

Studies on DNA Binding and Biological Activities of Copper (II) Complexes

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ABSTRACT

The two Cu (II) complexes $[\text{Cu}(\text{L}^1)_2]\text{Cl}_2$ (**1**) and $[\text{Cu}(\text{L}^2)_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**2**) (where $\text{L}^1 = 1\text{-amidino-}O\text{-methylurea}$; $\text{L}^2 = 1\text{-amidino-}O\text{-}i\text{-propylurea}$) have been synthesized and characterized. The DNA binding properties of these complexes have been investigated under physiological conditions (pH 7.2, $25 \pm 0.2^\circ\text{C}$) by absorption spectroscopy, cyclic voltammetry and viscosity measurements. The results indicate that the complexes bind with CT DNA by non-intercalative mode with intrinsic binding constant in the order of 10^4M^{-1} . Further, the complexes have been screened for their antimicrobial activities against *Escherichia coli*, *Klebsiella pneumoniae* sub *sp. pneumoniae* and *Proteus mirabilis* bacteria.

Keywords: Cu (II) complex, CT DNA, non-intercalative, antimicrobial activities.

INTRODUCTION

The study of DNA interaction with transition metal complexes becomes increasingly important as it can elucidate how genetic information is expressed. Such study has been motivated not only by a desire to understand the basics of these interaction modes but also by the development of metal complexes into anti-inflammatory, antifungal, antibacterial or anti-cancer agents¹. Besides, copper is a bio-essential element in all living systems. Because of its biological activity and compatibility at normal concentrations, copper has been used in a number of medications throughout the history of present day man². The ligand (1-amidino-*O*-alkylurea) is a nitrogen donor system having many hydrogen bonding sites, which may give the opportunity to form hydrogen bonding with the DNA bases. Numerous research groups have reported the biological importance of the hydrogen bonding interactions, their ability to interact with DNA bases and even showing the antimicrobial properties against several pathogenic microbes³⁻⁷. In view of the above respect, in the present work, $[\text{Cu}(\text{L}^1)_2]\text{Cl}_2$ (**1**) and $[\text{Cu}(\text{L}^2)_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**2**) complexes (where $\text{L}^1 = 1\text{-amidino-}O\text{-methylurea}$; $\text{L}^2 = 1\text{-amidino-}O\text{-}i\text{-propylurea}$), have been studied for their DNA binding properties by electronic absorption spectroscopy, cyclic voltammetry and

viscosity measurements. Further, the antimicrobial activities of these complexes have been screened using *Escherichia coli*, *Klebsiella pneumoniae* sub *sp. pneumoniae* and *Proteus mirabilis* bacteria.

EXPERIMENTAL

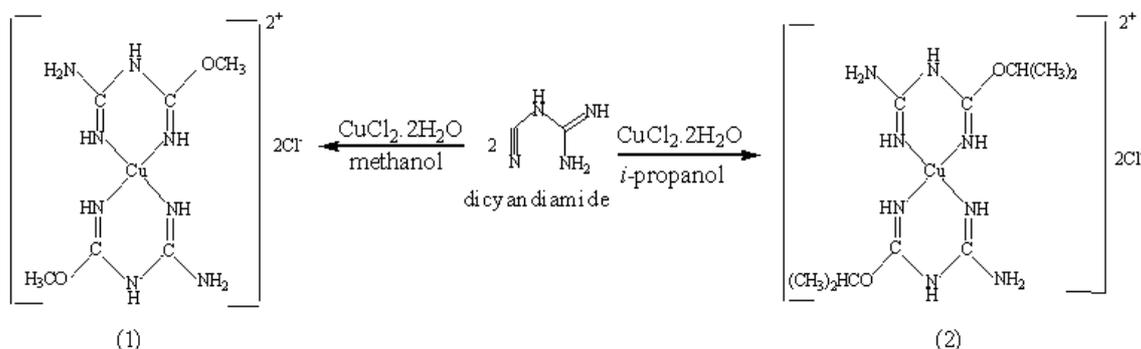
All the chemicals and reagents used were of analytical grade and used without further purification. $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, dicyandiamide and ethidium bromide (EB) (Merck), Calf thymus CT DNA fibrous type (Calbiochem) were used. Organic solvent used was absolute alcohol.

