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## DNA interaction and antimicrobial studies of novel copper (II) complex having ternary Schiff base

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### Abstract

A novel ternary Schiff base ligand (HL) of ONO type and its copper (II) complex were synthesised using 2-aminophenol and *o*-acetoacetotoluidide. They have been characterised by the usual analytical and spectral methods. The interaction of the complex with calf-thymus (CT) DNA has been investigated by UV-Vis, viscosity measurement, cyclic voltammetry and differential pulse voltammetry studies. The results show that the complex binds to CT DNA by intercalating way. The CT DNA cleavage study was monitored by gel electrophoresis method. From this study, it was found that the complex cleaves CT DNA in presence of the oxidant, hydrogen peroxide. The *in vitro* antimicrobial activities of the synthesized compounds have been tested against the bacteria *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putita* and *Klebsiella pneumoniae* and fungi *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Candida albicans* and *Rhizoctonia bataicola* by well diffusion method. The Minimum Inhibitory Concentration (MIC) value against the growth of the above microorganisms is much larger for copper complex than the ligand.

**Keywords:** Ternary Schiff base, DNA binding constant, Intercalative binding mode, Redox properties, CT DNA cleavage, Antimicrobial assay

### 1. Introduction

Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biology [1, 2]. Hence, current burgeoning interest in small molecules that are capable of binding and cleaving DNA is related to their utility in the design and development of synthetic restriction enzymes, new drugs, DNA foot printing agents *etc*, and also to their ability to probe the structure of DNA itself [3]. In this regard, multidentate Schiff base ligands and their metal complexes have been extensively studied for many years [4, 5]. These complexes play an important role in the development of coordination chemistry [6] and possess important properties, such as biological activity [7, 8], catalytic activity [9] and photochromic properties [10]. They serve as models to emulate activity of proteins [11]. These complexes are also used as model compounds in the study of reversible oxygen carrier. The essential requirement of Schiff base which can act as model to mimic the oxygen carrying property of the natural system is that it should possess at least two nitrogen, oxygen, sulphur or

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combinations of these three. Moreover, copper (II) complexes have been found to be particularly useful because of their potential to bind DNA *via* a multitude interaction and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities [3].

Hence, we are tempted to design and synthesis a biologically important ternary Schiff base containing two oxygen atoms and one nitrogen atom and its copper complex. Herein, it is reported that the synthesis and spectral characterization of ternary Schiff base and its copper complex and the interaction of this complex with DNA. The antimicrobial studies of the ligand and its copper (II) complex have also been investigated.

## 2. Experimental

### 2.1. Chemicals

All reagents and chemicals were procured from Merck products. Solvents used for electrochemical and spectroscopic studies were purified by standard procedures [12]. CT DNA was purchased from Bangalore Genei (India). DNA solution in Tris-HCl/NaCl (pH 7.2) buffer medium gave a ratio of  $A_{260}/A_{280}$ , of *ca.* 1.9:1, indicating that the DNA was sufficiently free from protein concentration [13]. Stock solutions were stored at 4 °C and used within 4 days. Agarose (molecular biology grade), ethidium bromide (EB), tetrabutylammonium perchlorate were obtained from Sigma (USA). Tris-HCl/NaCl buffer solution was prepared using deionized, sonicated triply distilled water.

### 2.2. Instrumentation

Elemental analyses (C, H and N) were carried out with a Carlo Erba 1108 analyzer at the Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute (SAIF, CDRI), Lucknow, India. IR spectra of the samples were recorded in the region 4000-400  $\text{cm}^{-1}$  using KBr pellets and a Perkin-Elmer 783 spectrophotometer at Madurai Kamaraj University, Madurai, Tamil Nadu, India.  $^1\text{H-NMR}$  spectra (300 MHz) of the samples were recorded in  $\text{CDCl}_3$  by employing TMS as internal standard on a Bruker Avance DRX 300 FT-NMR spectrometer at the same institution. Fast atomic bombardment mass spectra (FAB-MS) were obtained using a VGZAB-HS spectrometer in a 3-nitrobenzylalcohol matrix at the Sophisticated Analytical Instrumentation Facility, Central Drug Research Institute (SAIF, CDRI), Lucknow, India. The X-band ESR spectra of the complexes were recorded at RT (300 K) and LNT (77 K) using TCNE (tetracyanoethylene) as the g-marker at Indian Institute of Technology, Mumbai. Electronic absorption spectra were recorded using a Shimadzu UV-1601 spectrophotometer.

