SUST JOURNAL OF SCIENCE AND TECHNOLOGY

SHAHJALAL UNIVERSITY OF SCIENCE AND TECHNOLOGY

VOL 27, NO. 1, 2017

www.sust.edu

SUST Journal of Science and Technology, Vol. 27, No. 1, 2017, P: 9-20

Antibacterial Activity and DNA Interaction of Chelating Cobalt and Copper Complexes

K. M. Anis-Ul-Haque, Mahmudul H. Suhag and Muhammad Younus*

Department of Chemistry, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh
Corresponding author:* **E-mail: *myounus-che@sust.edu*

Abstract

Bis(ethylenediamine)cobalt(II) chloride and ethylenediaminecopper(II) chloride chelating complexes have been synthesized by the reaction of ethylenediamine with corresponding chloride salt in methanol. The complexes were purified by recrystallization from methanol-water solvent mixture. The synthesized complexes have been characterized by elemental analysis, FT-IR and UV-Visible spectroscopy. Ethylenediaminecopper(II) chloride has previously been reported [3]. The antibacterial activity against some pathogenic bacteria *Escherichia coli*, *Klebsiella oxytoca* and *Staphylococcus aureus* has been studied by disk diffusion method. The complexes were found to inhibit the growth of bacteria. The DNA binding constant of these complexes was studied using extracted DNA from lemon by UV-Vis spectroscopy. The calculated binding strengths were found to be 1.18×10^3 and 6.3×10^6 for $[Co(en)_2Cl_2]$ and $[Cu(en)Cl_2]$ complexes, respectively.

Keywords: Chelating complex, DNA binding, antibacterial activity, disk diffusion method

1. Introduction

Metal ions play important roles in the synthesis and transport of organic molecules and in catalyzing redox processes of biological systems [1]. Metal chelate complexes containing gadolinium, cobalt, lithium, bismuth, iron, calcium, lanthanum, gallium, tin, arsenic, rhodium, copper, zinc, aluminum and lutetium have all been used in medicine [2]. Metal chelates are commonly prepared from the previously synthesized ligands with metal salt or by the template synthesis [3-6]. The interaction of transition metal complexes with DNA is a subject of intensive investigation with the perspective of development of newer materials for application in biotechnology and medicine. Metal complexes bind to DNA by non-covalent interaction such as electrostatic binding, groove binding and intercalative binding [7]. Absorption titration method is one of the well-known methods to monitor the interaction of metal complexes with DNA by UV-Vis spectrophotometry. In this method, a UV–Vis absorption band of metal complex with DNA can be monitored carefully in a region where a free DNA does not show absorption band. In general, hypochromism and red shift are associated with the intercalative binding of the complex to the helix of DNA where strong stacking interactions take place between them [8].

Antibacterial properties of a number of metal complexes have already been established by different research groups [3, 7]. Disk diffusion method is widely used for testing antibacterial activity of compounds and determining its bacterial susceptibility as an antimicrobial. This method refers to the dispersion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the solid culture medium. The result of this experiment is based on the determination of an inhibition zone which is proportional to the bacterial susceptibility of the antimicrobial present in the disk [9].

Many works related to synthesis of metal complexes using chelating ligands, and studies of antibacterial activity and DNA interaction of these complexes could be found in the literature. For instance, Kannan *et al.* reported the synthesis, DNA-binding studies and antimicrobial activity of 1,10-phenanthroline, l-tyrosine and urea containing copper(II) complexes [7]. Nagababua *et al.* studied the DNA binding and photo cleavage of ethylenediamine, phenanthroline and bipyridine containing cobalt complexes [8]. Osinsky *et al.* synthesized a tetradentate aliphatic Schiff base containing compounds or their analogs as axial ligands containing cobalt (III) complexes as potential antitumor agents [10]. Nagababu *et al.* reported the synthesis, DNA binding and cytotoxicity studies of ethylenediamine and pyrazole containing cobalt (II) complex [11].