Carbon, hydrogen and nitrogen were determined by using a Perkin-Elmer-2400 Series II, CHNS/O elemental analyzer. Infrared spectra were obtained on a Shimadzu-8400S, FTIR spectrometer in wavelength region $4000\text{-}400\text{ cm}^{-1}$. The spectra were recorded as KBr pellets. UV-Vis spectra were obtained on a Shimadzu 2450 UV-Vis spectrophotometer in wavelength region 800-200 nm. Cyclic voltammetric measurements were performed on a CH602C Electrochemical analyzer.

The complexes $[\text{Cu}(\text{L}^1)_2]\text{Cl}_2$ (**1**) and $[\text{Cu}(\text{L}^2)_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (**2**) were synthesized by refluxing cupric chloride dihydrate and dicyandiamide in a 1:2 stoichiometric ratio with the appropriate alcohols for 1-3hr, as previously reported⁸. The schematic diagram

for the synthesis of complexes is shown in (Scheme 1). The amount of copper was determined by decomposing the complexes

with a mixture of HNO_3 and H_2SO_4 ; and finally by performing an iodometric titration.



Scheme 1: Schematic diagram for the synthesis of complexes 1 and 2

All experiments involving CT DNA were performed in Tris buffer solution (50 mM NaCl/5mM Tris-HCl, pH 7.2) at $25 \pm 0.2^\circ\text{C}$. Double distilled water was used to prepare the buffer solution. The concentration of CT DNA was determined from the intensity of absorbance at 260 nm with a known extinction coefficient value ($\epsilon_{260} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$)⁹. The ratio of the absorbance of CT DNA at 260 nm and 280 nm was found as 1.9. Therefore, no further purification was attempted¹⁰.

Absorption titration measurements were carried out by varying the concentration of CT DNA from 0 to $45 \times 10^{-6} \text{ M}$, while keeping the metal complex concentrations constant at $25 \times 10^{-6} \text{ M}$. Samples were incubated at $25 \pm 0.2^\circ\text{C}$ for 24 hr before recording each spectrum. The intrinsic binding constant (K_b) for the interaction of the two complexes with CT DNA was determined using the following equation¹¹.

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

where [DNA] is the concentration of CT DNA, the apparent absorption coefficients ϵ_a , ϵ_f and ϵ_b correspond to $A_{\text{obsd}}/[\text{Cu}]$, the extinction coefficient for the free copper (II) complex and the extinction coefficient for the Cu (II) complex in the fully bound form, respectively. A plot of $[\text{DNA}] / (\epsilon_a - \epsilon_f)$ versus [DNA] gave a slope of $1/(\epsilon_b - \epsilon_f)$ and a Y-intercept equal to $1/K_b (\epsilon_b - \epsilon_f)$, K_b is the ratio of the slope to the Y-intercept.

Cyclic voltammetric measurements were performed on a CH602C Electrochemical analyzer in Tris buffer at $25 \pm 0.2^\circ\text{C}$, pH 7.2. A standard three electrode system comprising platinum electrode working electrode, platinum wire auxiliary electrode and an Ag/AgCl reference electrode were used.

Solutions were deoxygenated by purging with nitrogen gas for 20 minutes prior to the measurements.

Viscosity measurements were carried out by using Ubbelodhe viscometer maintained at a constant temperature of $25 \pm 0.2^\circ\text{C}$ in a thermostatic bath. The CT DNA concentration was kept constant ($0.5 \times 10^{-3} \text{ M}$) and the concentration of the copper (II) complexes were varied to give [complex]/[DNA] (r) ratios in the range of 0.00-0.157. Flow time was measured with a stopwatch. The experiment was carried out in triplicates and an average flow time was calculated. Viscosity values were calculated from the observed flow time of DNA-containing solutions corrected for the flow

time of the buffer alone (t_0): $\eta = (t - t_0) / t_0^{1.2}$. Data were presented as relative viscosity $(\eta/\eta_0)^{1/3}$ versus binding ratio (r) $[\text{DNA}]/[\text{sample}]$, where η is the viscosity of CT DNA in the presence of sample and η_0 is the viscosity of CT DNA alone.

