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Spectroscopic, microbial and DNA binding affinities of some copper metal complexes using 1,10-phenanthroline as a co-ligand

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ABSTRACT

A new Schiff base ligand is synthesized from 9,10-Phenanthrenequinone and 4-bromoaniline. The synthesized ligand is complexed with Cu(II) metal ions and its structural arrangements, geometry are confirmed by ^{13}C NMR, EI Mass, FT-IR, EPR, Electronic spectra and molar conductance values. The electronic spectra confirms the octahedral geometry for Co(II) and Ni(II) complexes and square planar geometry for the Cu(II) complex which is further supported by EPR spectrum. Affinities of the metal complexes towards CT-DNA are studied in detail using absorption and emission spectral techniques. It has been revealed that the complexes could bind to calf thymus (CT)-DNA via intercalation mode. Anti bacterial activity of the complexes are carried out using disc diffusion method against various bacterial strains like Staphylococcus aureus, Salmonella typhi, Pseudomonas aerugenosa and Escherichia coli.

Keywords: Schiff base, 9,10-Phenanthrenequinone, calf thymus DNA, disc diffusion method.

INTRODUCTION

The Chemistry of coordination compounds is at present undergoing rapid development in diverse disciplines like biological, biochemical, electrochemical studies. A significant rising interest in the design of metal complexes as drugs and diagnostic agent is currently observed in the area of scientific inquiry, appropriately termed medicinal chemistry [1]. DNA is a biomacromolecule that contains all the genetic information essential for the cellular efficacy and functioning of all living organism [2]. The dissimilar encodes present in the DNA are implicated in various regulatory processes such as gene expression, gene transcription, mutagenesis, carcinogenesis *etc*. The above mentioned processes can be modified by the interaction of drugs with specific regions of DNA [3,4], leading to variety of pathological changes in living organisms. It has been reported that the tumor cells can be destroyed by stopping the replication of DNA [5]. Metal-based anticancer drug discovery remains as one of the advanced areas of pharmaceutical research.

Metal complexes which can efficiently bind and cleave DNA under physiological conditions are considered as potential candidates for utility as therapeutic agents in medicinal applications and for genomic research[6-11].It is a well known fact that cisplatin is arguably the most successful anticancer drug in the world. But, it exhibits high toxicity to normal cells leading to undesirable side-effects, although minimized by careful administration protocols, and also it is inactive against many cancer cell lines and metastasis (secondary) cancers [12]. Therefore, attempts are being made to replace cisplatin with suitable alternatives and hence numerous transition metal complexes have been synthesized and tested for their anticancer activities. Investigation of the DNA-metal complexes interaction is very important for the development of new chemotherapy drugs, because a large percentage of chemotherapeutic

anticancer drugs include a compound that binds to DNA and modifies DNA within cells [13]. Also, bioactive metal complexes are useful biochemical tools for the detection of DNA both *in vitro* and inside the cell, as DNA probes [14, 15].

Hence, we report synthesis and characterization of novel Schiff base ligand (L) and its complexes with Cu(II). The binding affinities of these complexes with calf thymus DNA are also reported. Also the antibacterial activity of these complexes against several bacterial strains like *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aerugenosa* and *Escherichia coli* are studied.

MATERIALS AND METHODS

The chemicals involved in this work were of AnalaR grade and the solvents used were of 99% purity. The ligands employed in our present investigation, viz. 9,10-Phenanthrenequinone (Aldrich), 4-bromoaniline (Loba Chemie) and 1,10-Phenanthroline (Loba Chemie) were purchased in pure form and used as such. CT DNA (calf thymus) was purchased from Bangalore

Genei (India). Ethidium bromide (EB) was obtained from Sigma (USA). Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution were prepared by using triple distilled water.

The elemental analysis (C, H and N) data were analyzed using Carlo Erba 1108 model elemental analyzer. The ^{13}C NMR spectra were recorded on a Bruker Advance DRX 300 FT-NMR spectrometer in $CDCl_3$ solution using TMS as standard. EI mass spectra were recorded on a JEOL DX-303 EI mass spectrometer. FT-IR spectra of the synthesized ligand and its complexes were recorded on a JASCO FT IR / 4100 Spectrometer, in the wave number region of 4000-400 cm^{-1}. The electronic spectra of the complexes were computed in 200-800 nm wavelength range on a Perkin Elmer Lambda 35 spectrophotometer using DMSO as the solvent. Molar conductivity of the complexes was measured by using a Elico model SX 80 conductivity-bridge in DMF. EPR spectrum was recorded at liquid nitrogen temperature using a JEOL TES 100 EPR spectrometer by means of DPPH as the g-marker.