In this work, cobalt and copper complexes $[Co(en)_2Cl_2]$ and $[Cu(en)Cl_2]$ have been synthesized by the adaption of the literature method [3]. The UV–Vis absorption properties of these complexes have been used to monitor the interaction of individual complex with DNA. Antibacterial activity of these complexes has been studied against one gram-positive bacterium (*Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli* and *Klebsiella oxytoca*) by disk diffusion method.

2. Materials and Methods

2.1. Materials

Cobalt(II) chloride and copper(II) chloride were purchased from Sigma-Aldrich and used as supplied. 1,2-diaminoethane was purchased from MERCK and were used without further purification. Methanol, ethanol, ethyl acetate, dichloromethane and chloroform solvents were pre-dried from appropriate drying agent and freshly distilled before use. Nutrient agar, beef extract, peptone, sodium chloride were purchased from Sigma-Aldrich. DNA was extracted by salt extraction method from lemon [12]. DNA-binding experiments were carried out in tris–HCl buffer solution (50 mM NaCl, 5 mM *Tris*–HCl, pH 7.1). *Tris*–HCl buffer was prepared using deionized double distilled water. Solutions of extracted DNA in buffer gave a ratio of UV–Vis absorbance of 1.5:1 at 260 and 280 nm, which indicated that DNA was mixed with some amount of protein. The concentration of DNA was determined spectrophotometrically (ε_{260} = 6600 M^{-1} cm^{-1}) [11].

2.2. Apparatus and Measuring Techniques

Elemental analyses were performed in elemental Analyzer System Vario EL III Element Analyzer, at the Analytical Service Cell, BCSIR Laboratories, Dhaka, Bangladesh. The infrared spectra of complexes were recorded on KBr pellets with a Shimazdu IR spectrometer (Prestige 21). UV-Visible spectra of the complexes and absorption titration experiments of DNA and complexes were studied in a Shimazdu UV-Visible spectrophotometer (UV-1800 PC), from 200 nm to 800 nm ranges. All apparatus were used in antibacterial test after sterilizing. Melting points of the complex was obtained with an electro thermal melting point apparatus (Gallenkamp, England). Bacteria were cultured and antibacterial test were performed in an incubator.

2.3 Synthetic Procedure

2.3.1 Synthesis of Cobalt Complex, $[Co(en)_2Cl_2]$

To a warm methanol solution (30 cm^3) of the metal salt $CoCl_2.6H_2O$ (1.189 g, 5 mmol), 1,2-diaminoethane (0.601 mL, 10 mmol) was added. The resulting mixture was stirred overnight at 55°C to yield solid material which was collected by filtration, washed with methanol and dried in vacuum. The complex was purified by recrystallization using methanol-water solvent mixture. The product was obtained as a reddish yellow colored solid in 54% yield (1.22 g). The decomposition point of the product was 226-228°C. IR (KBr): ν(Co-N), 578 cm^{-1}; ν(Co-Cl), 410 cm^{-1}; ν(C-H), 2980 cm^{-1}; ν(C-N), 1061 cm^{-1}; ν(N-H, bending), 3510 cm^{-1}, and ν(N-H, stretching), 1619 cm^{-1}. UV-Vis (H_2O): (λ_{max}) = 338 nm and 466 nm. Anal. Found: C, 18.50; H, 7.40; N, 21.01%. Calc. for $C_4H_{16}Cl_2CoN_4$: C, 19.21; H, 6.45; N, 22.41%.