### 2.3. DNA binding experiments

The DNA binding experiments were carried out in Tris-HCl/NaCl (pH 7.2) buffer using the copper complex in DMF. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient ( $6600 \text{ M}^{-1}\text{cm}^{-1}$ ) at 260 nm [14]. Absorption titration experiments were performed by varying the concentration of the DNA with the complex. All UV-Vis. spectra were recorded after equilibration. The intrinsic binding constant  $K_b$  was determined from a plot of  $[\text{DNA}] / (\epsilon_a - \epsilon_f)$  versus  $[\text{DNA}]$  equation (1).

$$\frac{[\text{DNA}]}{(\epsilon_a - \epsilon_f)} = \frac{[\text{DNA}]}{(\epsilon_a - \epsilon_f)} + \frac{1}{K_b(\epsilon_b - \epsilon_f)} \quad (1)$$

where  $\epsilon_a$ ,  $\epsilon_f$  and  $\epsilon_b$  are the extinction coefficients of apparent, free and bound form of the complex to DNA respectively.

Viscosity experiments were carried on an Ostwald viscometer, immersed in a thermostated water-bath maintained at a constant temperature at  $30.0 \pm 0.1$  °C. DNA samples of approximately 0.5 mM were prepared by sonicating in order to minimize complexities arising from DNA flexibility [15]. Flow time was measured with a digital stopwatch three times for each sample and an average flow time was calculated. Data were presented as  $(\eta/\eta^0)^{1/3}$  versus the concentration of the Cu(II) complex, where  $\eta$  is the viscosity of DNA solution in the presence of complex, and  $\eta^0$  is the viscosity of DNA solution in the absence of complex. Viscosity values were calculated after correcting the flow time of buffer alone ( $t_0$ ),  $\eta = (t - t_0) / t_0$  [16].

Cyclic voltammetry and Differential pulse voltammogram studies were performed on a CHI620C electrochemical analyzer with three electrode system of a glassy carbon (GC) electrode as the working electrode, a platinum wire as auxiliary electrode and Ag/AgCl as the reference electrode. All the electrochemical measurements were carried out in a 10 mL electrolytic cell. Solutions were deoxygenated by purging with N<sub>2</sub> prior to measurements. The freshly polished GC electrode was modified by transferring a droplet of 2  $\mu$ L of  $5 \times 10^{-5}$  M of DNA solution on to the surface, followed by air drying. Then the electrode was rinsed with distilled water. Thus, a DNA-modified GC electrode was obtained.

#### 2.4. CT DNA cleavage study

The cleavage of CT DNA was determined by agarose gel electrophoresis. The gel electrophoresis experiments were performed by incubation of the samples containing 30  $\mu$ M CT DNA, 50  $\mu$ M copper complex and 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> in Tris-HCl/NaCl buffer (pH 7.2) at 37 °C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1  $\mu$ g cm<sup>-3</sup> ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature unless otherwise mentioned.

#### 2.5. Antimicrobial activity

The *in vitro* antibacterial activity of the ligand and the complex were tested against the against bacteria *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putita* and *Klebsiella pneumoniae* by well diffusion method using nutrient agar as the medium. The antifungal activity was evaluated by well diffusion method against the fungi *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataicola* and *Candida albicans* cultured on potato dextrose agar medium. Streptomycin and nystatin were used as standards for bacteria and fungi respectively. The stock solution ( $10^{-2}$  mol L<sup>-1</sup>) was prepared by dissolving the compound in DMF and the solution was serially diluted in order to find Minimum Inhibitory Concentration (MIC) values. In a typical procedure, a well was made on the agar medium inoculated with microorganisms in a petri plate. The well was filled with the test solution and the plate was incubated for 24 h for bacteria and 72 h for fungi at 35 °C. During the period, the test solution diffused and the growth of the inoculated microorganisms was affected. The inhibition zone was developed, at which the concentration was noted.

