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Binding of Copper (II) Complexes with DNA and Heterocyclic Bases, L-Methionine and Thiosemicarbazide: Studies on Anti-Microbial Activities

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ABSTRACT

Two copper (II) complexes, $[Cu(L\text{-}Met)(Phen)(TSC)](NO_3)$, L-Ser = L-Methionine, TSC= Thiosemicarbazide), have been synthesized and characterized by Infra-red, EPR spectral and elemental analysis methods. The one-electron paramagnetic complexes display a d–d band near 600 nm in water. Binding modes of the complexes with calf-thymus DNA have been studied by electronic absorption spectroscopy, emission spectroscopy, viscosity, and cyclic voltammetry. We continued to study the antimicrobial activities of complexes 1 & 2 and they were found to have good antimicrobial activities.

1. INTRODUCTION:

The interaction of metal complexes with DNA is associated with interesting chemical and biological properties of the resulting supramolecular system. In fact, the cytotoxic activity of metallodrugs has often been correlated to their DNA binding properties[1–3]. For this reason, we have been recently involved in the synthesis of novel metal complexes and organic compounds as potential DNA binders[4–5]. The interaction modes of a small molecule with the DNA macromolecule have been broadly categorized into the following three types:[4,6,7] (1) covalent binding DNA-alkylators,[8,9] or with Lewis acid metal ions, such as platinum in many clinically used anticancer drugs;[10–12] (2) major or minor groove binding;[13,14] and (3) intercalation[15-16].The latter two categories are classified as non-covalent interactions. Grooves binding involves the contributions of electrostatic interaction of polar or cationic molecules with the negatively charged phosphate groups, and/or the formation of hydrogen bonds with the oxygen and/or nitrogen atoms of the DNA bases or of the sugar fragment, as both donor and acceptor atoms. Intercalation involves specifically the π–π interaction of the planar aromatic section of the small molecules with the stacked aromatic planes of the nitrogen bases. Mixed modes of interactions have been also advised. For example, many intercalators, such as anthracyclines, also interact by electrostatic attraction or by hydrogen bonding with the atoms exposed in the grooves[17–19]. Moreover, it has been recently shown that transition metal complexes of planar aromatic ligands may combine coordination with intercalation in their DNA-binding[20-21]. Such a feature confers them enhanced anticancer properties. The fine structural details of the molecule–DNA binding mode in solution cannot be completely obtained by techniques such as UV-vis absorption, CD and viscometry. Nonetheless, the above-mentioned techniques are interesting because they can be routinely applied to solution samples under physiological conditions.

On the other hand, detailed local structural information can be experimentally obtained by X-ray crystallography or by NMR spectroscopy only of drug–DNA supramolecular complexes involving small synthetic DNA oligonucleotides, typically in the range 2–12-mer oligonucleotides[22–23]. Luckily, the analysis of the X-ray absorption spectrum allows detecting changes in the coordination structure of metal complexes, in particular, the formation of new covalent bonds, when a metal complex interacts with biomolecules in aqueous solution[24]. For such reasons, in the present paper, we have exploited also this technique. Even though the extended X-ray absorption fine structure (EXAFS) has been widely used to characterize the active site of metalloproteins[25–27], to the best of our knowledge, this is the first time this spectroscopic technique is used to detect the bonds between native DNA and a metal complex in water solution.

In this work, we focused synthesis, characterization, DNA binding antimicrobial activities of mixed ligand copper(II) complexes using thiosemicarbazide.

2. MATERIALS AND METHODS:

2.1 MATERIALS:

We purchased the reagents like $Cu(NO_3)_2.3H_2O$, NaOH, $NaClO_4.H_2O$, 2,2'-bipyridyl, L-methionine, thiosemicarbazide, 1,10-Phenanthroline, CT DNA, pBR322 DNA, Tris-HCl, NaCl and ethidium bromide(EtBr) from Aldrich. The spectroscopic titration was carried out in the buffer (50 mMNaCl–5 mMTris–HCl, pH 7.1) at room temperature. We recorded absorption spectra on a UV/VIS Shimadzu 2450 Spectrophotometer using cuvettes of 1-cm path length and emission spectra were recorded on JASCO FP 770 spectrofluorimeter. FT-IR spectra were recorded on an FT-IR Perkin Elmer spectrophotometer with samples prepared as KBr pellets. EPR spectra were recorded on Varian E-112 EPR spectrometer at room temperature, the field being calibrated with DPPH = 1, 10-diphenyl-2-picrylhydrazyl (g =2.0037).

