect of increasing amounts of EB and complex on the relative viscosity of CT DNA at 25 ±0.2^{0}C; [DNA] = 0.5x10^{-3}M, [complex]/[DNA] (r) ratio= (0.00 - 0.157)

Biological Activity

The results of biological activities of the synthesized Cu (II) complex and parent metal salt are presented in the Table 2. The complex possessed moderate antibacterial property. The complex is active against all the three tested bacteria as compared with the reference antibiotic, gentamycin sulfate. It can be further depicted that the complex showed the more antibacterial activity than the metal salt. The increase in antibacterial activity of the complex than the parent metal salt may be explained on the basis of chelating effect of the ligand [28]. Chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and possible π electron delocalization within the whole chelate ring. Also chelation increases the lipophelic nature of the central atom, which subsequently favors its permeation through the lipid layer of the cell membrane. It will affect the enzymes which are involved in the metabolic processes of the cell. Then, it will ultimately affect the growth of the bacteria.

Table 2. *In vitro* antibacterial activities of the complex and parent metal salt with reference to gentamycin sulfate

[a]Diameter of inhibiton zone									
Complex	*Escherichia coli* (mg/ml)			*Klebsiella pneumonia* sub sp. *pneumonia* (mg/ml)			*Proteus mirabilis* (mg/ml)		
	0.1	1.0	2.0	0.1	1.0	2.0	0.1	1.0	2.0
$CuCl_2.2H_2O$	8.0±0.7	8.3±0.6	9.0±0.3	7.0±0.3	8.6±0.6	9.5±0.6	6.8±0.7	6.8±0.9	7.8±0.0
Complex	7.8±0.6	8.4±0.9	9.2±0.9	9.3±0.9	9.5±0.3	11±0.3	9.7±0.6	11.7±0.6	13.3±0.9
Gentamycin sulfate	–	–	50.0±0.3	–	–	42.0±0.3	–	–	46.7±0.7

Mean value of the three replicates of three repeated experiments of each test. The diameter of inhibition zone induced by DMSO in Escherichia coli, Klebsiella pneumoniae sub sp. pneumoniae and Proteus mirabilis were 6.0 ± 0.3 ,7.0 ± 0.7 and 8.0 ± 0.6 respectively. Statisti cal analyses were performed using SPSS software and the data were subjected to one way analysis of variance followed by Tukey's test. Values are presented as means ± SE.

[a]Represents the diameter of inhibition zone after subtracting the inhibition zone induced by DMSO. Values of $p < 0.05$ were considered significant.

CONCLUSION

The $[CuL_2]Cl_2.H_2O$ complex crystallizes in the orthorhombic system with crystallite size of 67nm. The complex exhibits good redox property. The complex was researched the interaction with CT DNA by absorption spectroscopy, cyclic voltammetry and viscosity measurements. The results show that the complex is bound to CT DNA by non-intercalative mode. Further, the complex exhibited fair antibacterial activity toward *Escherichia coli*, *Klebsiella pneumoniae* sub sp. *pneumoniae* and *Proteus mirabilis.*

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