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Copper(II) complexes with neutral Schiff bases: Syntheses, crystal structures and DNA interactions

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This work is our tribute to Sir P.C. Rây on the eve of his 150th birth anniversary

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ABSTRACT

The synthesis and X-ray structural characterisation of a new Cu(II) complex, $[Cu(L^1)Cl](ClO_4)\cdot CH_3OH$ (**1**) [L^1 = *N*,*N*′-bis((pyridine-2-yl)phenylidene)-1,3-diaminopropan-2-ol], has been described in this work. The structural study reveals that the Cu(II) centre in **1** has a square pyramidal geometry with a trigonality index $\tau = 0.43$, being coordinated by the organic ligand and a chloro group. The interaction of complex **1** and another complex previously reported by our group, $[Cu(L^2)](ClO_4)_2$ (**2**) [L^2 = *N*-(1-pyridin-2-yl-phenylidene)-*N*′-[2-({2-[(1-pyridin-2-ylphenylidene)amino]ethyl}amino)ethyl]ethane-1,2diamine], with calf thymus DNA (CT-DNA) has been investigated using absorption and emission spectral studies. The binding constant (K_b) and the linear Stern–Volmer quenching constant (K_{sv}) have been determined.

1. Introduction

The design and synthesis of new cationic complexes that interact with DNA are of considerable interest nowadays as the affinity and selectivity of molecules in binding to nucleic acids are valuable for the rational design of new diagnostic and therapeutic agents [1–5]. These metal complexes are known to bind to DNA through a series of interactions, such as π-stacking interactions associated with intercalation of aromatic heterocyclic groups between the base pairs, hydrogen-bonding and van der Waals interactions in the case of binding to the groove of the DNA helix [6]. Copper, with its relevant oxidation states +1 and +2, has been extensively used in metal mediated DNA cleavage [2–5]. Copper(II) complexes under certain ligand environments have also been shown to bring about photocleavage of DNA as well as RNA [2–4]. In this present report, we determine the binding constants (K_b) and the linear Stern–Volmer quenching constants (K_{sv}) for the interaction of the new Cu(II) complex $[Cu(L^1)Cl](ClO_4)\cdot CH_3OH$ (**1**) [L^1 = *N*,*N*′-bis((pyridine-2-yl)phe nylidene)-1,3-diaminopropan-2-ol, Scheme 1] and a previously reported complex, $[Cu(L^2)](ClO_4)_2$ (**2**) [L^2 = *N*-(1-pyridin-2-yl-phenylidene)-*N*′-[2-({2-[(1-pyridin-2-ylphenylidene)amino]ethyl}amino) ethyl] ethane-1,2diamine, Scheme 1] [7] with CT-DNA by absorption and emission spectral studies.

2. Experimental

2.1. Materials

High purity 2-benzoylpyridine (Fluka, Germany), copper(II) chloride dihydrate (E. Merck, India), sodium perchlorate (Alfa Aesar, Germany) and 1,3-diaminopropan-2-ol (Aldrich, UK) were purchased from the respective concerns and used as received. The calf thymus (CT) DNA and ethidium bromide (EB) were obtained from Sigma.

Caution! Perchlorate salts of metal ions are potentially explosive, especially in the presence of organic ligands. Only a small amount of material should be prepared and handled with care.

2.2. Physical measurements

Elemental analyses (carbon, hydrogen and nitrogen) were performed on a Perkin-Elmer 2400 CHNS/O elemental analyzer. IR spectra (KBr discs, 4000–300 cm^{-1}) were recorded using a Perkin-Elmer FT-IR model RX1 spectrometer. Ground-state absorption and steady-state fluorescence measurements were made with a

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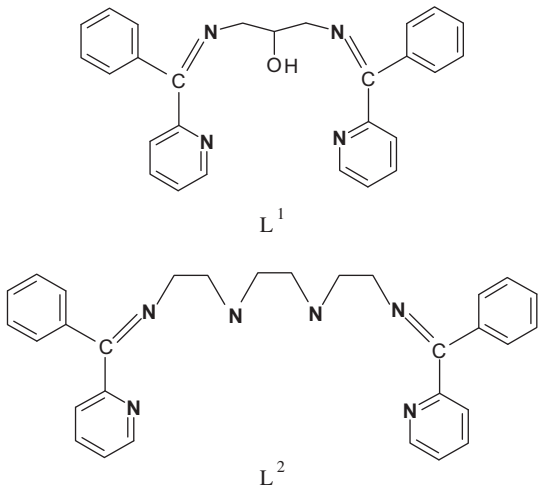

Scheme 1.