#### 2.6. Synthesis of ligand (HL)

An ethanolic solution (10 mL) of 2-Aminophenol (1.09 g, 10 mmol) was mixed with *o*-acetoacetotoluidide (1.91 g, 10 mmol) dissolved in ethanol (10 mL). The resulting reaction mixture was refluxed for ca. 3 h. The dark red precipitate of Schiff base obtained was filtered, washed with distilled water, recrystallised in ethanol and dried *in vacuo*. Yield: 80% (m.p . 83 °C); CHN analysis: Found C, 71.9; H, 6.1; N, 9.7; Calc C, 72.3; H, 6.3; N, 9.9. <sup>1</sup>H NMR, 6.8 - 7.2  $\delta$  (phenyl multiplet), 2.2  $\delta$  (methyl proton), 3.6  $\delta$  (-C-CH<sub>2</sub>), 10.4  $\delta$  (phenolic proton), 7.9  $\delta$  (-PhNH).

### 2.7. Synthesis of copper complex

The Schiff base HL (2.97 g, 10 mmol) dissolved in hot ethanol (50 mL) was added to a hot ethanolic solution (25 mL) of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  (0.85 g, 5 mmol). The pH of the reaction mixture was adjusted to 6-7 by the addition of aqueous ammonia. The solution was reduced to one third on a water bath and cooled. The solid complex separated was filtered, washed with ethanol and dried *in vacuo*. Yield: 52%. CHN analysis: Found M, 9.7; C, 64.8; H, 5.1; N, 8.5; Calc. M, 9.9; C, 65.2; H, 5.4; N, 8.9. Molar Conductance  $\Lambda_m = 2.10 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ;  $\mu_{\text{eff}} = 1.74 \text{ BM}$ .

## 3. Results and discussion

The Schiff base ligand and its copper complex are found to be air stable. The ligand is soluble in chloroform, DMF and DMSO but the complex is soluble only in DMF and DMSO. The analytical data of the complex correspond well with the formula  $\text{CuL}_2$ . The low conductance of this complex supports its non-electrolytic nature.

### 3.1. Mass spectra

The mass spectra of the ligand and complex were recorded and compared for the stoichiometric composition. The molecular ion peak for the ligand observed at 282 m/z is supported by the "Nitrogen Rule", since the compound possesses two nitrogen atoms. For copper complex, the molecular ion peak appeared at 625 m/z confirms the stoichiometry of the complex as  $\text{CuL}_2$ .

### 3.2. IR spectra

The IR spectrum provides valuable information regarding the nature of the functional group attached to the metal atom. The ligand exhibits bands around  $2900 \text{ cm}^{-1}$  (a broad and weak band due to the intra-molecular hydrogen bonding between hydrogen of the phenolic group and nitrogen of the azomethine group),  $1630 \text{ cm}^{-1}$  (C=N) and  $1280 \text{ cm}^{-1}$  (C-O) which are in good agreement with the earlier reports [17,18]. The absence of  $\nu_{\text{OH}}$  mode in the complex indicates the utilization of phenolic -OH in metal-oxygen bond formation [19].

$\nu_{\text{C-O}}$  and  $\nu_{\text{C=N}}$  modes occur at  $1398 \text{ cm}^{-1}$  and  $1591 \text{ cm}^{-1}$  respectively in the complexes. The shifting of  $\nu_{\text{C-O}}$  towards higher frequency as compared to the ligand ( $1269 \text{ cm}^{-1}$ ) is due to the conversion of hydrogen bonded structure into a covalent metal bonded structure. Lowering of  $\nu_{\text{C=N}}$  in the complexes as compared to the ligand [20] ( $1630 \text{ cm}^{-1}$ ) is due to reduction of double bond character of carbon-nitrogen bond of the azomethine group. Thus, it is suggested that the coordination of ligand to metal is through the phenolic-OH and nitrogen of the azomethine group. The band observed at  $1668 \text{ cm}^{-1}$ , characteristic of the carbonyl group of the toluidide of free Schiff base, is shifted to lower frequency in the copper complex indicating the coordination of the above group with copper ion. In addition, the new band appearing at  $450 \text{ cm}^{-1}$  in the complex which is due to  $\nu_{\text{Cu-O}}$  [21] confirms the presence of metal coordination.