The biological activities of the two complexes and parent salt $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ were carried out by standard filter paper disc diffusion method. Overnight grown bacteria (1 O.D.) were spread on nutrient agar and kept for about half an hour to allow the bacterial cells to rest. Filter discs (about 6 mm in diameter) were placed on the inoculated plates into which 0.01 cm^3 each of the test solutions (0.1 mg/ml, 1 mg/ml and 2 mg/ml) were loaded and sterile, kept for about 1hr to enable diffusion of the test solutions into the medium. They were incubated at $37 \pm 0.2^\circ\text{C}$. Inhibition of microbial growth was determined by measuring the diameter of the inhibition zone after 24-hr incubation. The antibacterial activities of the synthesized complexes and parent metal salt were compared with the reference antibiotic, gentamycin sulfate.

RESULTS AND DISCUSSION

The analytical data of the complexes are presented in Table 1. The IR spectra of ligand dicyandiamide show a strong nitrile $\nu_{\text{C}=\text{N}}$ band at 2165 cm^{-1} . In the IR spectra of the complexes there is the absence of a

band around 2165 cm^{-1} and also there is no C=O stretching band around 1740 cm^{-1} indicating that the complexing ligand is not substituted guanyurea. Instead the two copper complexes have a very strong ν_a (C–O–C) stretch at ca. $1218\text{--}1222 \text{ cm}^{-1}$ and ν_s (C–O–C) at ca. $955\text{--}985 \text{ cm}^{-1}$ to support 1-amidino-O-alkylurea^[13]. Further the IR spectra of dicyandiamide showed a band at around $1558\text{--}1570 \text{ cm}^{-1}$ for azomethine C=N stretching, this had a downward shift ($20\text{--}35 \text{ cm}^{-1}$) in all the two complexes showing that the azomethine nitrogen is coordinated to the metal ion. The increase in value of $\nu_{(\text{C}=\text{N})}$ to $167\text{--}1662 \text{ cm}^{-1}$ range in the two complexes is presumably due to change in the C=N bond order, or coordination through the nitrogen atom, facilitated by the transfer of electron density from the C–O–C moiety. The electron density on the N=C–O–C fragment of 1-amidino-O-alkylurea is delocalized in the complexes and the =C–O– bond order is raised¹³⁻¹⁵. The presence of new bands in the $472\text{--}480 \text{ cm}^{-1}$ range is due to (Cu–N) in the two complexes.

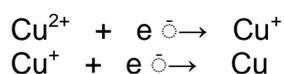
The UV-Vis spectra of the two complexes exhibited $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$ charge transfer bands at $44,444$ and $44,247 \text{ cm}^{-1}$, respectively. Another broad bands, observed at the range of $(18,869\text{--}18,519) \text{ cm}^{-1}$ were attributed to d-d transitions (${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$), typical for Cu (II) in square planar CuN_4 chromospheres¹⁶.

Table 1: Analytical data of the Cu (II) complexes

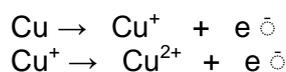
Complex	Color	Yield	Analytical found (%) (calculated) (%)			
			C	H	N	Cu
$[\text{Cu}(\text{L}^1)_2]\text{Cl}_2$ (1)	light pink	90	19.44 (19.65)	4.75 (4.39)	30.65 (30.56)	17.36 (17.32)
$[\text{Cu}(\text{L}^2)_2]\text{Cl}_2 \cdot \text{H}_2\text{O}$ (2)	Violet pink	82	26.20 (25.62)	6.07 (5.59)	24.96 (23.90)	13.70 (13.55)

L^1 = 1-amidino-O-methylurea; L^2 = 1-amidino-O-*i*-propylurea

Typical cyclic voltammetric behaviors of the two Cu (II) complexes ($0.5 \times 10^{-3} \text{ M}$) scan between 0.4 to -0.8 are shown in (Fig.1). In the cathodic scan two reduction peaks were observed, which are associated with the reduction of copper ions according to reaction:



In the reverse scans two prominent anodic peaks were observed at around -0.007 V and 0.15 V corresponding to oxidation of copper according to reaction:



The non-equivalent current intensities of the anodic and cathodic peaks as well as large separation between the anodic and cathodic peak potentials indicates the redox

processes are irreversible in nature¹⁷. The drastic decreased in the anodic peak currents from complex 1 to 2 might be due to the increase in bulkiness of the ligand.