Jasco model V-530 UV–Vis spectrophotometer and a Hitachi model F-4010 spectrofluorimeter, respectively.

2.3. Preparation of the Schiff bases and the complexes

The Schiff base L^1 was prepared following a reported method [8] with a little modification. The details are given below:

2-Benzoylpyridine (0.366 g, 2 mmol) was refluxed with 1,3-diaminopropan-2-ol (0.086 g, 1 mmol) in 30 ml dehydrated alcohol. After 10 h the reaction solution was evaporated under reduced pressure to yield a gummy mass, which was dried and stored *in vacuo* over $CaCl_2$ for subsequent use. Yield, 0.469 g (72%). *Anal.* Calc. for $C_{27}H_{23}N_4O$ (L^1): C, 77.23; H, 5.52; N, 13.34. Found: C, 76.52; H, 4.90; N, 12.92%. IR (KBr, cm^{-1}): 1632 (s), 3375 (s).

The synthetic procedure for complex **2** is described elsewhere [7]. Complex **1** was synthesized from the chloride salt of copper(II) using a 1:1 molar ratio of the metal salt and L^1 in MeOH at room temperature. X-ray quality single crystals of **1** separated out within a week.

2.3.1. Preparation of *1*

A methanolic solution (5 cm^3) of L^1 (0.419 g, 1 mmol) was added dropwise to a solution of $CuCl_2{\cdot}2H_2O$ (0.170 g, 1 mmol) in the same solvent (10 cm^3). $NaClO_4$ (0.122 g, 1 mmol) was then mixed in the reaction mixture. The deep green solution was filtered and the supernatant liquid was kept in air for slow evaporation.

Yield: 0.469 g (72% based on metal salt). *Anal.* Calc. for $C_{28}H_{28}N_4O_6Cl_2Cu$ (**1**): C, 51.66; H, 4.33; N, 8.60. Found: C, 51.30; H, 4.17; N, 8.06%. IR (KBr, cm^{-1}): 1618, 1593 ($\nu_{C=N}$), 472 (ν_{Cl}), 3224 (ν_{OH}), 1286, 1117, 1084, 625 (ν_{ClO_4}); UV–Vis (λ_{max}, nm): 268.

2.4. X-ray diffraction studies

Single crystal data of **1** were collected on a Bruker AXS KAPPA APEX II diffractometer using Mo Kα radiation (λ = 0.71073 Å) at 295(2) K. Systematically absent reflections led to the identification of space group *P*212121 for **1**. Of the 26597 total reflections for the complex, 7037 with [$I > 2\sigma(I)$] were used for structure solutions. The structures were solved by direct methods, and the structure solution and refinement were based on $|F^2|$. All non-hydrogen atoms were refined with anisotropic displacement parameters whereas hydrogen atoms were placed in calculated positions were possible and given isotropic *U* values 1.2 times that of the atom to which they are bonded. The final differences Fourier map showed the maximum and minimum peak heights at 0.775 and −0.680 e $Å^{-3}$ for **1** with no chemical significance. All calculations were carried out using SHELXL-97 [9] and ORTEP-32 [10]. The crystal data and data collection parameters are listed in Table 1.

Table 1
Crystal data and structure refinement parameters for **1**.

Empirical formula	$C_{28}H_{28}N_4O_6Cl_2Cu$
Formula weight	650.98
T (K)	295(2)
Wavelength (Å)	0.71073
Crystal system	orthorhombic
Space group	*P*212121
Unit cell dimensions	
a (Å)	8.3120(2)
b (Å)	9.8493(2)
c (Å)	34.7109(7)
α (°)	90
β (°)	90
γ (°)	90
V ($Å^3$)	2841.69(11)
Z	4
D_{calc} (mg/m^3)	1.522
Absorption coefficient (mm^{-1})	1.006
F(000)	1340
Crystal size (mm^3)	0.45 × 0.35 × 0.32
Theta range for data collection (°)	2.15–28.33
Index ranges	$-11 \leqslant h \leqslant 11$, $-13 \leqslant k \leqslant 13$, $-45 \leqslant l \leqslant 39$
Reflections collected	26597
Independent reflections	7037 [R_{int} = 0.0570]
Completeness to theta = 28.33°	99.4%
Absorption correction	semi-empirical from equivalents
T_{max} and T_{min}	0.725 and 0.662
Refinement method	full-matrix least-squares on F^2
Data/restraints/parameters	7037/1/371
Goodness-of-fit (GOF) on F^2	1.047
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0584, wR_2 = 0.1555
R indices (all data)	R_1 = 0.0875, wR_2 = 0.1691
Absolute structure parameter	0.013(19)
Largest difference in peak and hole (e $Å^{-3}$)	0.775 and −0.680