### 3.3. Electronic absorption spectra

The Cu(II) complex exhibits four bands at 14970, 22421, 34965 and  $39525 \text{ cm}^{-1}$ . The first band is broad and asymmetric which is assigned to  ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$  transition in an octahedral geometry. The second band is sharp and intense which may be due to ligand to metal (L  $\rightarrow$  M) charge transfer [22]. Third and fourth transitions are intra-ligand charge transfer (ILCT) bands. The electronic spectrum of Cu(II) complex suggests an octahedral geometry around central copper ion [23]. The observed magnetic moment of the Cu(II) complex (1.74 B.M) indicates the

monomeric nature of the complex which is further supported by the microanalytical and FAB mass spectral data.

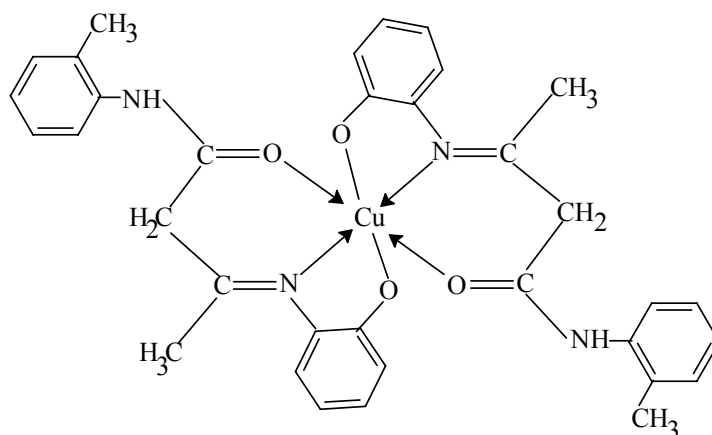
### 3.4. ESR Spectra

The ESR spectrum of the copper complex was recorded in DMSO at 300 and 77 K. The frozen solution spectrum shows a well resolved four line spectrum and no features characteristic for a dinuclear complex. This is also supported by the magnetic moment of copper complex (1.74 B.M.) which confirms the mononuclear nature of the complex. The spin Hamiltonian parameters for the copper complex were calculated from the spectrum. The observed order ( $A_{\parallel} = 115 > A_{\perp} = 27$ ;  $g_{\parallel} = 2.35 > g_{\perp} = 2.08$ ) indicates that the complex is axially elongated octahedral geometry [24]. Further, it is supported from the fact that the unpaired electron lies predominantly in the  $dx^2-y^2$  orbital, as was evident from the value of the exchange interaction term  $G$ , estimated from the expression:

$$G = \frac{g_{\parallel} - 2.0023}{g_{\perp} - 2.0023}$$

It is reported that  $g_{\parallel}$  is 2.4 for copper-oxygen bonds, 2.3 for copper-nitrogen bonds. For mixed copper-nitrogen and copper-oxygen systems, there is a small variation in the point of symmetry from octahedral geometry [25]. For the present copper complex, the  $g_{\parallel}$  value (2.35) is in between 2.3-2.4. This shows that the complex is having mixed copper-nitrogen and copper-oxygen bonds.

If  $G > 4.0$ , the local tetragonal axes are aligned parallel or only slightly misaligned. If  $G < 4.0$ , significant exchange coupling is present and the misalignment is appreciable. The observed value for the exchange interaction parameter for the copper complex ( $G = 4.4$ ) suggests that the local tetragonal axes are aligned parallel or slightly misaligned, and the unpaired electron is present in the  $dx^2-y^2$  orbital. This result also indicates that the exchange coupling effects are not operative in the present complex [26]. Based on the spectral data, the following structure (Fig.1) has been proposed for the copper complex.