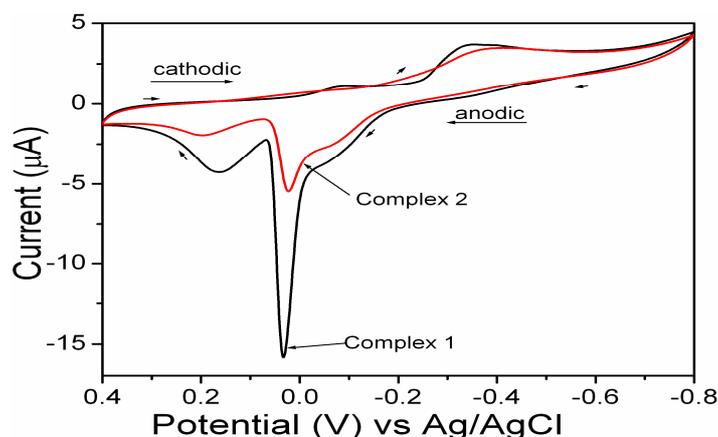


Fig. 1: Cyclic voltammograms of 0.5×10^{-3} M of the complex 1 and 2; Supporting electrolyte 50 mM NaCl/5 mM Tris-HCl (pH 7.2); Scane rate -0.01 V s^{-1}

The absorption spectra of the two complexes in the absence and presence of increasing amounts of CT DNA concentration are shown in (Fig.2). With increasing concentrations of DNA, the complexes exhibited hyperchromism with slight blue shifts of the absorption bands at 224.5 and 226 nm respectively. Since the complexes do not contain any fused aromatic ring to facilitate intercalation, classical intercalative interaction is excluded¹⁸. Based on the hyperchromism exhibited and shifts in absorbance upon addition of CT DNA, non-intercalative interaction probably by an electrostatic interaction between complex ions and negatively charge

phosphate groups of the CT DNA can be predicted¹⁹⁻²⁰. However, since DNA possesses several hydrogen bonding sites which are accessible both in the minor and major grooves, a favorable hydrogen bonding may be formed between the coordinated and non-coordinated amine – NH– groups of the complexes with the base pairs in CT DNA. Further, in order to compare quantitatively the CT DNA binding strengths of the two complexes, their intrinsic binding constant (K_b) were calculated by using Eq. (1). The binding constants (K_b) obtained for the complexes 1 and 2 are $1.2 \times 10^4 \text{ M}^{-1}$ and $3.4 \times 10^4 \text{ M}^{-1}$ respectively.

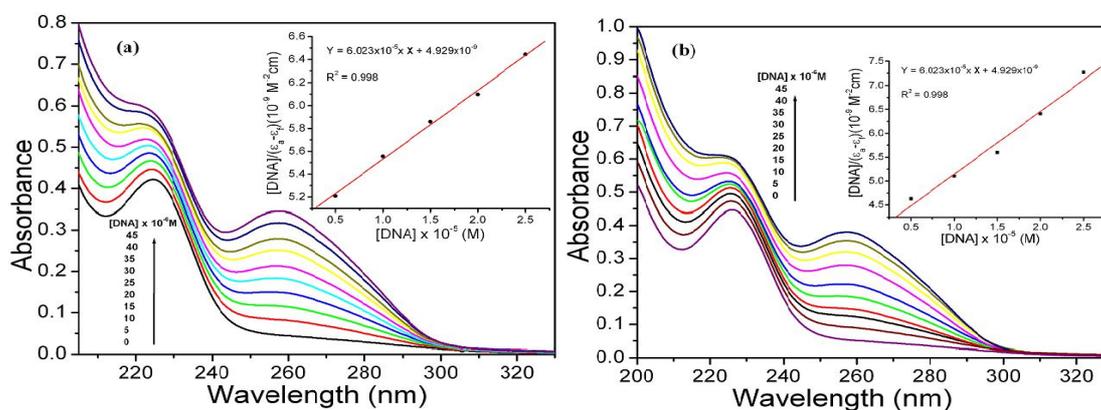


Fig. 2: Absorption spectral traces of complexes 1 (a) and 2 (b) in Tris-HCl buffer upon addition of CT DNA. Insets: plots of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs $[\text{DNA}]$ for the titration of complexes with CT DNA