3. Results and discussion

3.1. Synthesis and formulation

The ligand L^1 was synthesized by refluxing 1,3-diaminopropan-2-ol with 2-benzoylpyridine in 1:2 molar ratio in dehydrated alcohol. Compound **1** was prepared using the chloride salt of copper(II) and L^1 in a proper molar ratio as appeared in the compound. The compound was recrystallised from a methanol–water mixture. It was characterised using microanalytical, spectroscopic and other physicochemical results. The exact coordination sphere was determined by single crystal X-ray crystallography. In the IR spectrum the well resolved peak at 1592 cm^{-1} is attributed to ν(C=N) [11]. The compound shows weak bands in the range 2980–2900 cm^{-1} that are assignable to the aliphatic C–H stretching frequency.

3.2. X-ray structures

Oak Ridge Thermal Ellipsoid Plots (ORTEP) of **1** and **2** are represented in Figs. 1 and 2. The structure of **2** is described in our previous report [7]. Bond angles and distances for the structure of **1** are listed in Table 2. The coordination geometry at the copper(II) centre of **1** is best described as a square pyramid, as exemplified by its τ value (τ = 0.43) [12][2] with a CuN_4Cl chromophore. The imine

[2] $\tau = (\alpha - \beta)/60$; α = largest L–M–L bond angle, β = second largest bond angle; If the τ value is in between 0 and 0.5, then the geometry is square pyramid (Sq py) and if the value is in between 0.5 and 1.0, then the geometry is trigonal bipyramid (TBP).

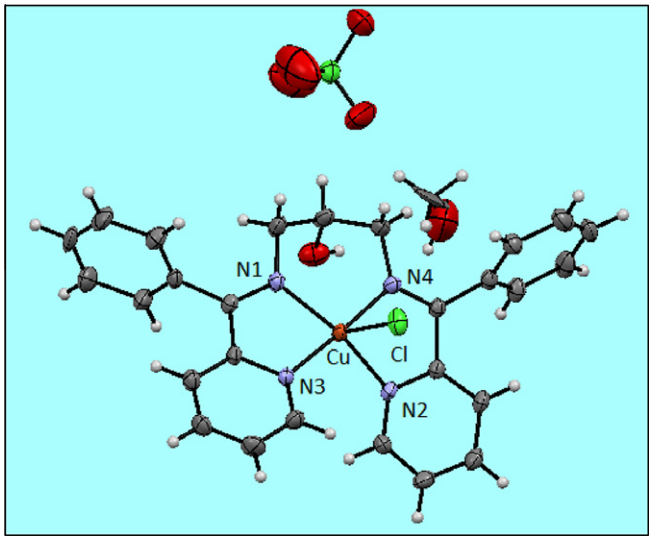

Fig. 1. ORTEP representation of **1** with 30% ellipsoid probability.

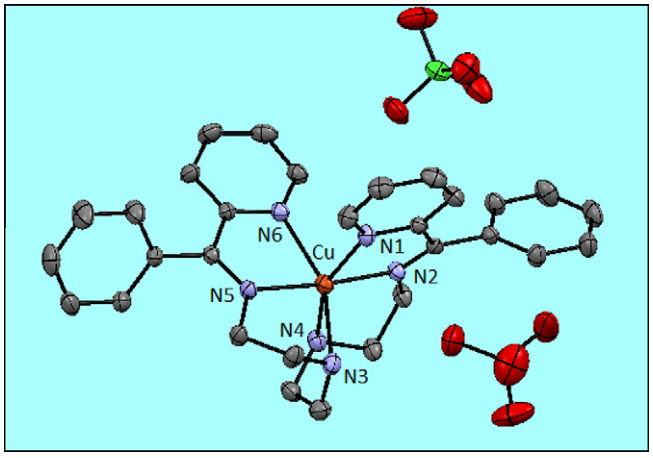

Fig. 2. ORTEP view of **2** with 30% ellipsoid probability [7].