2.1 Synthesis of ligand

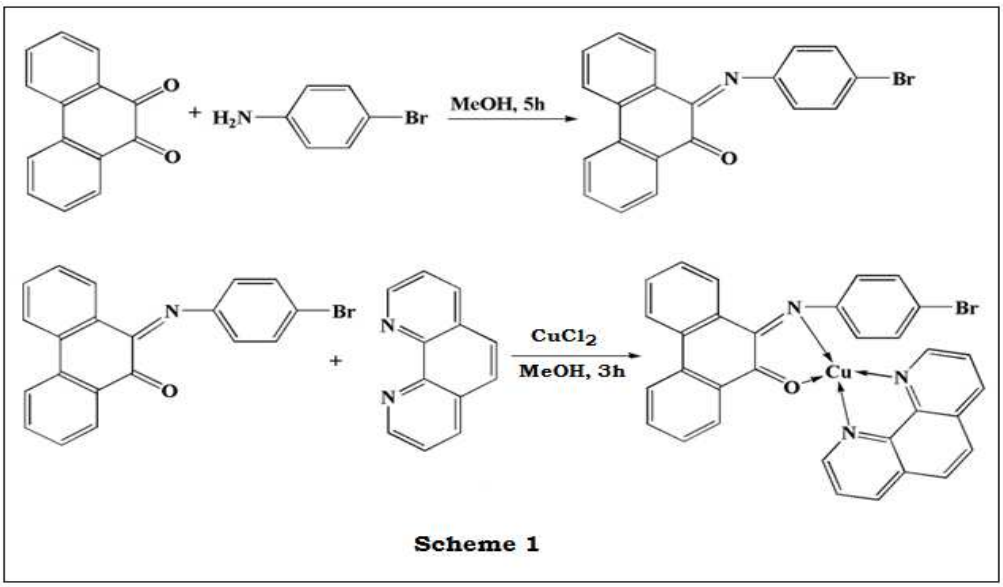
1 mmol of 4-bromo aniline in methanol is added to hot methanolic solution of 9,10-Phenanthrenequinone (1 mmol) and refluxed for 5 hours. The resultant precipitate was filtered and repeatedly washed with diethyl ether and dried (scheme I). The product obtained was characterized by IR, EI-MS and ^{13}C-NMR spectroscopy.

Yield: 74%, m.p: 165 ^0C, Anal. Found.(%): C, 66.32; H, 3.34; N, 3.87; Calc.: C, 66.35; H, 3.92; N, 4.11; EI-MS: m/z, 362; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1589 cm^{-1}, $\nu_{(CO)}$, 1672 cm^{-1}, ^{13}C NMR (δ, ppm in $CDCl_3$) 160.41 (C=N), 180.37 (C=O).

2.2 Synthesis of Cu(II) complex

Finely powdered ligand (1 mmol) is dissolved in 20 ml of methanol. To this 20 ml of methanolic solution of 1 mmol Copper (II) chloride and 20 ml of methanolic solution of 1 mmol 1,10-Phenanthroline was added drop wise. The mixture was refluxed for 12 hours. The resulting solution was slowly evaporated at room temperature and the product obtained is dried over anhydrous calcium chloride (Scheme-I).

Yield: 64%; Anal. Found.(%): C, 63.43; H, 3.33; N, 6.93 and Cu, 10.49; Calc.: C, 63.84; H, 3.52; N, 7.12; IR (KBr, cm^{-1}): $\nu_{(C=N)}$, 1579 cm^{-1}, $\nu_{(CO)}$, 1663 cm^{-1}, $\nu_{(M-O)}$, 533; $\nu_{(M-N)}$, 432; Λ_m ($Smol^{-1}cm^2$) 125.13; UV-Vis. in DMSO, nm (transition): 470 (d-d).



