DNA Binding/Cleavage Studies of Copper(II) Complexes Containing Heterocyclic Bases, L-Serine and Semicarbazide

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Abstract

*Two new copper(II) complexes, [Cu(phen)(L-Ser)(Sc)]NO_3 (**1**), [Cu(bpy)(L-Ser)(Sc)]NO_3 (**2**) [L-ser = L-serine, Sc = semicarbazide, phen = 1,10-phenanthroline, bpy = 2,2'-bipyridyl), were prepared and fully characterized using elemental analyses, electronic, FT-IR and EPR spectrum. The complexes (**1** & **2**) bind to calf-thymus DNA (CTDNA) revealed by UV-vis spectral, thermal denaturation, fluorescence spectral, viscosity and cyclic voltammetry methods. The obtained results indicated that binding activities of complex **1** to DNA was intercalatative mode and complex **2** to DNA was partial intercalative mode, also complexes **1** and **2** binding with supercoiled pBR322 DNA was observed.*

Keywords: *DNA binding, copper(II) complex, 1,10-phenanthroline, L-serine*

Introduction

DNA plays a vital role in the human life since it contains full genetic information for cellular function. However, DNA molecules are damaged under various conditions like interactions with some other molecules. This damage may cause various pathological changes in living organisms. The binding interaction of small molecules with DNA is of interest for both therapeutic and scientific reasons. The DNA binding of transition metal complexes have been paid much attention due to the importance in understanding the DNA-drug interactions and in designing new potential anticancer agents targeted to DNA [1]. Specially, transition metal complexes with DNA have been widely exploited for these applications due to their characteristic spectral behavior also, complexes which easily involve redox and ligand substitution reaction. Furthermore, the significant reason for this study is that by changing the ligand environment, can tune the DNA binding and cleaving ability of a metal complexes [2]. Copper complexes containing 1,10-phenanthrolineand its derivatives are of much interest due to their good biological activities. The copper(II) complexes with mixed ligand have been found to exhibit potential anticancer agents by inducing apoptosis and strongly bind and cleave DNA[3-5]. Reedijk and co-workers have found that pyrimol copper(II) complex brings about efficient DNA cleavage and cytotoxic effects [6]. Sadler and coworkers have observed [7] bis(salicylato)copper(II) complexes exhibit cytotoxic and antiviral activities.

Copper complexes containing amino acids have been studied as models for the behavior of copper enzymes [8] and some copper complexes with amino acid were reported to exhibit potent anti-tumor and nuclease activities [9–11]. However, molecular assemblies possessing a helical structure formed by amino acids have not been reported. On the other hand, it has been documented that amino acids and their first-row transition metal complexes exhibit fungicidal, bactericidal, antiviral, and

antitubercular activity [12–14]. Copper is one of the first-row transition metals and a bioessential element with relevant oxidation states, and its amino acid complexes have received much attention, specially aminoacid containing complexes having very good binding activity with CT-DNA, also potentially cleave pBR322 DNA. [15-18]. Already our group synthesized some of copper complexes and discussed anticancer and DFT properties [19-21]. In this communication, we report the synthesis and characterization of new copper (II) complexes containing heterocyclic bases, amino acid and semicarbazide as ligands, DNA binding/cleavage and cytotoxicity also carried out for these complexes.

Experimental

Materials and methods

Calf thymus DNA was obtained from Sigma–Aldrich, Germany, and was used as such. Copper(II) chloride dihydrate, 1,10-phenanthroline, L-serine and semicarbazide were purchased from Merck, Plasmid pBR322 DNA was purchased from Genei, India. The DNA binding titration was carried out in the buffer (50 mM NaCl–5 mM Tris–HCl, pH 7.1) at room temperature. Absorption spectra were recorded on a UV/VIS Shimadzu 2450 Spectrophotometer using cuvettes of 1-cm path length. FT-IR spectra were recorded on a 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). Emission spectra were recorded on a JY Fluorolog-3-11 spectrofluorometer. Cyclic voltammetry experiments were recorded on CHI 602D (CH Instruments Co., USA) electrochemical analyzer under oxygen free conditions using a three-electrode cell in aqueous solution with KCl (0.1 M) as the supporting electrolyte. A pt wire, glassy carbon, and the Ag/AgCl (in saturated KCl solution) electrodes were used as counter, working and reference.