Table 2
Bond lengths (Å) and angles (°) for **1**.

Bond distances			
Cu(1)–N(1)	1.970(4)	Cu(1)–N(3)	2.020(3)
Cu(1)–N(2)	1.971(4)	Cu(1)–N(4)	1.987(4)
Cu(1)–Cl(1)	2.5293(15)		
Bond angles			
N(1)–Cu(1)–N(2)	177.10(16)	N(2)–Cu(1)–N(3)	102.18(15)
N(1)–Cu(1)–N(4)	96.06(16)	N(4)–Cu(1)–N(3)	151.13(16)
N(2)–Cu(1)–N(4)	81.58(15)	N(1)–Cu(1)–Cl(1)	88.81(13)
N(1)–Cu(1)–N(3)	80.70(15)	N(2)–Cu(1)–Cl(1)	90.67(11)
N(4)–Cu(1)–Cl(1)	115.04(13)		
N(3)–Cu(1)–Cl(1)	93.65(11)		

nitrogens (N1, N4) and pyridine nitrogens (N2, N3) occupy the equatorial plane of the square pyramid. The chloride atom resides in the axial position. Here the Cu–N bond distances range from 2.020(3) to 1.970(4) Å. The Cu–Cl bond distance is 2.5293(15) Å.

3.3. DNA interactions

3.3.1. Spectrophotometric investigation

The mode in which the complexes are bound to DNA was investigated by absorption spectra. Hyperchromic and hypochromic effects are spectral features concerning changes in the double-helix structure of DNA. These spectral changes reflect the changes in the conformation and structure of DNA after the complex binds to it [6,13]. The addition of **1** and **2** separately to DNA caused an appreciable increase in the absorption intensity, which is a typical hyperchromic effect (Figs. 3 and 4). Both the compounds show a maximum absorbance at 268 nm in absence of DNA. They have no labile reaction centre (replaceable H_2O or anything else) and hence cannot coordinate with nucleotide bases. Spectrophotometric investigations also support this. Complexation of Cu(II) with DNA bases is expected to show appreciable changes to the electronic spectrum. However, no marked changes were observed in the electronic spectra of the complexes (λ_{max} for both **1** and **2** appear at 274 nm in presence of DNA) on addition of DNA, ruling out the possibility of coordinative binding to DNA donor atoms. It has been shown that the intercalators will shift the wavelength of the maximum absorbance towards longer wavelengths and virtually show hypochromism [14]. In the present case, however, with the addition of DNA these Cu(II) systems exhibit a small increase in λ_{max}. The λ_{max} values for the complexes change from 268 to 274 nm. These small shifts in λ_{max} are accompanied by a small increase in the absorbance intensity. So these changes in λ_{max} cannot account for coordinative binding. It is more in keeping with groove binding, leading to small perturbations [6]. Such small increases in λ_{max} and hyperchromicity have been observed in the case of some porphyrin and copper complexes on their interaction with DNA [15]. This hyperchromism in the absorption intensity may probably be due to the dissociation of ligand aggregates [16] or due to its external contact (surface binding) with the duplex [17].

The binding constants, K_b, for complexes **1** and **2** have been determined from the plot of [DNA]/($\varepsilon_A - \varepsilon_F$) versus [DNA] and have been found to be 5.47×10^4 and 1.33×10^4 M^{-1}, respectively (Figs. 5 and 6). The K_b values are lower than those reported for typical intercalators (for ethidium bromide and $[Ru(phen)_2(dppz)]_2^{2+}$) [6a]. The observed value of the binding constant is more in keeping with the groove binding of the Cu(II) complex **2** with DNA, as observed in the case of the binding of tris(1,10 phenanthroline)ruthenium(II) to DNA [6a].