2.3.2 Synthesis of Copper Complex, [Cu(en)Cl_2]

To a warm methanol solution (50 cm^3) of the metal salt $CuCl_2.2H_2O$ (0.500 g, 2.93 mmol), 1,2-diaminoethane (352 µL, 5.86 mmol) was added. The resulting mixture was stirred overnight at 55°C to yield solid material which was collected by filtration, washed with methanol and dried in vacuum. The product was obtained as an indigo colored solid in 39.3% yield (0.330 g). IR (KBr): 681 cm^{-1}, ν(Cu-N); 527 cm^{-1}, ν(Cu-Cl); 2967 cm^{-1}, ν(C-H); 1042 cm^{-1}, ν(C-N); 3299 cm^{-1}, ν(N-H, stretching) and 1571 cm^{-1}, ν(N-H, bending). UV-Vis (H_2O): (λ_{max}) = 229 nm. Anal. Found: C, 12.66; H, 3.92; N, 14.49% Calc. for $C_2H_8Cl_2CuN_2$: C, 12.35; H, 4.14; N, 14.40%.

2.4 Test of Antibacterial Activity

2.4.1 Preparation of the culture media (nutrient broth media)

Culture media was prepared by the mixture of beef extract (30gL^{-1}), peptone (50gL^{-1}) and NaCl (50gL^{-1}) in distilled water. The solution was heated on an oil bath for 15 minute for complete dissolution. The solution will be sterilized by an autoclave for 15 minute at 120°C. Then single colony of individual bacteria (*Escherichia coli, Staphylococcus aureus* and *Klebsiella oxytoca*) was added in this solution (20 ml) separately and incubated at 37°C for 24 hours.

2.4.2 Preparation of sample disk

A stock solution of 20 mg mL^{-1} was made by dissolving compound in distilled water. Paper discs of Whatman filter paper (0.45 micro phore) of uniform diameter (5 mm) and thickness (1mm) were sterilized. 10 micro liters of stock solution (200µg sample) were socked in each disk.

2.4.3 Preparation of agar plates

The media was made by dissolving bacteriological nutrient agar (32gL^{-1}) in distilled water. The mixture was autoclaved for 15 min at 120°C and then dispensed onto sterilized Petri dishes, allowed to solidify and then used for inoculation.

2.4.4 Procedure of inoculation

Inoculation was done with the help of micropipette with sterilized tips; 25 µl of activated strain was placed onto the surface of an agar plate, and spread evenly over the surface by means of a sterilized bent glass rod [9].

2.4.5 Application of disks

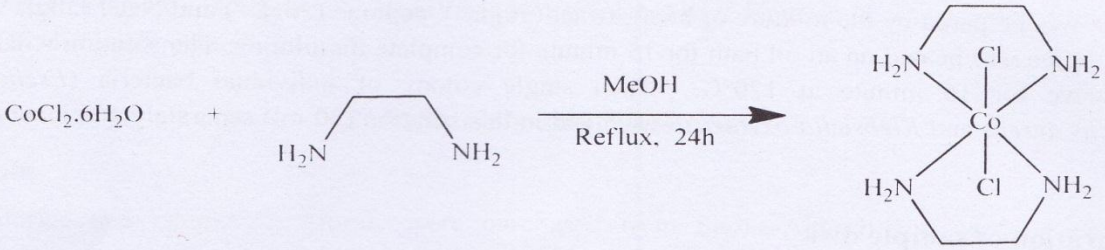
Sample disks and antibiotic disks were applied in each earlier inoculated agar plates and incubated at 37°C for 24 h. The zone of inhibition (diameter) was then measured (in mm) around the sample and standard antibiotic disk. Antibiotic imipenem (IPM) was used against *Escherichia coli, Staphylococcus aureus* and *Klebsiella oxytoca* bacteria as standard antibiotic disk. The antibacterial results of the compounds were compared with the standard antibiotic disk.

2.5. DNA binding studies by absorption titration

Absorption titration experiments were carried out by varying the DNA concentration at 6.5, 13.1, 19.6, 26.2, 32.7, 39.2 and 45.8 μM and maintaining the complexes concentration constant at 184 μM for $[Co(en)_2Cl_2]$ complex and 288 μM for $[Cu(en)Cl_2]$ complex. The reference solution was the *tris*–HCl buffer solution. The sample solution was scanned in the range of 200–800 nm. The absorption data were analyzed for an evaluation of the intrinsic binding constant, K_b of the complexes with DNA.