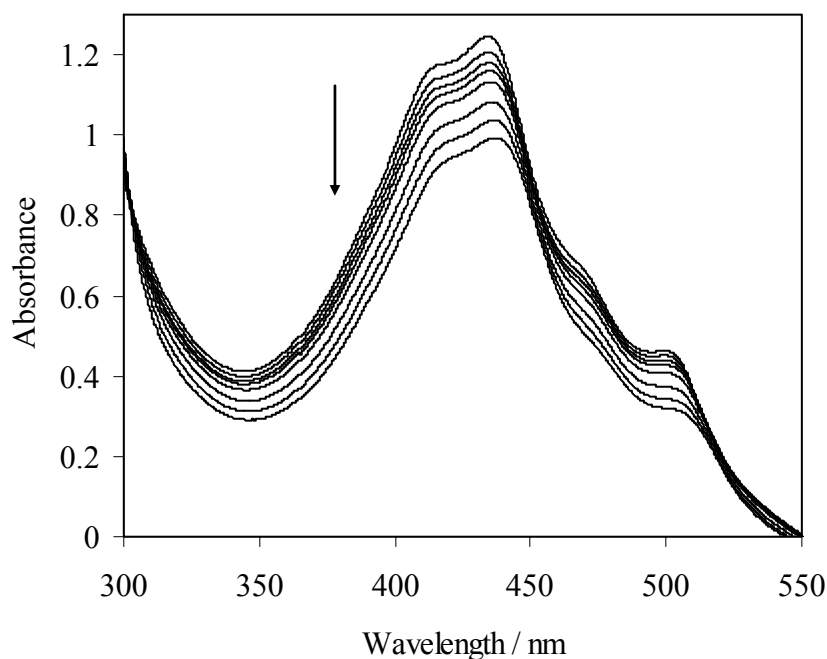
**Fig. 1** Proposed structure of the Schiff base copper (II) complex

### 3.5. DNA binding studies

#### 3.5.1. Electronic absorption spectra

The absorption spectral traces of the copper complex with increasing concentration of DNA are shown in Fig. 2. We have observed a minor bathochromic shift of 1-3 nm along with

significant hypochromicity. When the amount of DNA is increased, a decrease of 70% in the intensity of the charge transfer band is observed. These results indicate that the copper(II) complex binds to DNA through intercalating way due to a strong stacking interaction between the planar aromatic chromophore and base pairs of DNA. The intrinsic binding constant ( $K_b$ ) of the copper complex is  $4.941 \times 10^4 \text{ M}^{-1}$  and it suggests that it reasonably binds with DNA.



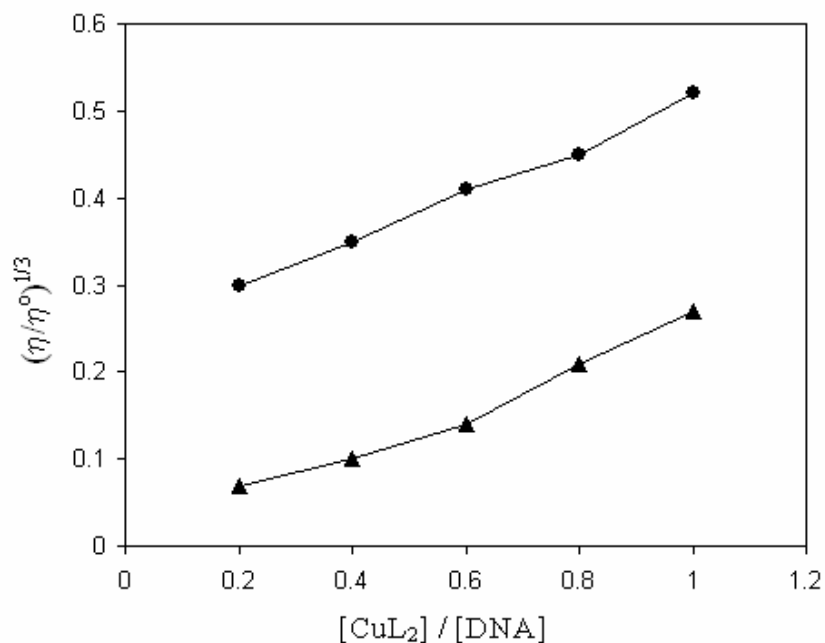
**Fig. 2** UV-Vis spectra for the titration of CT DNA with  $10^{-3} \text{ M}$  solution of copper (II) complex in Tris-HCl/NaCl buffer solution (pH 7.2)