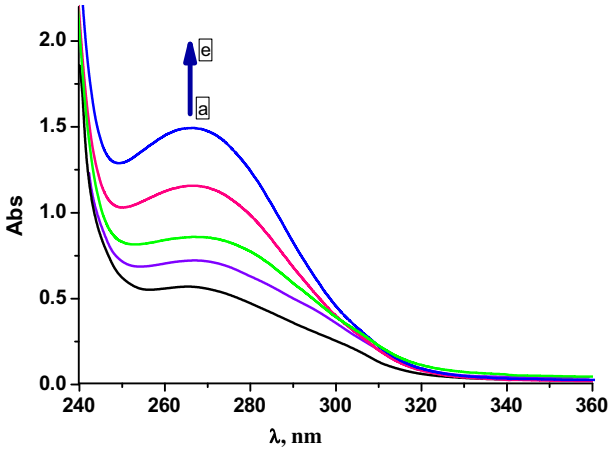

Fig. 3. Electronic spectra of **1** through titration with CT-DNA in Tris–HCl buffer; [Complex] = 1.0×10^{-4}; [DNA]: (a) 0.0, (b) 2.0×10^{-6}, (c) 4.0×10^{-6}, (d) 6.0×10^{-6}, (e) 8.0×10^{-6} (mol L^{-1}). The increase of DNA concentration is indicated by an arrow.

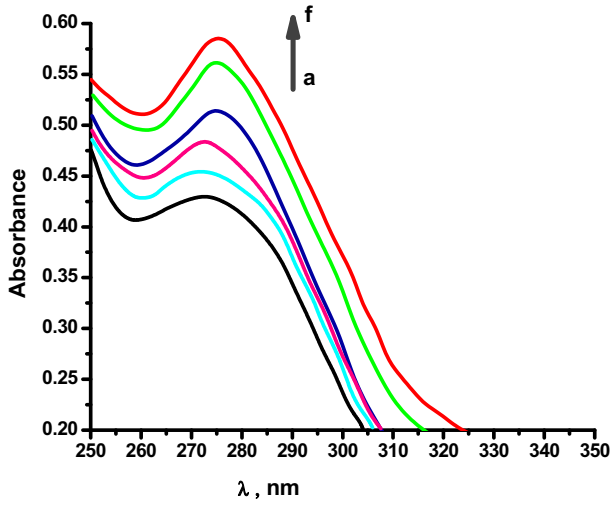

Fig. 4. Electronic spectra of **2** through titration with CT-DNA in Tris–HCl buffer; [Complex] = 1.97×10^{-5} (M); [DNA]: (a) 0.0, (b) 2.0×10^{-6}, (c) 4.0×10^{-6}, (d) 6.0×10^{-6}, (e) 8.0×10^{-6}, (f) 1.0×10^{-5} (mol L^{-1}). The increase of DNA concentration is indicated by an arrow.

3.3.2. Ethidium bromide fluorescence quenching experiments

Ethidium bromide (EB) is chemically known as 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide. It is a large, flat basic molecule that resembles a DNA base pair. Because of its chemical structure, it can intercalate into a DNA strand. EB has been used to probe the interaction of a complex with DNA. To evaluate the interaction mode of complexes **1** and **2**, EB fluorescence intensity quenching experiments were carried out. In fact the EB fluorescence intensity will be enhanced in the presence of DNA because of its intercalation into the helix, and it is quenched by the addition of another molecule that displaces EB from DNA [18]. Here, a significant decrease of the fluorescence intensity of EB bound to DNA was recorded by increasing the concentration of the complexes (Figs. 7 and 8). This observation of EB fluorescence quenching leads us to deduce that the copper(II) complexes may interact with DNA through a groove binding mode, releasing some EB molecules from the EB–DNA system [19]. The quenching of EB bound to DNA by the copper(II) complexes is in agreement with the linear Stern–Volmer equation:

$$I_0/I = 1 + K_{sv}[\text{complex}]$$

where I_0 and I represent the fluorescence intensities in the absence and presence of quencher, respectively. K_{sv} is the linear

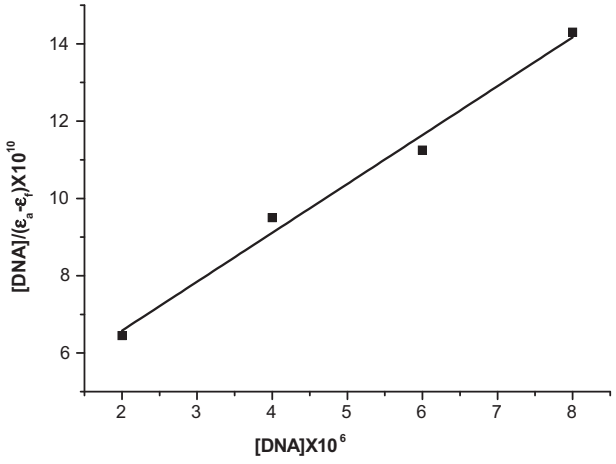

Fig. 5. Plot of $[DNA]/(\varepsilon_A - \varepsilon_F)$ vs. [DNA] for the titration of CT-DNA with **1** in Tris–HCl buffer, binding constant $K_b = 5.47 \times 10^4$ M^{-1} ($R = 0.98432$ for five points).