Schematic representation of the synthesized ligand and metal complex

2.3 CT-DNA binding studies

2.3.1 Absorption Spectral Method

The electronic absorption spectral titrations were carried out in (50 mM Tris-HCl buffer, pH 7.5) to investigate the binding affinity between CT-DNA and complexes. The concentration of calf thymus (CT) DNA was determined from the absorption intensity at 260 nm with a ε value[44] of 6600 M^{-1} cm^{-1}. Absorption titration experimentations were achieved by altering the concentration of the CT-DNA (0-250 µM) keeping the complexes concentration (10 µM) as constant. The intrinsic binding constant, K_b for the complexes were determined from the spectral titration results *via* the following equation [16] (1),

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

Where ε_a is the extinction coefficient observed for the charge transfer absorption at a given DNA concentration, ε_f, the extinction coefficient at the complex free in solution, ε_b, the extinction coefficient of the complex when fully bound to DNA, K_b, the equilibrium binding constant, and [DNA] the concentration in nucleotides. A plot of [DNA]/($\varepsilon_a - \varepsilon_f$) versus [DNA] gives K_b as the ratio of the slope to the intercept. The non-linear least squares analysis was done using Origin lab, version 6.1.

2.3.2 Fluorescence Spectral Method

The relative binding affinities of complexes to CT-DNA was studied with an EB-DNA solution in Tris-HCl buffer (pH 7.5). Fluorescence intensities at 610 nm (excited at 510 nm) were measured at different complex concentrations. The reduction of emission intensity gives a measure of binding property of complex to CT-DNA. Stern-Volmer quenching constant K_{sv} of the complexes to CT-DNA was determined from the equation (2)

$$\mathbf{I_o/I = 1 + K_{sv}r} \quad \text{------ (2)}$$

Where I_0, is the ratio of fluorescence intensities of the complex alone, I is the ratio of fluorescence intensities of the complex in the presence of CT-DNA. K_{sv} is a linear Stern–Volmer quenching constant and r is the ratio of the total concentration of quencher to that of DNA, [M] / [DNA]. In the plot of I_0 / I *vs.* [complex]/[DNA], K_{sv} is given by the ratio of the slope to the intercept. The apparent binding constant (K_{app}) was calculated using the equation K_{EB}[EB] / K_{app}[complex], where the complex concentration was the value at a 50% reduction of the fluorescence intensity of EB and K_{EB} = 1.0 x 10^7 M^{-1} ([EB] = 3.3 µM). [17]

1.4 Anti bacterial activity

The anti bacterial activity of the Cu(II) complex was studied against various bacterial strains such as *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aerugenosa* and *Escherichia coli* by disc diffusion method.

RESULTS AND DISCUSSION

3.1. ^{13}C NMR spectrum of ligand

The ^{13}C NMR spectrum of the ligand shows the signals in the range δ 121-136 which are due to aromatic carbons. The signal at δ 152.08 corresponds to C-N carbon atom and the signal at δ 160.41 corresponds to imino carbon of the Schiff base ligand. The signal at δ 180.37 is due to the carbonyl carbon atom. This confirms the effective condensation of 4-Bromoaniline and 9,10-Phenanthrenequinone.

3.2 Electron Impact Mass spectral analysis

The EI-mass spectrum of ligand shows the molecular ion (M^+) peak at m/z = 362 corresponding to the molecular weight of the ligand. The peaks at m/z = 209, 179, 151, 92 and 75 corresponding to various fragments C_8H_4BrNO, $C_{l4}H_8$, C_6H_4Br, C_6H_4N and Br respectively which confirms the structure of the ligand.