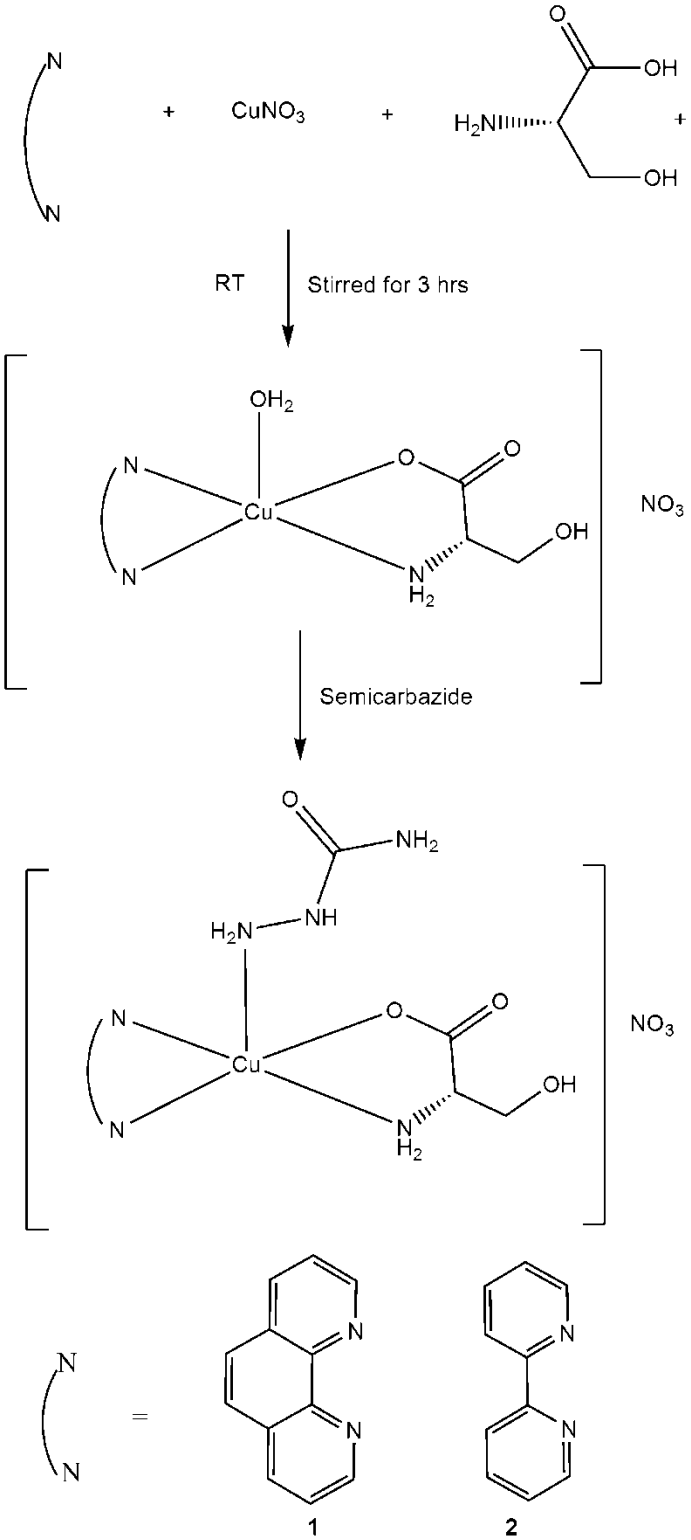
Preparation of [Cu(phen)(L-Ser)(Sc)]NO_3