Calf thymus DNA solution in the buffer gave a ratio of UV absorbance 1.8 – 1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. We used Milli-Q water to prepare the solutions. Cyclic voltammetry studies were verified on CHI 602D (CH Instruments Co., USA) electrochemical analyzer under oxygen-free conditions using a three-electrode cell in DMF solution with TBAP (0.1 M) as the supporting electrolyte. A Pt wire, glassy carbon, and the Ag/AgCl (in saturated KCl solution) electrodes were used as a counter, working, and reference.

2.2 METHODS:

2.2.1 Synthesis of $[Cu(L\text{-}Met)(Phen)(TSC)](NO_3)$(1)

The complex $[Cu(L\text{-}Met)(Phen)(H_2O)](NO_3)$ was prepared by literature method[43]. To the aqueous solution of the parental complex (1 mmol), thiosemicarbazide (1 mmol) was added and stirred for 4 hrs resulting in the color of the solution change from blue to bluish green.

The resulting solution was filtered. The filtrate was kept for slow evaporation after two weeks bluish green complex was separated out.

Yield: 67%; Anal. (%) Calc. for $C_{18}H_{23}CuN_7O_5S_2$: C, 39.66; H, 4.25; N, 17.99. Found: C, 38.07; H, 4.16; N, 17.90.IR (KBr pellet): 3419, 2960, 2192, 1641, 1383, 1157, 777 cm^{-1}. UV-Vis (λ_{max} nm): 273, 615 nm.

2.2.2 Synthesis of [Cu(L-Met)(bpy)(TSC)](NO_3) (2)

Synthesis was described in complex **1**, using [Cu(L-Met)(bpy)(H_2O)](ClO_4) (1 mmol) and thiosemicarbazide (1 mmol). Yield: 55%; Anal. (%) Calc. for $C_{16}H_{23}CuN_7O_5S_2$: C, 36.88; H, 4.45; N, 18.82. Found: 35.98; H, 4.27; N, 18.44. IR (KBr pellet): 3421, 2960, 1612, 1518, 1430, 1384, 1089,850, 744 cm^{-1}. UV-Vis (λ, nm): 300 and 610 nm.

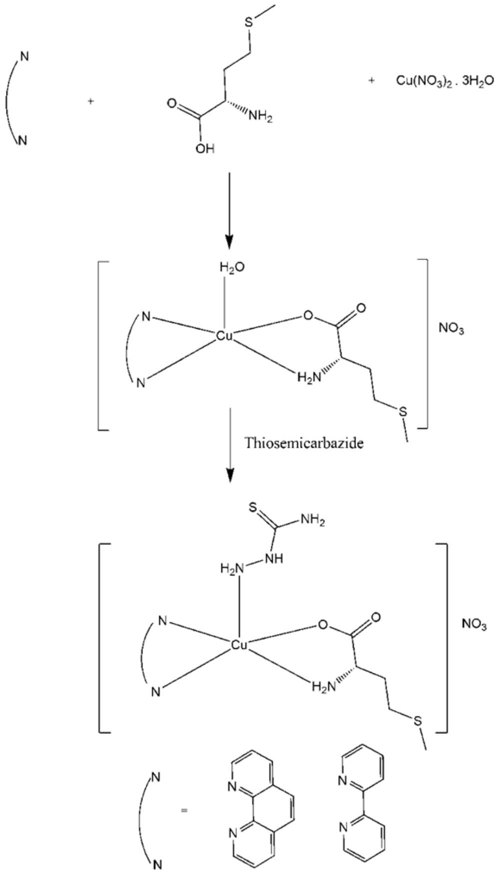
The DNA binding and antimicrobial activity experimental details were given in the literature.

2.2.3 Copper analysis

Copper content in the copper(II) complexes was estimated by the following method[28]. A known weight of the complex (0.02 g) was dissolved in 2 ml of concentrated nitric acid. The solution was gently warmed. After being cooled, the pH of the solution was adjusted to > 5 with 6M NaOH. The solution was transferred into 10 ml volumetric flask and made up to the mark with aqueous NH_3. The absorbance of this solution was measured against a reagent blank. From the absorbance, the concentration of copper was calculated.