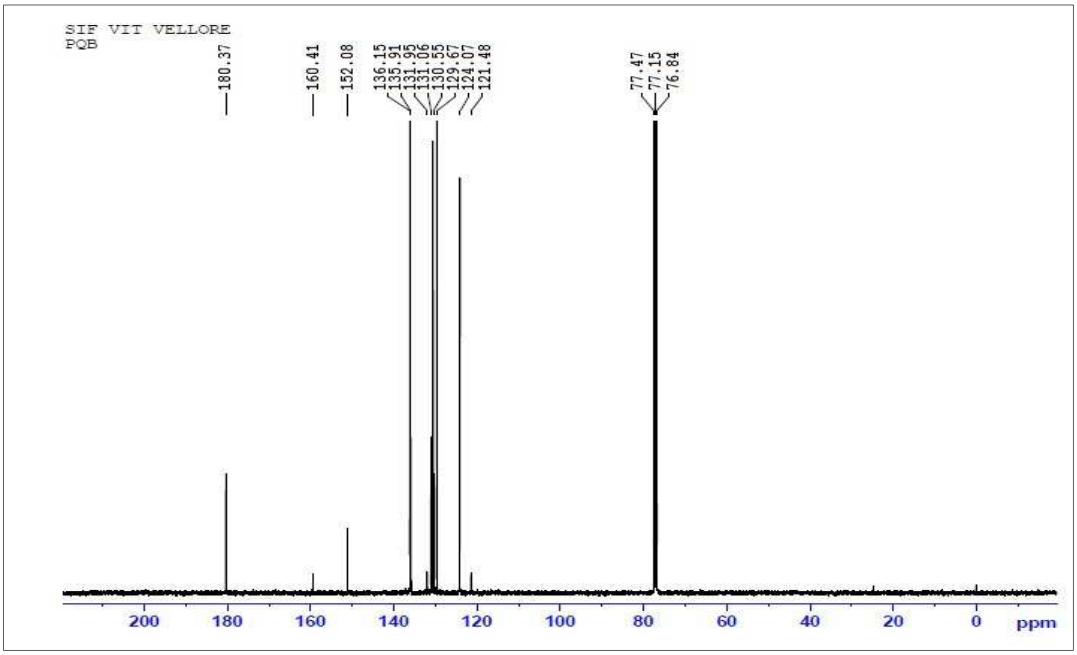


Fig. 1 ^{13}C NMR spectrum of the Schiff base ligand

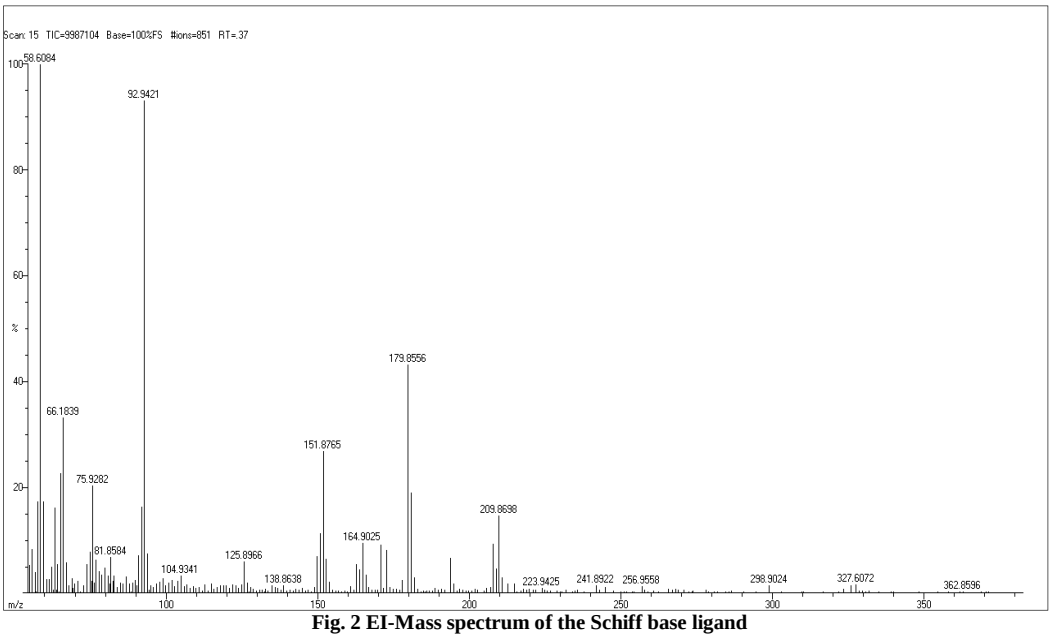


Fig. 2 EI-Mass spectrum of the Schiff base ligand

3.3 FT-IR spectral analysis

The FT-IR spectra of the ligand exhibits strong peak at 1589 cm^{-1} which corresponds to C=N stretching vibration [18]. The peak at 1672 cm^{-1} is due to C=O stretching [19], the peak at 764 cm^{-1} is assigned to C-Br stretching, the peaks at 1517 cm^{-1} and 2361 cm^{-1} correspond to C-C and C-H aromatic stretching vibrations**.**

In the IR spectra of complex the imino (C=N) stretching frequency has been shifted to lower frequency regions of 1579 cm^{-1} and the C=O stretching has been shifted to 1663 cm^{-1}. This indicates the coordination of imino nitrogen [20] and carbonyl oxygen [21] to Cu(II) ion. This is also confirmed by the formation of M-N and M-O bands at 432 cm^{-1} and 533 cm^{-1} respectively [22].