The application of electrochemical methods in the study of complex-DNA interactions provides a useful complement to the previously used UV-Vis method of investigation. Pure CT DNA is electrochemically inactive under our experimental conditions in the potential range of +0.4 to -0.8V which is not shown in the figure. Cyclic voltammograms of the complex 1 in the absence and presence of CT DNA are shown in (Fig.3). A prominent reduction peak were not appeared in the cathodic scan where as cathodic current increases from -0.3V onward due to reduction of Cu^{2+} . So, only the oxidation peaks were used to investigate the CT DNA binding nature of the complexes. The anodic

peak potential (E_{pa}) values for the two complexes in the absence and presence of CT DNA are summarized in Table 2. In all the cases, the addition of CT DNA caused the peak currents of the anodic waves to diminish considerably as compared to the solutions without CT DNA. This decrease in peak currents is due to the decrease in the concentration of unbound Cu (II) complexes. This is again due to the formation of CT DNA-complex system. Similar observation was reported by Shah *et al.*²¹. Further, with increasing concentration of CT DNA, the anodic peak potentials of the two complexes shifted to more negative values indicating the non-intercalative binding natures of the two Cu (II) complexes with CT DNA²².

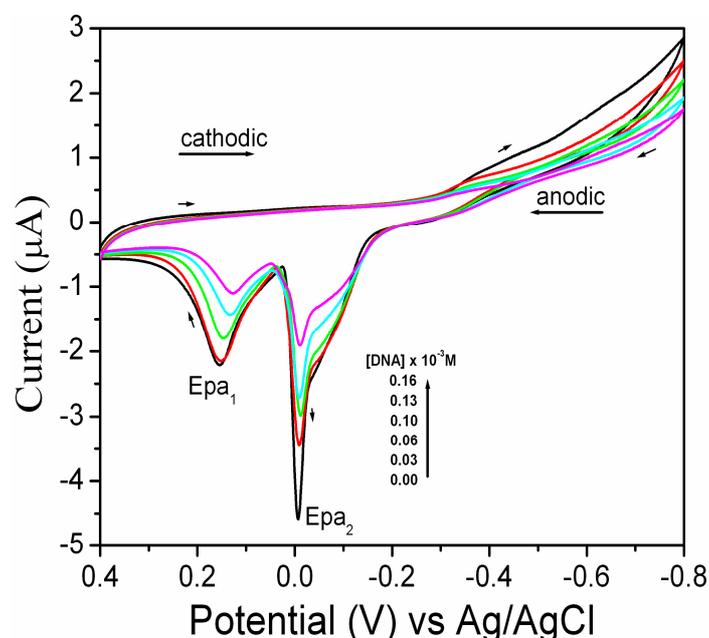


Fig. 3: Cyclic voltammograms of complex 1 in the absence and presence of CT DNA; where [complex] = 2×10^{-3} M. Supporting electrolyte, 50 mM NaCl/5 mM Tris-HCl (pH 7.2); Scan rate- 0.01 V s^{-1}

Table 3: The changes in the anodic peak potential values of the complexes 1 and 2 with increasing CT DNA concentration

[DNA] (10^{-3}M)	Complex 1		Complex 2	
	E_{pa1} (V)	E_{pa2} (V)	E_{pa1} (V)	E_{pa2} (V)
0	0.154	-0.006	0.092	-0.08
0.03	0.152	-0.008	0.088	-0.1
0.06	0.146	-0.009	0.086	-0.102
0.13	0.132	-0.010	0.086	-0.114
0.16	0.127	-0.011	0.084	-0.115

Supporting electrolyte, 50 mM NaCl/5 mM Tris-HCl (pH 7.2); scan rate- 0.01 V s^{-1}

Viscosity measurements were carried out for further clarifying the CT DNA binding nature of the Cu (II) complexes. According to the classical intercalation concept put forward by Lerman²³, the presence of the intercalation bond between a drug and the base pairs of DNA forces these base-pairs away from each other and therefore, unwinding the double helix and lengthening a given amount of DNA which in turn, increases the viscosity of the DNA solution. In contrast, groove-face or electrostatic interactions typically cause

less pronounced (positive or negative) or no change in the DNA solution viscosity²⁴. Figure 4 shows the changes in viscosity of the CT DNA upon addition of complexes 1 and 2 as well as EB (a well known intercalating agent). With increasing concentrations of Cu (II) complexes, relative viscosity of the CT DNA solutions deviates from the nature of the EB. This result supports the non-intercalative binding nature of the two studied Cu (II) complexes with CT DNA.