3. RESULTS AND DISCUSSION:

3.1 General Aspects



Scheme 1: Synthesis of complexes 1 and 2

These complexes are synthesized by ligand substitution method; the synthetic strategy of the complexes is outlined in Scheme **1**. The synthesized complexes are more stable and they are soluble in water and in other organic solvents. The elemental analysis data of the copper(II) complexes agree with the theoretical values.

3.2 Electronic absorption spectra

In the UV region, the complex presented in Fig.1, bands around 273nm and 300 nm which can be attributed to π→π* transition of the coordinated phenanthroline ligand, and the complexes **1**and**2**exhibits d-d band at UV-Visible spectra. The complexes are in good agreement with the previously reported square pyramidal geometry of the complexes[29-31].

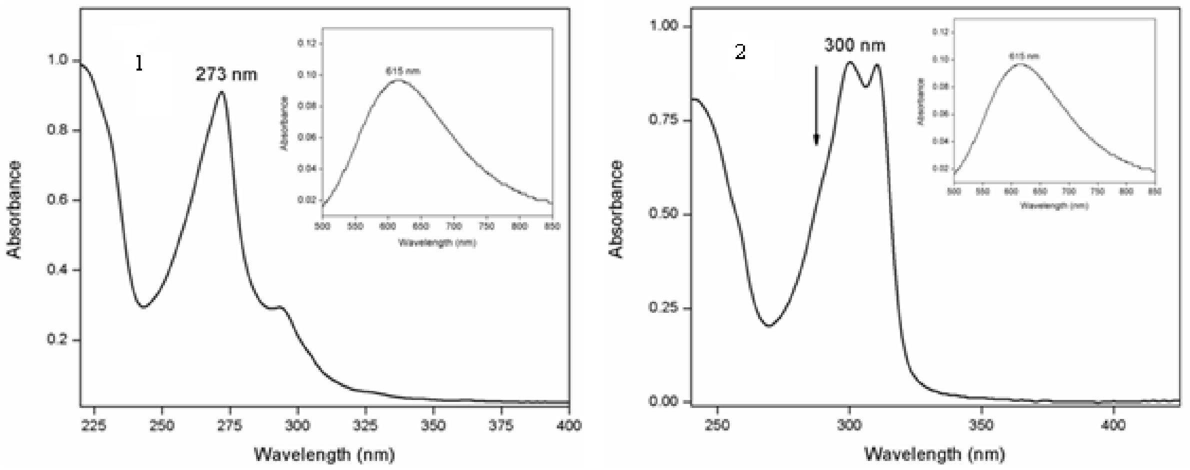


Fig.1: UV-Visible spectra of complexes 1 and 2

3.3 Infrared spectra

In the IR region, for complexes, the band around 3419 cm^{-1} (1) and 3421cm^{-1}(2) can be assigned to γ (N-H) stretching frequency of amino acid. The coordination of nitrogen atoms of heterocyclic base with copper metal ion can be examined by δ(C-H) for phenanthroline 853 cm^{-1} and 737 cm^{-1} is shifted to 825 cm^{-1} and 777 cm^{-1} and the band around 1383 cm^{-1} (1) and 1384 cm^{-1} (**2**) has been assigned for γ(N-O) of nitrate ion (Fig.2).