Complex [Cu(phen)(L-Ser)(H_2O)]NO_3 was prepared in 72% yield from a reaction of copper nitrate (1 mmol) in water with a solution of phenanthroline (1 mmol) in methanol and L-Serine (1 mmol) in NaOH solution. The reaction mixture was stirred for 5 hrs at room temperature. After the slow evaporation blue colored [Cu(phen)(L-Ser)(H_2O)]NO_3 was obtained. The aqueous solution of complex (1.0 mmol) is treated with semicarbazide and stirred for 4 hrs, the brown color solution was filtered after the solvent evaporation, the brown colored complex **1** was washed with methanol and ether. Yield 61%; Anal. (%) Calc. for $C_{16}H_{19}CuN_7O_7$: C, 39.63; H, 3.95; N, 20.49. Found: C, 38.63; H, 3.79; N, 20.20. IR (KBr pellet): 3385, 3385, 3217, 3057, 1739, 1622, 1606, 1585, 1365, 1346, 1321, 1226, 1201, 1145, 852, 719 cm^{-1}. UV-Vis (λ, nm): 271 and 607 nm.

Preparation of [Cu(bpy)(L-Ser)(Sc)]NO_3

The preparation of complex **2** was described as above. Yield 62%; Anal. (%) Calc. for $C_{14}H_{19}CuN_7O_7$: C, 36.48; H, 4.16; N, 21.27. Found: C, 35.20; H, 4.02; N, 20.88. IR (KBr pellet): 3390, 3290, 3197, 3028, 2337, 1739, 1724, 1598, 1367, 1352, 1319, 1215, 1047, 852, 717 cm^{-1}. UV-Vis (λ, nm): 300 and 621 nm.

DNA binding, cleavage and anticancer experiment details are given in literature [22].



Scheme: Preparation of complexes **1** and **2**.

Results and Discussion

Synthesis and general aspects

The ternary copper(II) complexes (**1** & **2**) was synthesized (Scheme 1) from the complex $[Cu(phen)(L\text{-}Ser)(H_2O)]NO_3$ by ligand substitution method. The copper(II) complexes are soluble in water and most of the organic solvents. The complexes have one electron paramagnetic behavior at room temperature due to d^9 electronic configuration for the copper(II) center. The elemental analysis data confirms that the metal-ligand ratio is 1:1:1 in complexes, which is consistent with the obtained elemental analysis. In the UV–Vis region, the intense absorption bands appeared at 271 nm for complex **1** and 300 nm for complex **2**, both intense band due to intraligand charge transfer. The d-d transitions band centered at 607 and 621 nm for complexes **1** and **2** respectively, indicating a square-pyramidal geometry in the metal center. In infrared spectra, asymmetric and symmetric COO- stretching vibrations for complex **1** was observed at 1620 and 1319 cm^{-1}, the differences indicate that carboxylate ion coordinate with copper(II) by monodentate fashion. The solid state EPR spectra of the copper (II) complexes were recorded in X-band frequencies. At room temperature the complexes exhibit well defined single isotropic feature g = 2.15 for complexes **1** and **2** respectively. Such isotropic lines are usually the results of intermolecular spin exchange, which broaden the lines.

DNA binding properties

The binding of the complexes to calf thymus DNA has been studied by different spectroscopic and hydrodynamic techniques like absorption, emission, DNA thermal denaturation and viscosity measurements.

Electronic spectral studies

The absorption spectral technique is used to determine the intrinsic binding constants (K_b) of the complexes along with the binding site size (s) to CT DNA by monitoring the absorption intensity of the charge transfer spectral band of the complexes near 300 nm with increasing concentration of CT DNA keeping the complex concentration as a constant. Complex **1** binds with DNA through intercalation, shows hyperchromism and a red shift of the absorption band due to strong stacking interactions between the aromatic chromophore of the complex **1** and the DNA base pairs[23]. Complex 2 binds with DNA through electrostatic interaction, shows hypochromism and a red shift of the absorption band (Fig 1). The binding constant (K_b) values are 11.3 X 10^4 mol^{-1}, 2.437 X 10^4 mol^{-1} for complexes **1** and **2** respectively. The phen complex **1** shows comparatively higher binding propensity to CT DNA possibly due to the extended planar and π-conjugation.