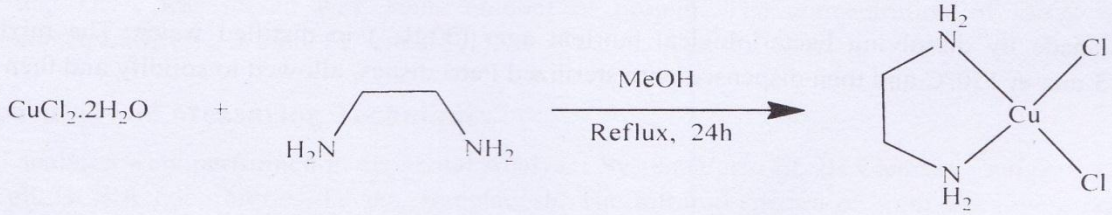
3. Results and Discussion

The reaction between 1,2-diaminoethane and metal salt $CoCl_2.6H_2O$ in methanol leads to the formation of *trans*-$[Co(en)_2Cl_2]$ (Scheme 1).



Scheme 1 Synthesis of *trans*-$[Co(en)_2Cl_2]$ complex

Chelating complex *cis*-$[Cu(en)Cl_2]$ was formed similarly (Scheme 2) [3].



Scheme 2 Synthesis of *cis*-$[Cu(en)Cl_2]$ complex

Physical properties and elemental analysis data of the prepared complexes are given in Table 1.

Table 1 Physical properties and elemental analysis data of the prepared complexes

Complex	Color	Elemental Analysis Found (Calculated %)		
		C	H	N
$C_4H_{16}Cl_2CoN_4$	Reddish Brown	18.50 (19.21)	7.4 (6.45)	21.01 (22.41)
$C_2H_8Cl_2CuN_2$	Indigo	12.66 (12.35)	3.92 (4.14)	14.49 (14.40)

3.1. IR Spectroscopy

The IR spectrum of cobalt complex *trans*-$[Co(en)_2(Cl)_2]$ shows bands at 3510 cm^{-1} and 1619 cm^{-1} corresponding to stretching and bending frequency of ν(N-H), respectively. The band at 2980 cm^{-1} corresponding to ν(C-H) vibrations (Fig. 1). A band at 578 cm^{-1} has been assigned for ν(Co-N) in the cobalt complex which indicates that the nitrogen atoms of ethylenediamine group are coordinated to the metal atom [13]. The band at 410 cm^{-1} has been assigned for ν(Co-Cl) vibrations in cobalt complex. However, in the IR spectrum of bidentate schiff base complexes of cobalt, Kurzak *et al.* assigned ν(Co-N) at 500 cm^{-1}, and ν(Co-O) at 337 cm^{-1} [14].