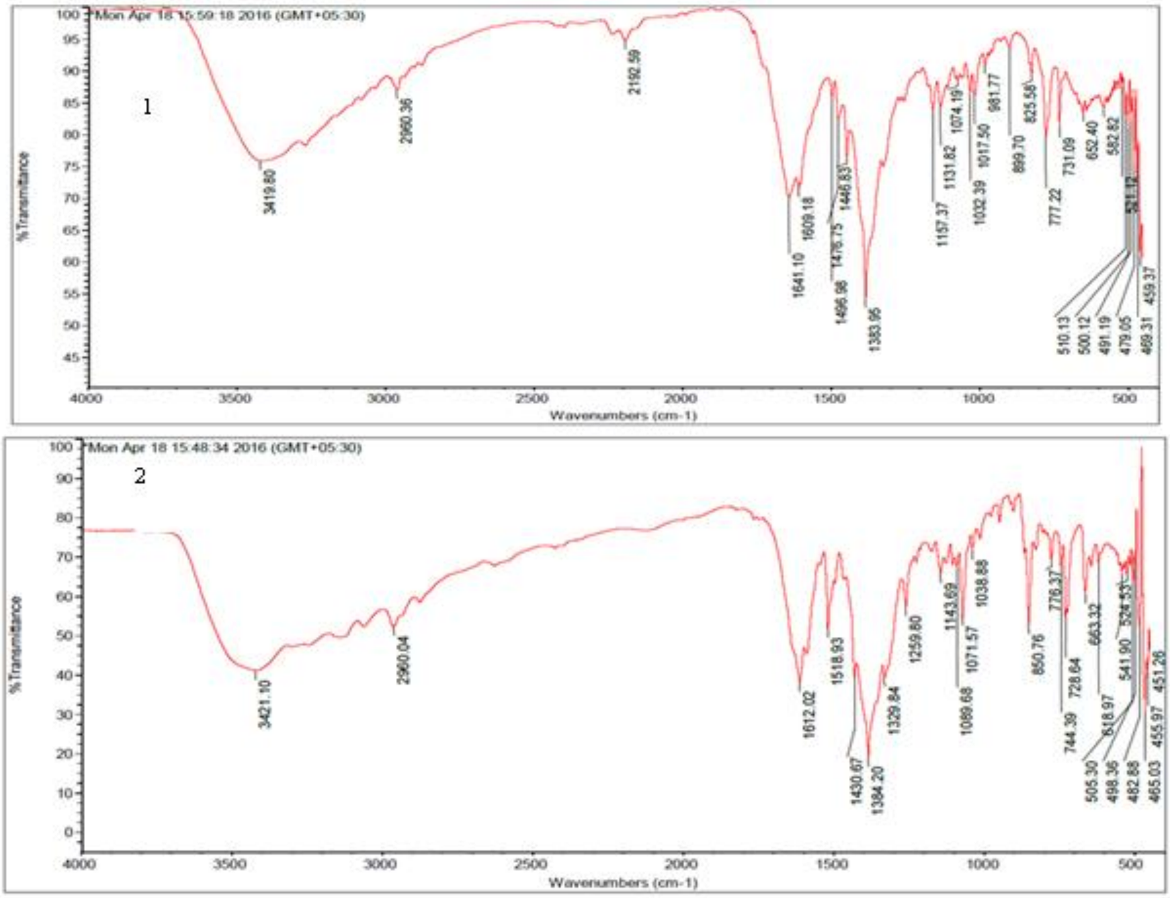


Fig.2: Infrared spectra of complexes 1 and 2

3.4 Electron Paramagnetic Resonance

The solid state EPR spectra of the copper (II) complexes were recorded in X-band frequencies (Fig .3). At room temperature, complexes 1and 2 exhibits well defined single isotropic lines. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines. This intermolecular type of spin exchange is caused by the strong spin coupling which occurs during coupling of two paramagnetic species. EPR spectra of mononuclear complexes copper(II) species with S=1/2, those with two signals ($g_\perp$and $g_\parallel$), on comparing these two signals $g_\perp(x,y) > g_\parallel(z)$ ($B_\perp(x,y) < B_\parallel(z)$) representing the elongated axial symmetry of the spin tensor.