### 3.5.2. Viscosity measurements

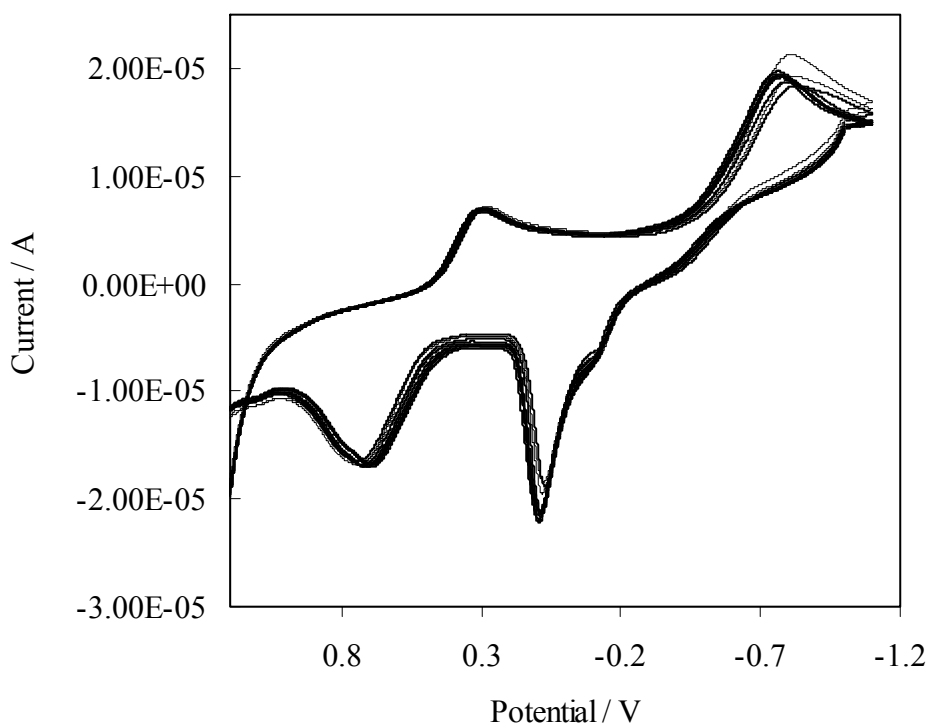
To further clarify the interactions between the investigated compounds and DNA, viscosity measurements were carried out. Hydrodynamic measurements that are sensitive to length change (i.e. viscosity and sedimentation) are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data [27]. A classical intercalation model results in lengthening the DNA helix as base pairs were separated to accommodate the binding ligand, leading to the increase of DNA viscosity. In contrast, a partial and/or nonclassical intercalation of ligand could bend (or kink) the DNA helix, reducing its effective length and concomitantly its viscosity [27, 28]. The effects of the ligand and complex on the viscosity of CT DNA at  $25.0 \text{ }^\circ\text{C}$  are shown in Fig. 3. Viscosity experimental results clearly show that copper complex can intercalate between adjacent DNA base pairs, causing an extension in the helix, and thus increases the viscosity of DNA. The copper (II) complex intercalates more strongly and deeply than the free ligand, leading to the greater increase in viscosity of the DNA with an increasing concentration of complex.

### 3.5.3. Cyclic voltammogram study

The application of electrochemical methods in the study of metallointercalation with DNA provides a useful complement to the previously used methods of investigation such as UV-Vis spectroscopy and viscosity measurement. In the present study these methods have been used to understand the nature of DNA binding with copper complex. Typical CV curves for  $5 \times 10^{-3} \text{ mol dm}^{-3}$  of the copper complex in Tris-HCl/NaCl buffer (pH 7.2) in the varying amount of DNA are shown in Fig. 4.