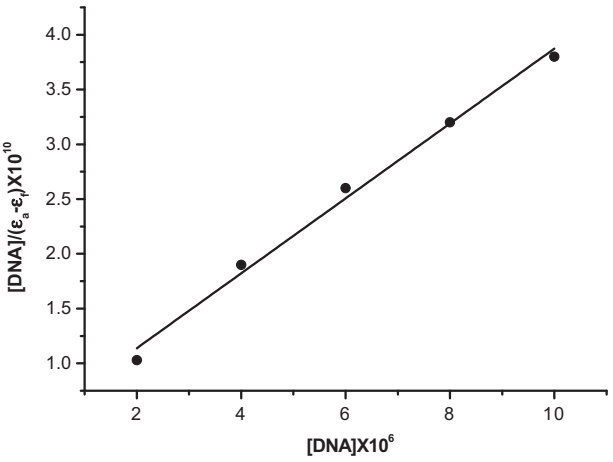

Fig. 6. Plot of $[DNA]/(\varepsilon_A - \varepsilon_F)$ vs. [DNA] for the titration of CT-DNA with **2** in Tris–HCl buffer, binding constant $K_b = 1.33 \times 10^4$ M^{-1} ($R = 0.9909$ for five points).

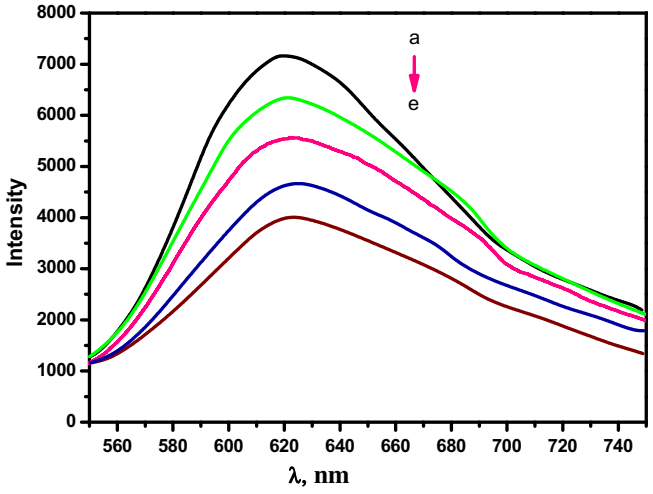

Fig. 7. Emission spectra of the CT-DNA–EB system in Tris–HCl buffer based on the titration of **1**. $k_{ex} = 524$ nm; [DNA] = 1.0×10^{-5}; [complex]: (a) 0.0, (b) 0.97×10^{-5}, (c) 1.94×10^{-5}, (d) 2.91×10^{-5}, (e) 3.88×10^{-5} (mol L^{-1}). The arrow indicates the increase of the complex concentration.

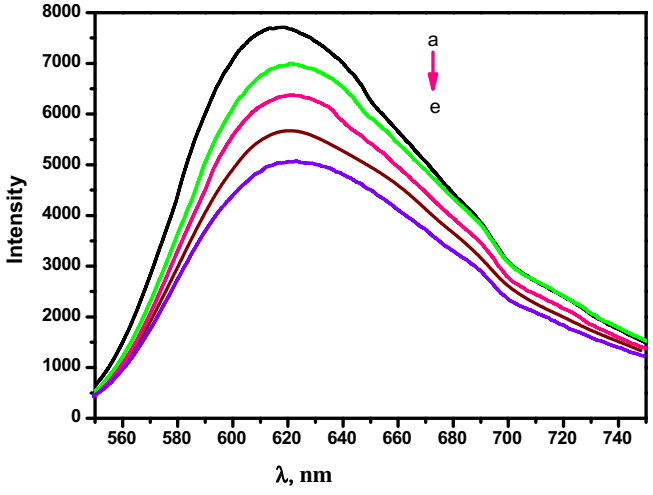

Fig. 8. Emission spectra of the CT-DNA–EB system in Tris–HCl buffer based on the titration of copper(II) complex **2**. $k_{ex} = 522$ nm; [DNA] = 1.0×10^{-5}; [complex]: (a) 0.0, (b) 1.0×10^{-5}, (c) 2.0×10^{-5}, (d) 3.0×10^{-5}, (e) 4.0×10^{-5} (mol L^{-1}). The arrow indicates the increase of the complex concentration.