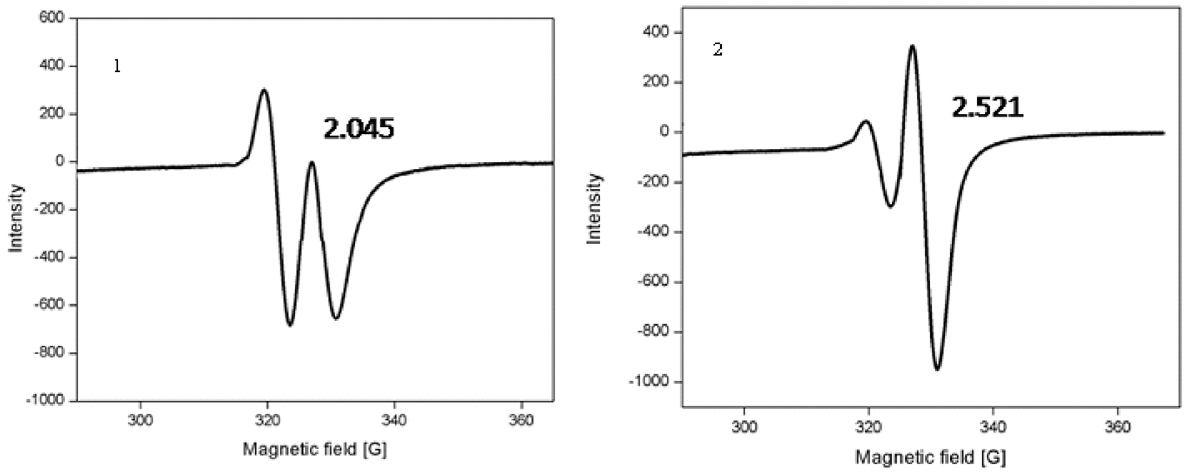


Fig. 3: EPR spectra of complexes 1 and 2

4. DNA binding studies

4.1 Electronic spectral studies

DNA Binding. Preparation of a CT DNA Soln. A conc. CT DNA stock soln. was prepared in 5 mm Tris ·HCl/50 mm NaCl in H2O at pH 7.5, and the concentration of the DNA soln. was determined by UV absorbance at 260 nm. The molar absorption coefficient was taken as 6600 m^{-1} cm^{-1}[32]. The absorption titration was carried out keeping the concentration of complex constant (15 µM) and varying the concentration of DNA from 50 to 150 µM. The absorbance was recorded after each addition of DNA. The intrinsic binding constant *K*b was determined according to the following equation:

[DNA] / (εa–εf) = [DNA] / (εb–εf) + 1 / *K*b (εb–εf) (1)where, εa, εb, and εf correspond to A_{obs}/[Cu], the extinction coefficient for the free copper(II) complex and the extinction coefficient for the copper(II) complex in the fully bound form, respectively. A plot of [DNA] / (εb–εf) versus [DNA] gives *K*b, as the ratio of the slope to intercept.

The binding of ternary copper(II) complexes 1 and 8 to the calf thymus (CT) DNA has been studied by electronic absorption spectrum. The absorption spectral traces of the complex 2 with increasing concentration of CT DNA are shown in Fig. 4. We have observed minor bathochromic shift with significant hypochromicity for complex 2. We observed that the bipyridyl complex shows weak binding to the DNA due to less extended planarity compared to complex which is consistent with the observed trend in hypochromism. Kb value of 1 and

2 is 6.24 X 10^4 $M^{-1}$and 4.55 X 10^4 M^{-1} respectively. The higher binding propensity of the phen complex in comparison to its bpy analog could be due to the presence of the extended planar aromatic ringin phen. Earlier studies on bis-phen copper complex have shown that this complex binds to DNA either by partial intercalation or binding of one phen ligand to the minor groove while the other phen making favorable contacts within the groove[33–36]. The nature of binding of the phen complex is proposed to be similar as observed for the bis-phen species.

4.2 Fluorescent spectral studies

As the copper (II) complexes are non-emissive, competitive binding studies with EthBr were carried out to gain support for the mode of binding of the complexes with DNA. The study involves the addition of the complexes to DNA pretreated with EthBr ([DNA]/ [EthBr] = 1) and then the measurement of the intensity of emission. The observed enhancement in emission intensity of EthBr bound to DNA is due to intercalation of the fluorophore in between the base pairs of DNA and stabilization of its excited state (Fig. 5)[37]. Addition of all the complexes to CT-DNA incubated with EthBr decreases the DNA induced enhancement in emission to the same extent. This suggests that the complexes displace DNA-bound EthBr and bind to DNA at the intercalation sites with almost the same affinity, which is consistent with the above spectral results suggesting partial intercalation of the phenanthroline ring.