**Fig. 3** Effect of increasing amounts of Cu(II) complex and ligand on the relative viscosity of calf thymus DNA at 25 °C



**Fig. 4** Cyclic voltammogram of  $5 \times 10^{-3} \text{ mol L}^{-1}$  of copper complex with incremental addition of DNA in DMF- Tris-HCl/NaCl buffer solution (pH 7.2)

On the incremental addition of DNA to the complex, the current intensity is decreased and shifts the redox couple slightly towards the positive side. The ratio of cathodic and anodic peak currents decreases with increasing concentration of CT DNA, suggests the slow diffusion of the Cu(II) complex on the addition of CT DNA. The observed results of decreasing current intensity along with slight potential shift due to its intercalative binding with DNA indicate that the reaction of the complex with DNA on the GC electrode is a diffusion controlled quasi-reversible

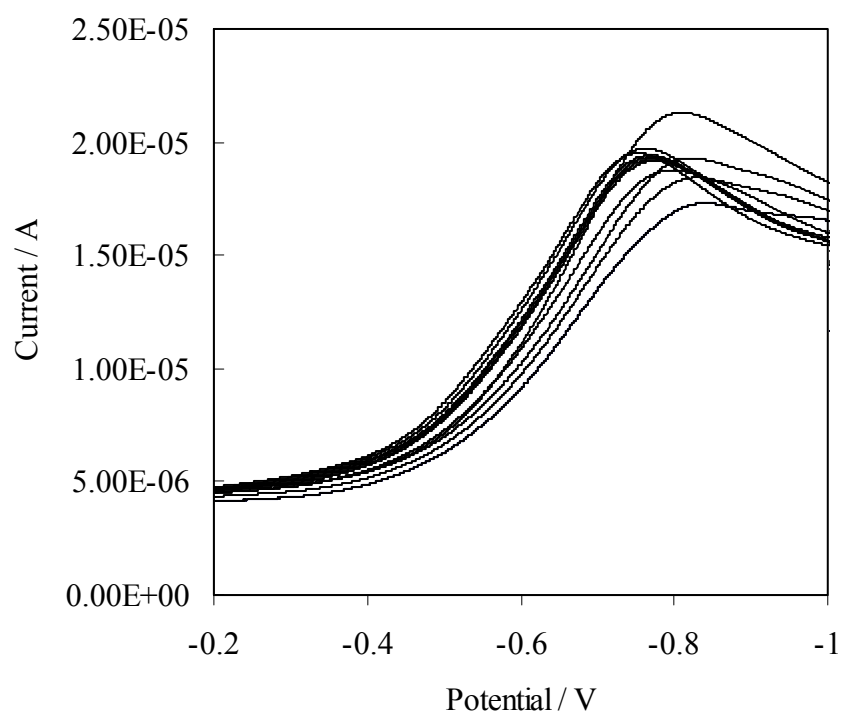
redox process. The separation of potential ( $\Delta E_p$ ) and average of potential ( $E_{1/2}$ ) of anodic and cathodic peak potential values are decreased ( $\Delta E_p = 240$  mV to 201 mV,  $E_{1/2} = 0.43$  V to 0.36 V) upon the addition of DNA to copper complex. These results clearly suggest that copper complex binds to CT DNA through intercalating way.

### 3.5.4. Differential pulse voltammogram study

Differential pulse voltammogram for  $5 \times 10^{-3}$  mol dm<sup>-3</sup> of the copper complex in Tris-HCl/NaCl buffer (pH 7.2) in both absence and presence of varying amount of DNA is shown in Fig. 5. The peak potential and current intensity of the Cu(II) complex are changed in the presence of DNA. According to the equation,

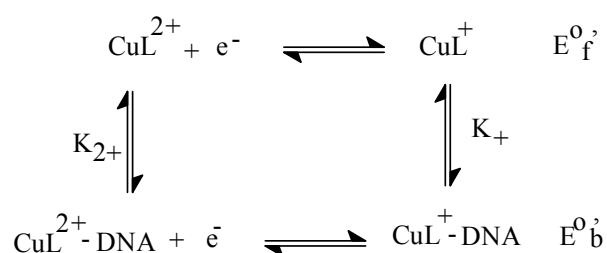
$$E_b - E_f = 0.0591 \log(K_+/K_{2+}) \quad (2)$$

where  $E_b$  and  $E_f$  are the formal potential of the Cu(II)/Cu(I) complex couple in the bound and free forms, respectively. The ratio of the binding constants ( $K_+/K_{2+}$ ) for DNA binding of the Cu(II)/Cu(I) complex was calculated and found to be less than unity.