To investigate the interaction of the complexes with CT-DNA, thermal denaturation study carried out. On increasing temperature of the CT-DNA solution, the complex **1** exhibits a hyperchromic effect on absorption spectra of DNA bases at 262 nm (λ_{max}). Generally, the intercalative mode of binding with DNA increases the DNA melting temperature (T_m) [24]. The T_m has been found to increase in presence of complex **1** suggesting an intercalative mode of binding. The binding of the complex **2** slightly decrease in melting temperature (T_m) of CT-DNA suggesting electrostatic binding nature of the complex (Fig. 2).

Fluorescent spectral studies

Ethidium bromide (EB) emits fluorescence light in the presence of DNA due to strong intercalation, and its fluorescence behavior quenched by addition of a second, competitive molecule. In

Fig. 3, the emission spectra of EB bound to DNA in the absence and presence of complexes **1** and **2** are shown, the addition of the complexes to DNA with EB produced significant decrease in emission intensity, indicating that the complexes bound with EB in DNA binding[26].

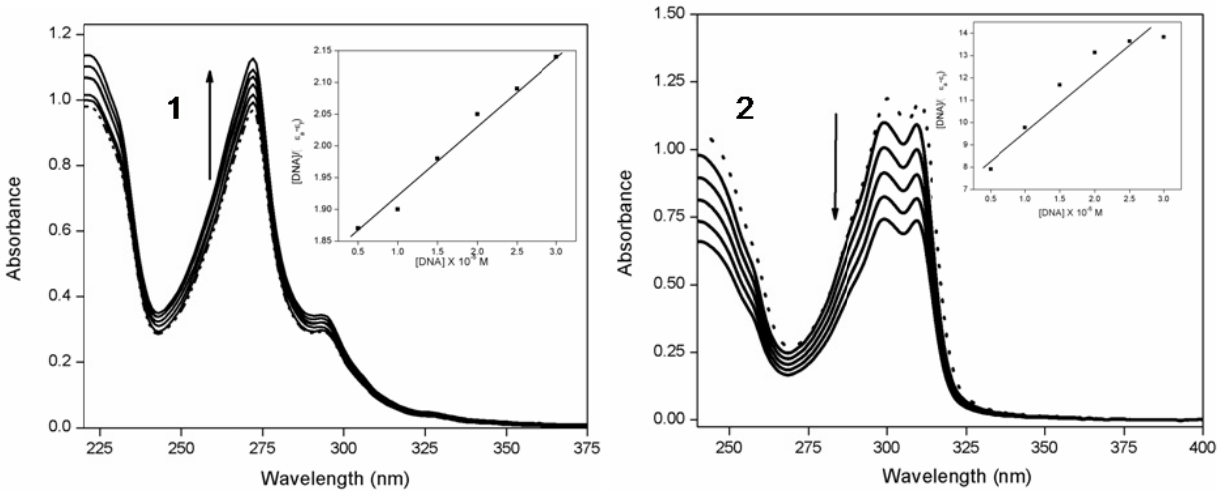


Fig. 1: Absorption spectral traces on addition of CT DNA to complexes **1** and **2** (shown by arrow). Inset plot of [DNA]/(ε_a-ε_f) vs [DNA] for absorption titration of CT DNA with complex at 271 nm.