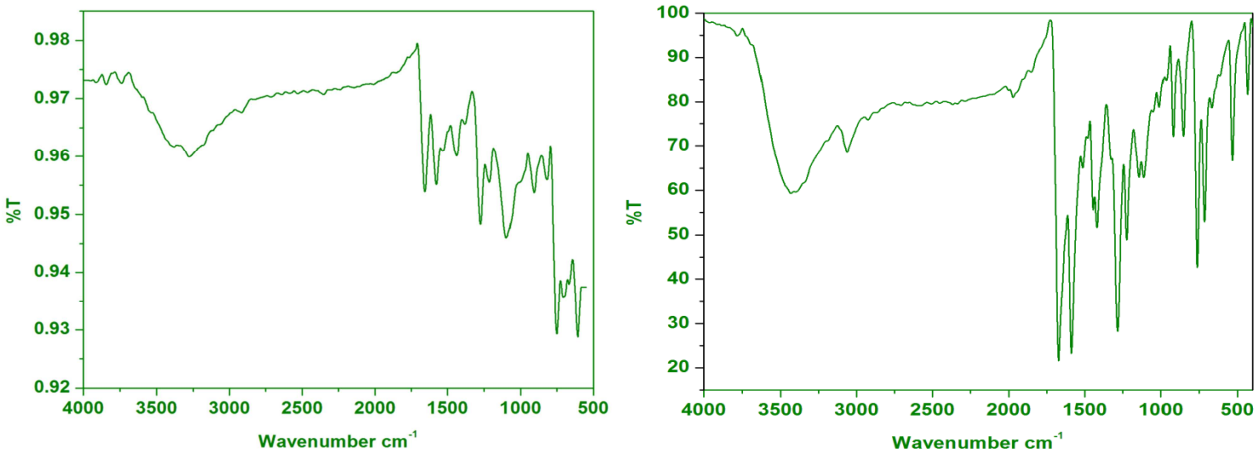


Fig. 3 FT-IR spectra of the Schiff base ligand and its Cu(II) complex

3.4 Electronic spectra and Molar conductance measurements

The electronic spectrum of Cu(II) complex exhibits three absorption bands. The bands at 220 nm and 310 nm correspond to ligand field transitions and the less intense d-d band at 470 nm corresponds to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition. This confirms the square planar geometry of the Cu(II) complex. The molar conductance value of Cu(II) complex is 125.13 which suggest the 1:2 electrolytic nature.

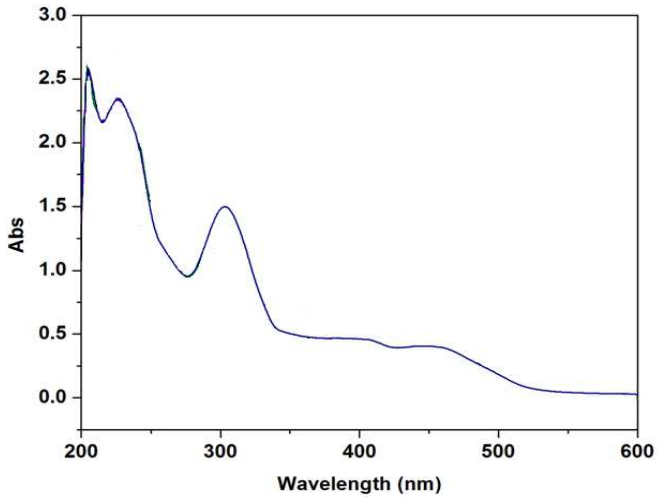


Fig. 4 Electronic spectrum of the Cu(II) complex

3.5 EPR spectral analysis

The liquid nitrogen temperature (LNT) X-band EPR spectrum of the Cu(II) complex is shown in Figure 5. The spectrum for the frozen solution shows anisotropic pattern for a powder sample. It shows four lines with nuclear hyperfine spin 3/2 due to hyperfine splitting. i.e., four well resolved peaks of low intensities in the low-field region and one intense peak in the high-field region resulting from the coupling of the unpaired electron with the nuclear spin of Cu(II). The trend $g_{\|}$ (2.237) > g_1 (2.171) > 2 observed in these complex shows that the unpaired electron lies in the $d_{x^2-y^2}$ orbital of the Cu(II) ion having $^2B_{1g}$ as the ground state characteristic of square-planar geometry and axially symmetric. Further, in an axial symmetry, the g-values are related by the expression,