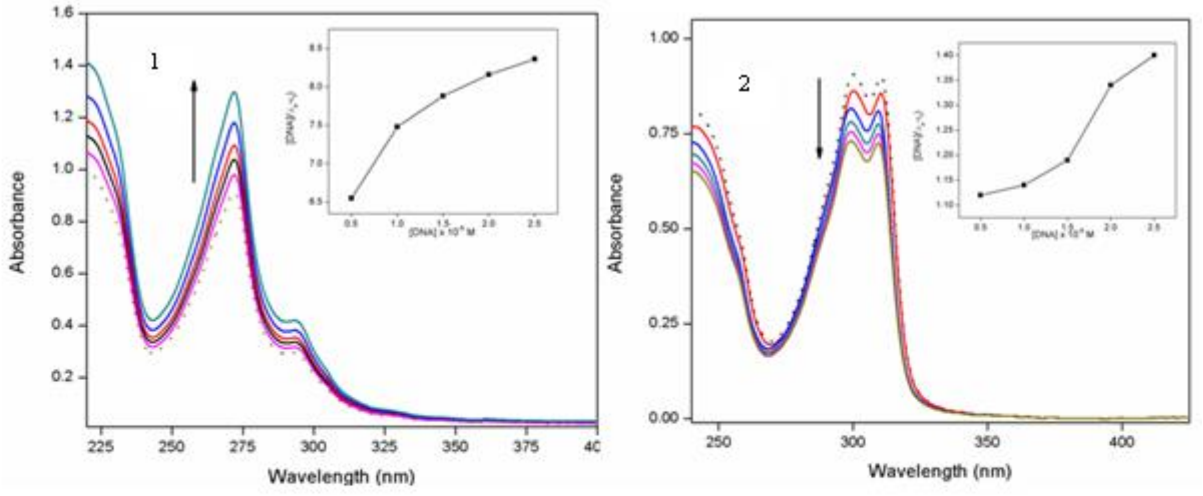


Fig.4: Absorption spectral traces on the addition of CT DNA to complexes 1and 2

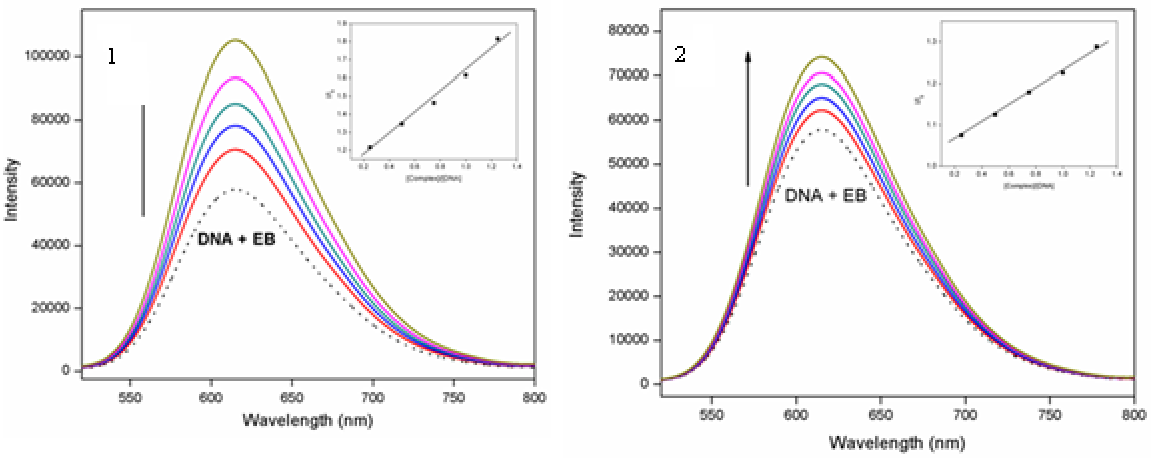


Fig. 5: Emission spectra of EB bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes **1** and **2**. The arrow shows the intensity changes upon increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.

4.3 Viscosity measurements

To further explore the binding mode of the copper(II) complex with DNA, viscosity measurements were carried out. Since the relative specific viscosity ($\eta/\ \eta_0$) (η and η_0 are the specific viscosities of DNA in the presence and absence of the complex, respectively) of DNA reflects the increase in contour length associated with separation of DNA base pairs caused by intercalation, a classical intercalator such as ethidium bromide could cause a significant increase in viscosity of DNA solutions. In contrast, a partial and/or non-classical intercalation of the ligand could bend or kink DNA, resulting in a decrease in its effective length with a concomitant increase in its viscosity[38,39], while the electrostatic and groove binding cause little or no effect on the relative viscosity of DNA solutions. Therefore viscosity measurements, which are sensitive to the changes in the contour length of DNA, are useful to probe for DNA intercalation by complexes.