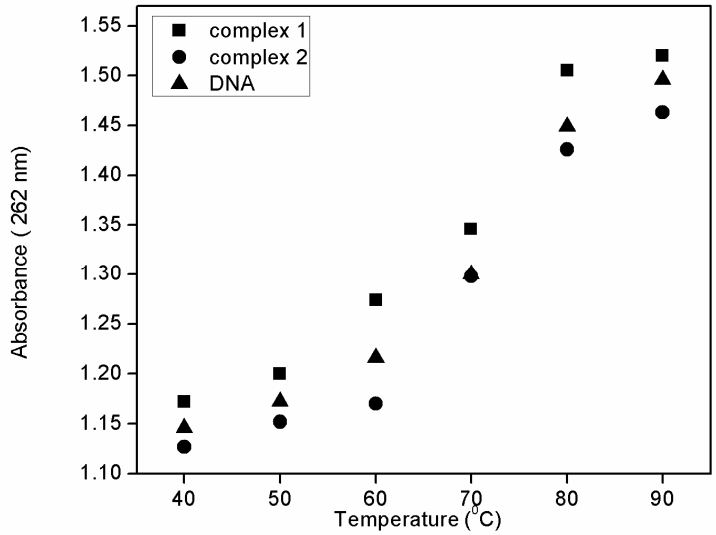


Fig. 2: Thermal denaturation profiles of CT DNA (140 μM) alone and in the presence of complexes **1** and **2** (40 μM) in 5 mM phosphate buffer (pH 6.85)

The quenching plots (inset in Fig. 3) demonstrate that the quenching of the EB fluorescence by complexes **1** and **2** is in good agreement with the linear Stern –Volmer relationship, which confirms that the two complexes (**1** & **2**) bind to DNA. In the plot of I_0/I vs. [complex]/[DNA], K_{sv} is given by the ratio of the slope to the intercept. From these data, K_{sv} values of 0.456 and 0.277 were determined for **1** and **2**, respectively.

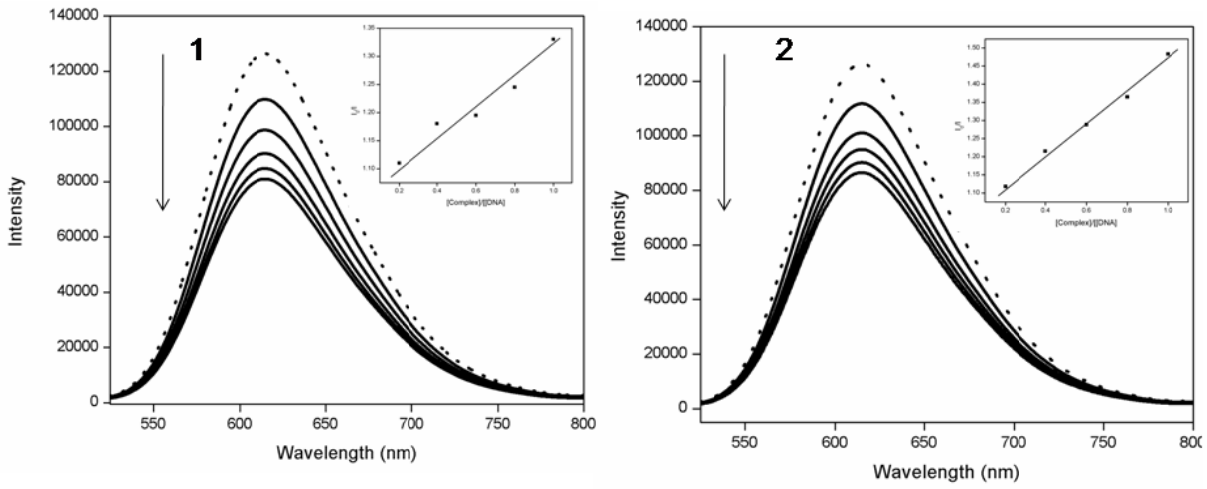


Fig. 3: Emission spectra of EB bound to DNA in the absence (dotted line) and the presence (dashed line) of complexes **1** and **2**. Arrow (↓) shows the intensity changes upon increasing the concentration of the complex. Inset: Stern–Volmer quenching curves.