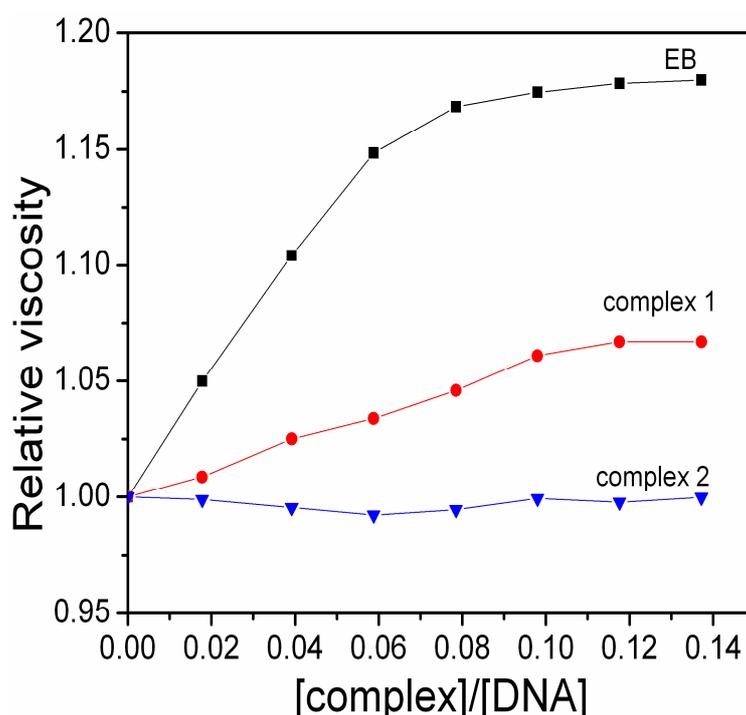


Fig. 4: Effect of increasing amounts of EB, complexes 1 and 2 on the relative viscosity of CT DNA at $25 \pm 0.2^\circ\text{C}$; $[\text{DNA}] = 0.5 \times 10^{-3}\text{M}$, $[\text{complex}]/[\text{DNA}]$ (r) ratio= (0.00 - 0.157)

The results of biological activities of the two synthesized Cu (II) complexes and parent metal salt were presented in the Table 3. The two complexes possessed moderate antibacterial properties. They were active against all the three tested bacteria as compared with the reference antibiotic, gentamycin sulfate. It can be further depicted that the complex 2 showed the more antibacterial activity than the complex 1 and metal salt. The increase in antibacterial activities of the two complexes than the parent metal salt may be explained

on the basis of chelating effect of the ligands²⁵. 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. Also chelation increases the lipophilic nature of the central atom, which subsequently favors its permeation through the lipid layer of the cell membrane. It will affect the enzymes which are involved in the metabolic processes of the cell. Then, it will ultimately affect the growth of the bacteria.

Table 3: *In vitro* antimicrobial activities of synthesized complexes with reference to gentamycin sulfate

^a Diameter of inhibition zone									
Complex	<i>Escherichia coli</i> (mg/ml)			<i>Klebsiella pneumonia</i> sub sp. <i>pneumonia</i> (mg/ml)			<i>Proteus mirabilis</i> (mg/ml)		
	0.1	1.0	2.0	0.1	1.0	2.0	0.1	1.0	2.0
CuCl ₂ .2H ₂ O	8.0±0.7	8.3±0.6	9.0±0.3	7.0±0.3	8.6±0.6	9.5±0.6	6.8±0.7	6.8±0.9	7.8±0.0
Complex 1	8.7±0.6	11.3±0.9	13.3±0.9	8.5±0.9	9.4±0.3	11.8±0.3	7.6±0.6	8.9±0.6	10.2±0.9
Complex 2	11.9±0.9	13.0±0.9	15.3±0.6	8.9±0.6	10.3±0.3	12.0±0.6	8.7±0.3	9.4±0.0	11.5±0.7
Gentamycin sulfate	-	-	50.0±0.3	-	-	42.0±0.3	-	-	46.7±0.7

Mean value of the three replicates of three repeated experiments of each test. The diameter of inhibition zone induced by DMSO in *Escherichia coli*, *Klebsiella pneumoniae* sub sp. *pneumoniae* and *Proteus mirabilis* were 6.0 ± 0.3 , 7.0 ± 0.7 and 8.0 ± 0.6 respectively. Statistical analyses were performed using SPSS software and the data were subjected to one way analysis of variance followed by Tukey's test. Values are presented as means \pm SE. ^aRepresents the diameter of inhibition zone after subtracting the inhibition zone induced by DMSO. Values of $p < 0.05$ were considered significant.

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