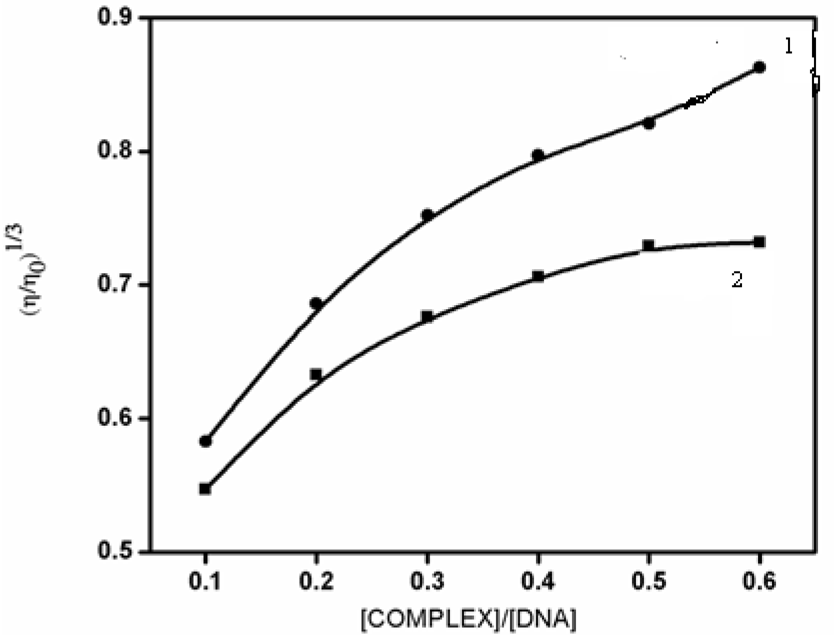


Fig.6: Effect of increasing amounts of complexes on the relative viscosities of CT DNA at 25^{0}C.

The plots of relative specific viscosities versus 1/R = ([Complex]/[DNA]) are shown in Fig.6. The relative specific viscosity increases with increasing concentration of the complex. However, the increase in the viscosity was much less compared to that of classical intercalators like ethidium bromide in the same DNA concentration range[40]. This observation supports the above spectral studies which suggest that the complex **1** intercalates with the DNA base pairs and complex **2**involve through groove binding. Intercalation results in lengthening of the DNA helix due to base pairs being separated to accommodate the binding ligand, leading to an increase in viscosity of the solution.

4.4 Cyclic voltammetry studies

The cyclic voltammetric (CV) response for complexes **1** and **2** in Tris–HCl buffer (pH 7.28) in the presence and absence of CT DNA is shown in Fig.7. In the forward scan, a single cathodic and anodic peak was observed, which corresponds to the reduction and oxidation of complexes, which indicates that the process is reversible. When CT-DNA is added to a solution of complexes, marked decrease in the peak current and potential values were observed. The cyclic voltammetric behavior was not affected by the addition of very large excess of DNA, indicating that the decrease of the peak current of complexes after the addition of DNA due to the binding of complex to the DNA[41]. When the concentration of

DNA increased, the changes in peak current and potential become slow. This reveals that the complexes interacted with Calf thymus - DNA.