Viscosity measurements

We have done viscosity measurements to gain better insights about the mode of binding of the complexes to DNA. Intercalation of a species between DNA base pairs causes a significant increase in the viscosity of the DNA solution due to an increase in the separation of the base pairs in order to accommodate the bound species [25]. In contrast, groove binding or partial intercalation only leads to a minor change in the viscosity[26]. A plot of the relative specific viscosity $(\eta/\eta_0)^{1/3}$ *vs.* [complex]/[DNA] ratio shows a significant change in the viscosity (Fig. 4) of DNA bound to the complex **1** and minor changes for the complex **2**. The results indicate a intercalative binding nature of the complex **1** and a primarily DNA groove binding propensity for the complex **2**.

Cyclic voltammetry studies

The application of CV to study binding of metal complexes to DNA is a useful complement. The typical cyclic voltammogram of complexes **1** and **2** in the absence and presence of varying amount of [DNA] is shown in Fig. 5. The cathode and anode peak currents changed in the presence of DNA. The change in current may be attributed to molecules bound to DNA. In the absence of DNA, the cathodic peak appears at 0.132V and anodic peak appears at -0.233V, the separation of the anodic and cathodic peak potential (ΔE_p) is 0.365V for complex **1,** for complex **2** the cathodic and anodic peak appears at 0.143V and -0.244V respectively, and the ΔE_p is 0.287V. The redox couples ratio of Ipc/Ipa is approximately unity, indicating that the reaction of the complexes on the working electrode surface is quasireversible. In the addition of DNA to the complex **1**, the considerable changes with redox couple causes a negative shift in $E_{1/2}$ (-0.010V) and complex **2** redox couple shift in $E_{1/2}$ (-0.087). The electrochemical potential shift after reacting with DNA is suggested that complexes interact with DNA.

DNA cleavage

DNA cleavage is also a useful method to probe DNA-complex interactions[31]. Supercoiled plasmid DNA cleavage by the Cu(II) complexes into nicked DNA (Form II) and linear DNA (Form III) was studied in the presence of gallic acid (Fig. 6). We found that the supercoiled DNA was completely

cleaved by complex **1** in the presence of gallic acid when increase ratio of complex/gallic acid, but complex **2** didn't cleave significantly compared to complex **1**. This result revealed that complex **1** was a potent DNA cleavage agent in the presence of GA as a reducing agent.

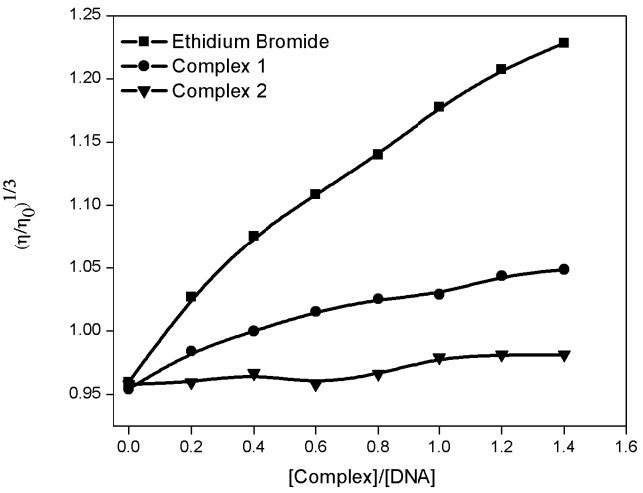


Fig. 4: Effect of increasing amounts of complexes on the relative viscosities of CT DNA at 25^0C.