$G = g_{\|} - 2.0023 / g_1 - 2.0023$

This measures the exchange interaction between the copper centers in polycrystalline solid. According to Hathaway, if the G value is larger than four, the exchange interaction is negligible because the local tetragonal axes are aligned parallel or are slightly misaligned. If the G value is less than four, the exchange interaction is considerable and the local tetragonal axes are misaligned. The observed G value for the copper complex indicates negligible exchange interaction of Cu–Cu in the complex. The present EPR results show that $g_{\|}$ is equal to 2.237 which are in conformity with the presence of mixed system of copper–nitrogen and copper–oxygen bonds in this complex [23].

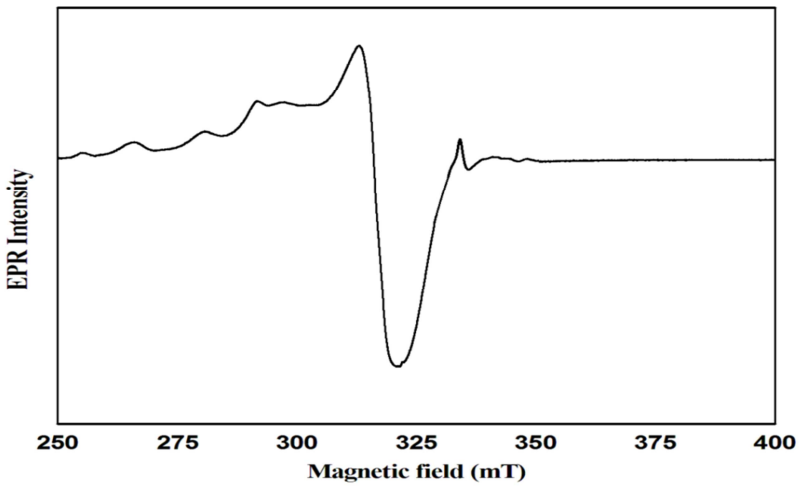


Fig. 5 X- band EPR spectrum of Cu(II) complex in LNT

4. DNA binding studies of Cu(II) complex

4.1 Absorption spectral studies

The binding of Cu(II) complex to CT-DNA was monitored characteristically through absorption titration method. Metal complexes bound to DNA through intercalation is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative binding mode involving a stacking interaction between the DNA base pairs [24]. The electronic absorption spectra of the Cu(II) complex is significantly perturbed by the addition of increasing amounts of DNA. With increasing concentration of CT-DNA (0–250 µM), hypochromism in the absorption bands around about 225-310 nm for the Cu(II) complex was observed accompanied by a red shift of not more than 3-7 nm, suggesting of stabilization of the DNA Helix. Further, a plot of DNA]/(ϵ_a-ϵ_f) versus [DNA] was drawn to elucidate the DNA binding affinities of the Cu(II) complex as represented in Figure 6. The intrinsic binding constants K_b of the Cu(II) complex is found to be 3.73 x 10^5 M^{-1}.

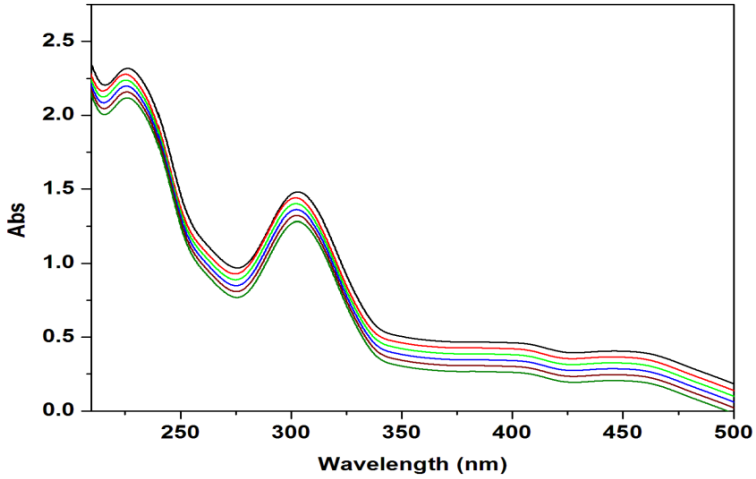


Fig. 6 Absorption spectra of Cu(II) complex (1 x 10^{-5} M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10^{-5} M) at room temperature in 50 mM Tris-HCl/NaCl buffer (pH = 7.5)