**Fig. 5** Differential pulse voltammogram of  $5 \times 10^{-3}$  mol L<sup>-1</sup> of copper complex with incremental addition of DNA in DMF-Tris-HCl/NaCl buffer solution (pH 7.2)

This indicates that the binding of the Cu(I) complex to DNA is small compared to that of Cu(II) complex. The above electrochemical experimental results indicate that Cu(II) complex binds to DNA molecules. The possible mechanism is shown below:



It is observed from Fig. 5, the positive potential shift along with significant decreasing of current intensity during the addition of increasing concentration of DNA clearly indicates that the copper complex binds to DNA through intercalative way.

### 3.6. DNA cleavage studies

The cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA-binding ability. The metal complex is able to convert super coiled DNA (Form-I) into open circular DNA (Form-II). The general oxidative mechanisms proposed account of DNA cleavage by hydroxyl radicals *via*, abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [29]. The cleavage is inhibited by the free radical scavengers implying that hydroxyl radicals or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metallocomplex bound hydroxyl radical or a peroxy species generated from the co-reactant  $H_2O_2$ .

In the present study, the CT-DNA gel electrophoresis experiment was conducted at 35°C using the synthesized complex in the presence of  $H_2O_2$  as an oxidant. As can be seen from the results in Fig. 6, at very low concentration, copper complex exhibits nuclease activity in the presence of  $H_2O_2$ . Control experiments using DNA alone do not show any significant cleavage of CT-DNA even on longer exposure time. From the observed results, it is concluded that the copper complex cleaves DNA as compared to control DNA. Further, the presence of a smear in the gel diagram indicates the presence of radical cleavage [30].



**Fig. 6** Changes in the agarose gel electrophoretic pattern of calf-thymus DNA induced by  $H_2O_2$  and Cu(II) complex, Lane 1, DNA alone; Lane 2, DNA + Cu(II) complex; Lane 3, Cu(II) complex +  $H_2O_2$ ; Lane 4, DNA + Cu(II) complex +  $H_2O_2$ .

### 3.7. Antimicrobial assay

The *in vitro* antimicrobial activity of the investigated compounds was tested against the bacteria *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putita* and *Klebsiella pneumoniae* and fungi *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Candida albicans* and *Rhizoctonia bataicola*. The minimum inhibitory concentration (MIC) values of the investigated compounds are summarized in Tables 1 and 2. From these tables, the observed MIC values indicate that the complex has higher antimicrobial activity than the free ligand. Such increased activity of the copper complex can be explained on the basis of chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of  $\pi$ -electrons over the whole chelate ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms [31].

**Table 1**

Minimum inhibition concentration of the synthesized compounds against growth of six bacteria (mg mL<sup>-1</sup>)

Compound	<i>S. typhi</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. putita</i>	<i>K. pneumoniae</i>
HL	60	70	75	80	70	65
CuL <sub>2</sub>	20	25	40	20	40	30
Streptomycin	18	12	10	14	18	12

**Table 2**

Minimum inhibition concentration of the synthesized compounds against growth of five fungi (mg mL<sup>-1</sup>)

Compound	<i>A. niger</i>	<i>A. flavus</i>	<i>R. stolonifera</i>	<i>C. albicans</i>	<i>R. bataticola</i>
HL	70	82	85	70	65
CuL <sub>2</sub>	38	36	35	22	32
Nystatin	10	8	16	12	14

#### 4. Conclusions

The synthesized ligand and its copper(II) complex have been characterized by microanalytical and spectral data. The complex exhibits an octahedral geometry. It is non-electrolyte and monomeric nature. It binds to DNA through intercalating mode. The intrinsic binding constant ( $K_b$ ) of the complex with CT DNA is  $4.941 \times 10^4 \text{ M}^{-1}$ . Gel electrophoresis experiment suggests that the complex cleaves DNA in the presence of hydrogen peroxide. The MIC value against the growth of microorganisms is much larger for the complex than the ligand.

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