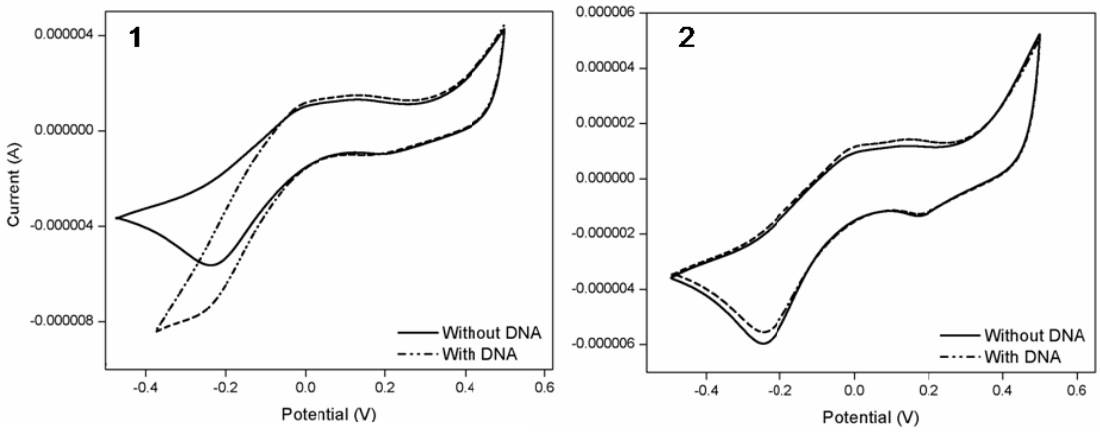


Fig. 5: Cyclic voltammogram of complexes (**1** and **2)** in the absence (dashed line) and presence (dotted line) CT DNA.

Antibacterial and antifungal activity

The copper (II) complexes were screened in vitro for its microbial activity against certain pathogenic bacterial and fungal species using disc diffusion method. The complexes were found to exhibit considerable activity against bacteria and the fungus. Our group recently, reported that aminoacid containing complexes have good antimicrobial activity[27]. Zoroddu et al[28] reported that copper complexes show considerable activity against the bacteria. Recently Patel et al[29] have indicated that the copper(II) complexes with L-phenylalanine has exhibited considerable activity against some human pathogens. In our biological experiments, using copper (II) complexes[30], we have observed antibacterial activity antifungal activity.

The complex **1** and **2** have shown high antibacterial activity (Table 1) against *Staphylocuccus aureus* and complex **1** has shown high antifungal activity against *Aspergillus fumicatus*. It may be concluded that our complexes **1** and **2** inhibit the growth of bacteria and fungi to a greater extent.

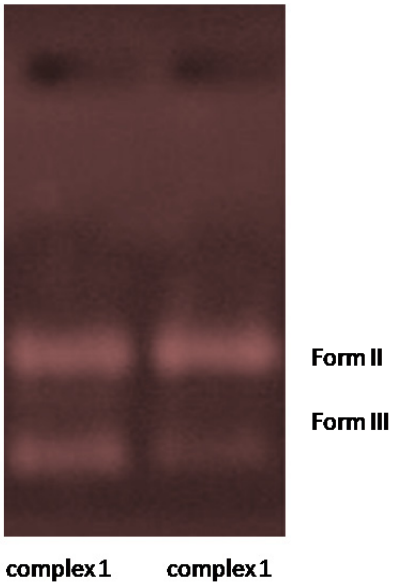


Fig. 6: Cleavage of supercoiled pBR322 DNA by complexes **1** and **2** at different concentrations in the presence of gallic acid.DNA + complex (60μM) + Gallic acid (1mM);

Table 1. Antimicrobial activity of copper(II) complexes

S.No	Micro Organisms	Complex 1	Complex 2	Copper Nitrate	Ciproflaxacin
		Zone of Inhibition (mm)			
Bacteria					
1	*Escherichia coli*	22	30	12	18
2	*Enterococcus faecalis*	29	32	18	30
3	*Staphylocuccus aureus*	35	39	33	34
Fungi					
S.No	**Micro Organisms**	**Complex 1**	**Complex 2**	**Copper Nitrate**	**Amphotericin-B**
		Zone of Inhibition (mm)			
5	*Aspergillus fumicatus*	19	20	15	12
6	*Mucor sps*	18	14	11	15

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