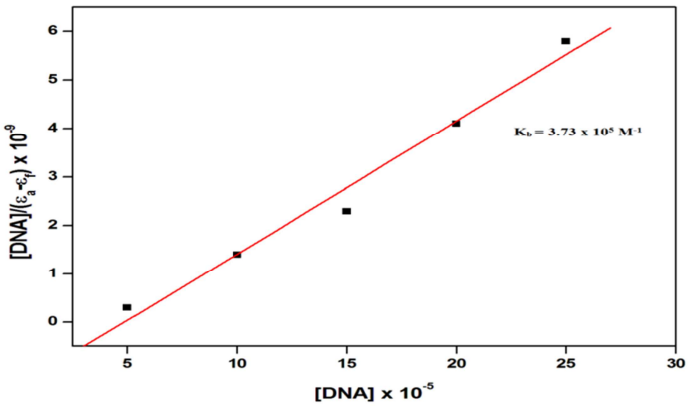


Fig. 7 The plot of [DNA]/($\varepsilon_a - \varepsilon_f$) vs [DNA] for absorption titration of CT-DNA and Cu(II) complex

4.2 Fluorescence spectral studies

The binding affinity of Cu(II) complex to CT-DNA have been examined by fluorescence spectral method. EB emits intense fluorescent light in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. The emission band around 612 nm of the DNA-EB system decreased in intensity with the increasing concentration of the complexes. The observed quenching of the DNA-EB fluorescence intensity for the complexes suggested that they can displace EB from the DNA-EB system and interact with DNA probably *via* the intercalative mode [25]. To find the DNA binding affinity of the Cu(II) complex, the emission spectra of EB-DNA in the absence and presence of Cu(II) complex has been carried out. And the plot of I_o/I *vs* [DNA]/[complex], was drawn and is given in Figure 16. From the graph the apparent binding constant (K_{app}) value of the Cu(II) complex is calculated and it is found to be 2.59 x 10^5 M^{-1}.

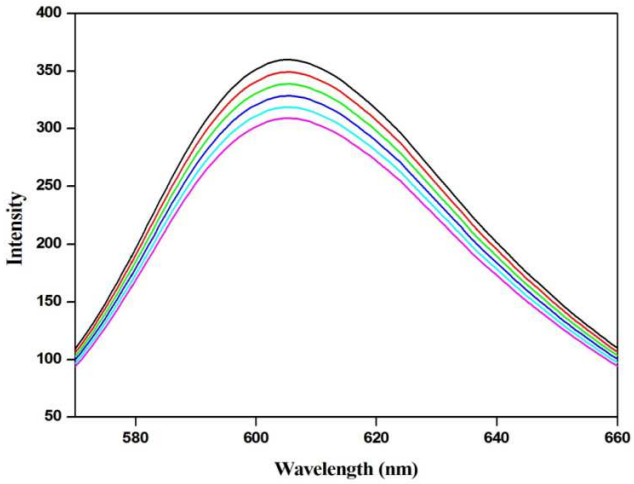


Fig. 8 Emission spectrum of EB bound to DNA in the presence of Cu(II) complex ([EB] = 3.3 µM, [DNA] = 40 µM, [complex] = 0-30 µM, λ_{ex}= 430 nm). Inset shows the plots of emission intensity I_o/I *vs* [DNA]/[complex]