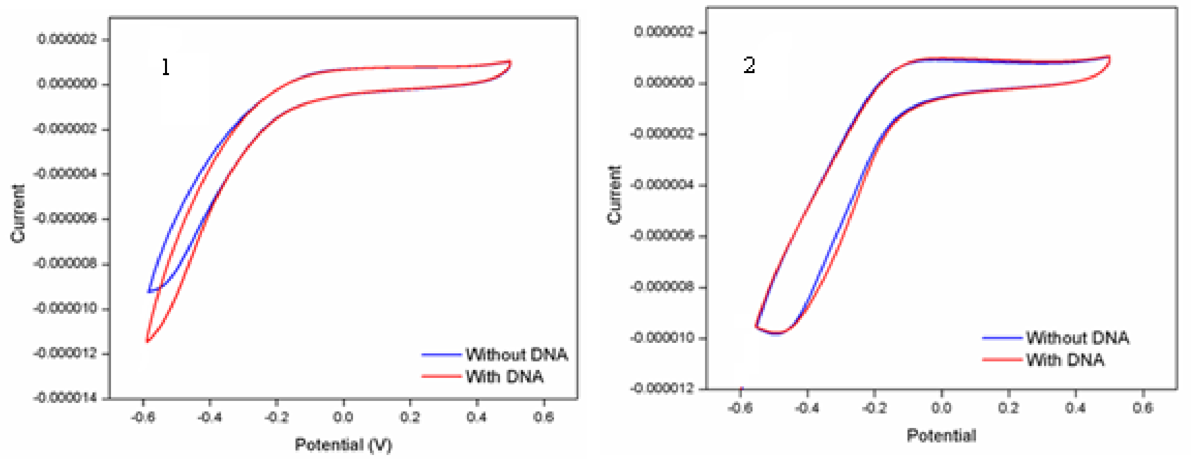


Fig. 7: Cyclic voltammogram of complexes 1 and 2in the absence (dashed line) and presence (dotted line) CT DNA.

4.5 Antibacterial and antifungal activity

Table. 1: Antimicrobial activity of complexes 1 and 2.

S. No	Name of microorganism	Complex 7	Complex 8	Disc (Ciprofloxacin/Amphotericin - B)
Antibacterial activity				
1	*Staphylococcus epidermidis*	18	24	14
2	*Streptococcus fecalis*	20	33	20
3	*Bacillus subtilis*	22	35	15
4	*Klebsiellapneumoniae*	23	29	20
5	*Escherichia coli*	24	21	21
6	*Proteus vulgaris*	20	34	22
Antifungal activity				
1	*Aspergillus niger*	8	11	12
2	*Aspergillus flavus*	7	10	9
3	*Candida tropicalis*	9	7	10
4	*Aspergillus fumigatus*	6	8	8
5	*Aspergillus terreus*	12	13	11
6	*Candida albicans*	10	11	10

The copper (II) complexes were screened *in-vitro* for its microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. The complex was found to exhibit considerable activity against bacteria and the fungus. The test solutions were prepared in double distilled water and the results of the antimicrobial activities are summarized in Table.1. Zoroddu et al., 1996[42] have reported that the copper complex show any significant activity against the Gram-positive and Gram-negative bacteria. Recently Patel et al[43], 2005, have indicated that the copper (II) complex with L-phenylalanine has exhibited considerable activity against some human pathogens. In our biological experiments, using copper(II) complexes, we have observed good antibacterial and antifungal activity (Fig.8). Particularly, complex 1 has very high biological activity against Staphylococcus epidermidis. Complex 1 and 2 have good antifungal activity against Aspergillus fumigatus. It may be concluded that our copper (II) complexes inhibit the growth of bacteria and fungi to a greater extent.

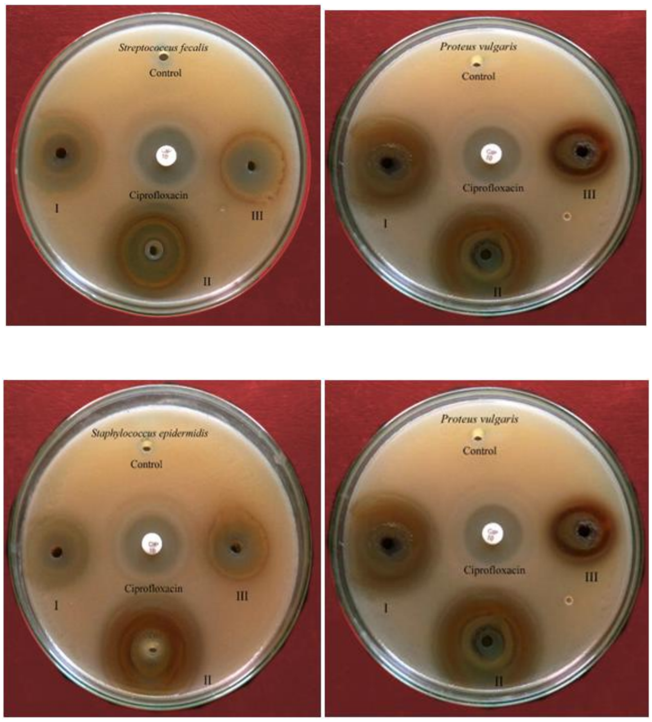


Fig. 8: Antimicrobial activity of complexes 1 and 2.

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