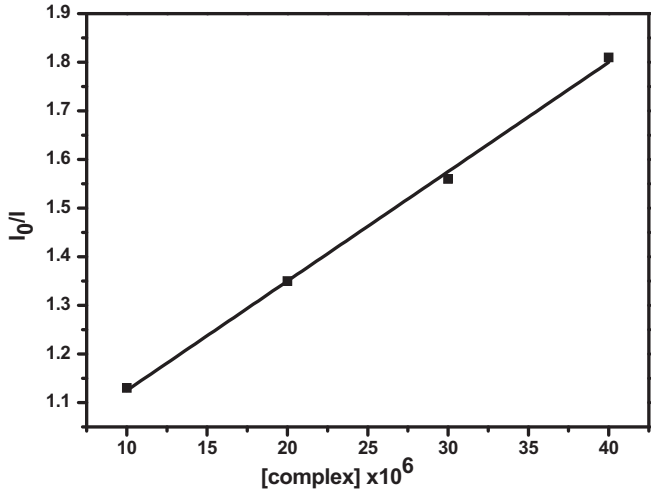

Fig. 9. Plot of I_0/I vs. [complex] for the titration of CT-DNA–EB system with **1** using a spectrofluorimeter; linear Stern–Volmer quenching constant (K_{sv}) for **1** = 1.92×10^4 ($R = 0.996$ for four points).

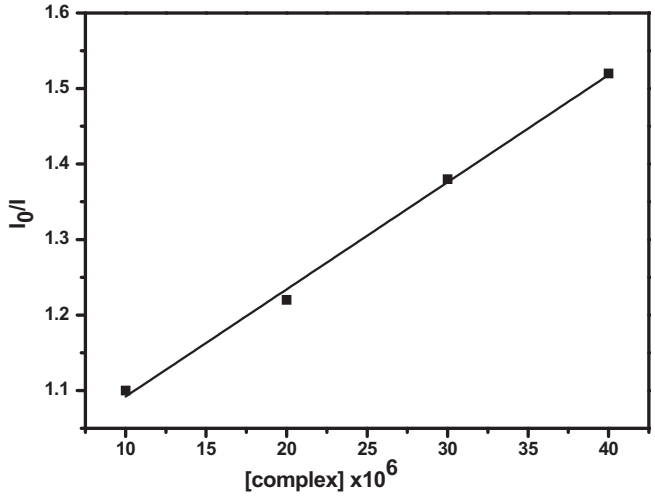

Fig. 10. Plot of I_0/I vs. [complex] for the titration of CT-DNA–EB system with **2** using a spectrofluorimeter; linear Stern–Volmer quenching constant (K_{sv}) for **2** = 1.49×10^4 ($R = 0.995$ for four points).

Stern–Volmer quenching constant and [complex] the concentration of the quencher. From the slope of the regression line in the derived plot of I_0/I versus [complex] (Figs. 9 and 10), the K_{sv} values for **1** and **2** were found to be 1.92×10^4 ($R = 0.996$ for four points) and 1.49×10^4 ($R = 0.995$ for four points), respectively, indicating a strong affinity of the complexes to CT-DNA.

4. Conclusion

Here in this work we have reported the synthesis and single crystal X-ray structural characterisation of a new Cu(II) complex, **1**, with a neutral Schiff base ligand. **1** and another complex **2**, which was reported very recently by our group [7], are found to interact with CT-DNA. The binding constants for the complexes are found to be 5.47×10^4 and 1.33×10^4 M^{-1}, respectively. The linear Stern–Volmer quenching constants were determined as 1.92×10^4 ($R = 0.996$ for four points) and 1.49×10^4 ($R = 0.99585$ for four points), respectively. The spectral features indicate a groove binding mode interaction of the complexes to DNA.

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Appendix A. Supplementary data

CCDC 835459 contains the supplementary crystallographic data for complex **1**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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