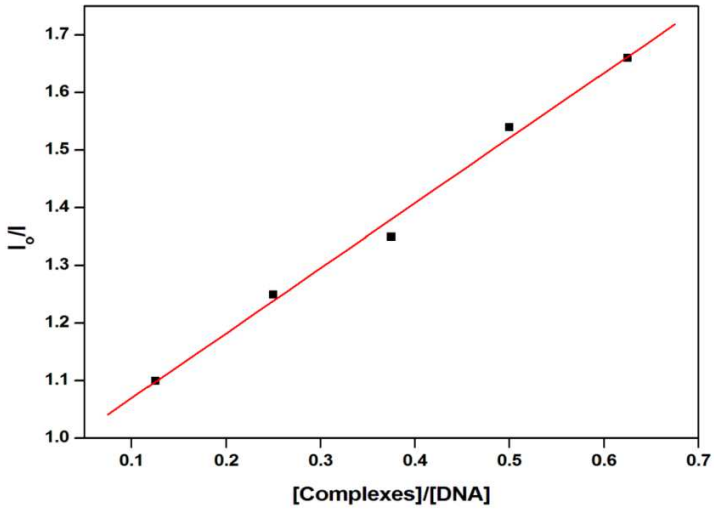


Fig. 9 The plot of I_0/I vs. [Complex]/[DNA] for fluorescence quenching curves of DNA-EB by Cu(II) complex

4.3 Antibacterial Studies

Antibacterial activities of the complex was studied by the well diffusion method using nutrient agar against bacteria strains such as *Staphylococcus aureus, Salmonella typhi, Pseudomonas aerugenosa and Escherichia coli.* It is suggested that the complexes having antibacterial activities inhibit multiplication process of the microbe by blocking their active sites. The mechanism of toxic activity of the complexes with the ligands can be ascribed to the increase in the lipophilic nature of the complexes arising from chelation. 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. The chelation also increases the lipophilic nature of the central metal atom, which subsequently favours the permeation through the lipid layer of cell membrane. The mode of action of complexes involves the formation of hydrogen bonds with the imino group by the active sites leading to interference with the cell wall synthesis. This hydrogen bond formation damages the cytoplasmic membrane and the cell permeability may also be altered leading to cell death. The screening results of the metal complex are displayed as follows: *Staphylococcus Aureus* (11mm), *Salmonella typhi* (8mm), *Pseudomonas aerugenosa* (10 mm), *Escherichia coli* (7 mm).

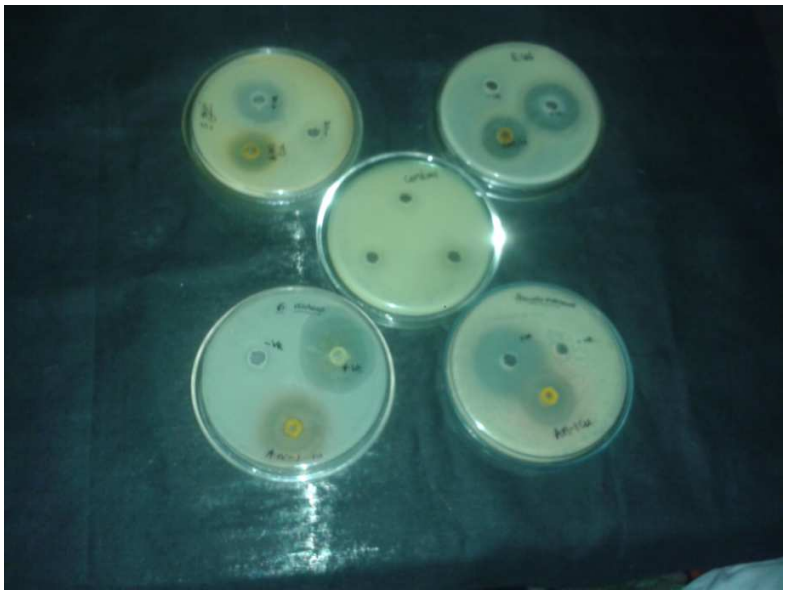


Fig. 10 Antibacterial screening image of Cu(II) complex

CONCLUSION

A Schiff base ligand and its Cu(II) complex have been synthesized using 1,10-Phenanthroline as co-ligand and characterized by electronic absorption spectra, IR, 13C NMR and mass spectral analysis. The metal ion is four coordinate and the geometry is deduced as square planar. The molar conductance value reveal that the complex is 1:2 electrolytic in nature. DNA binding property of the metal(II) complex with DNA have been investigated by UV-Vis spectra, and emission spectra. Results indicate that the complex bind to CT-DNA via an intercalative mode. Antibacterial activities of the complex was studied by the well diffusion method using nutrient agar against bacteria strains such as *Staphylococcus aureus, Salmonella typhi, Pseudomonas aerugenosa and Escherichia coli*. and was found to possess better activity.

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