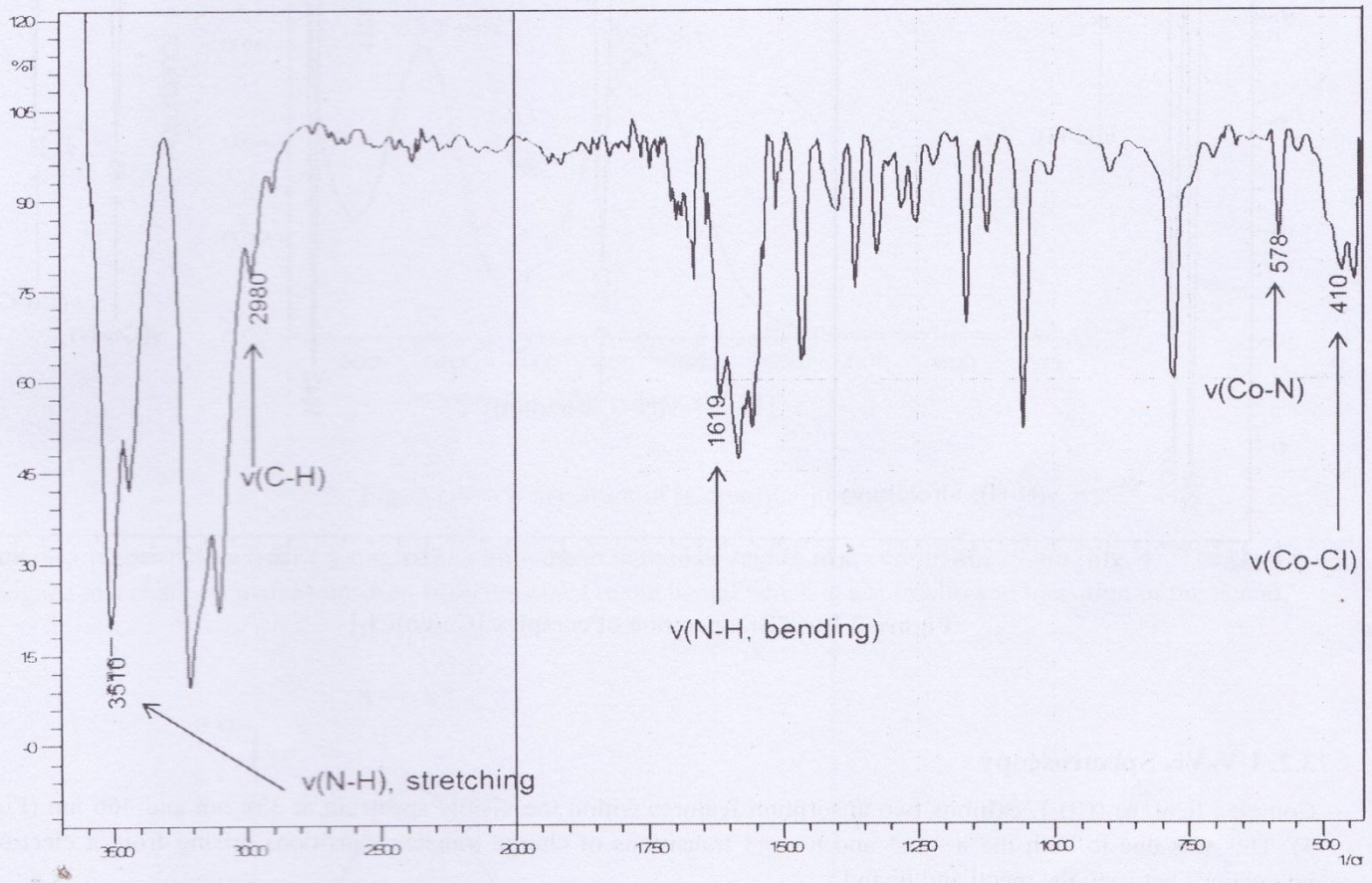


Figure 1 IR (KBr) spectrum of complex $[Co(en)_2Cl_2]$

In the IR spectrum of complex *cis*-$[Cu(en)Cl_2]$, the N-H stretching vibration is found to occur in the range of 3230-3300 cm^{-1}. The characteristic N-H bending vibration is obtained as a strong band at 1572 cm^{-1} which is a commonly observed fact for chelated ethylenediamine (en) complexes. The C-H stretching vibrations are shown up as two bands at 2967 and 2887 cm^{-1}. The sharp peak obtained at 1042 cm^{-1} have been assigned for ν(C-N). The signal obtained at 681 cm^{-1} and 527 cm^{-1} can be assigned for ν(Cu-N) and ν(Cu-Cl) bonds, respectively (Fig. 2). The IR spectral features, similar to this complex, have also been observed by Sarmah *et al.* [15]. However, Barman *et al.* assigned ν(Cu-N) at 640 cm^{-1} and ν(Cu-O) at 521 cm^{-1} in the IR spectrum of the polymeric compound $[Cu(C_6H_5O_2N)Cl]_n$ [16].

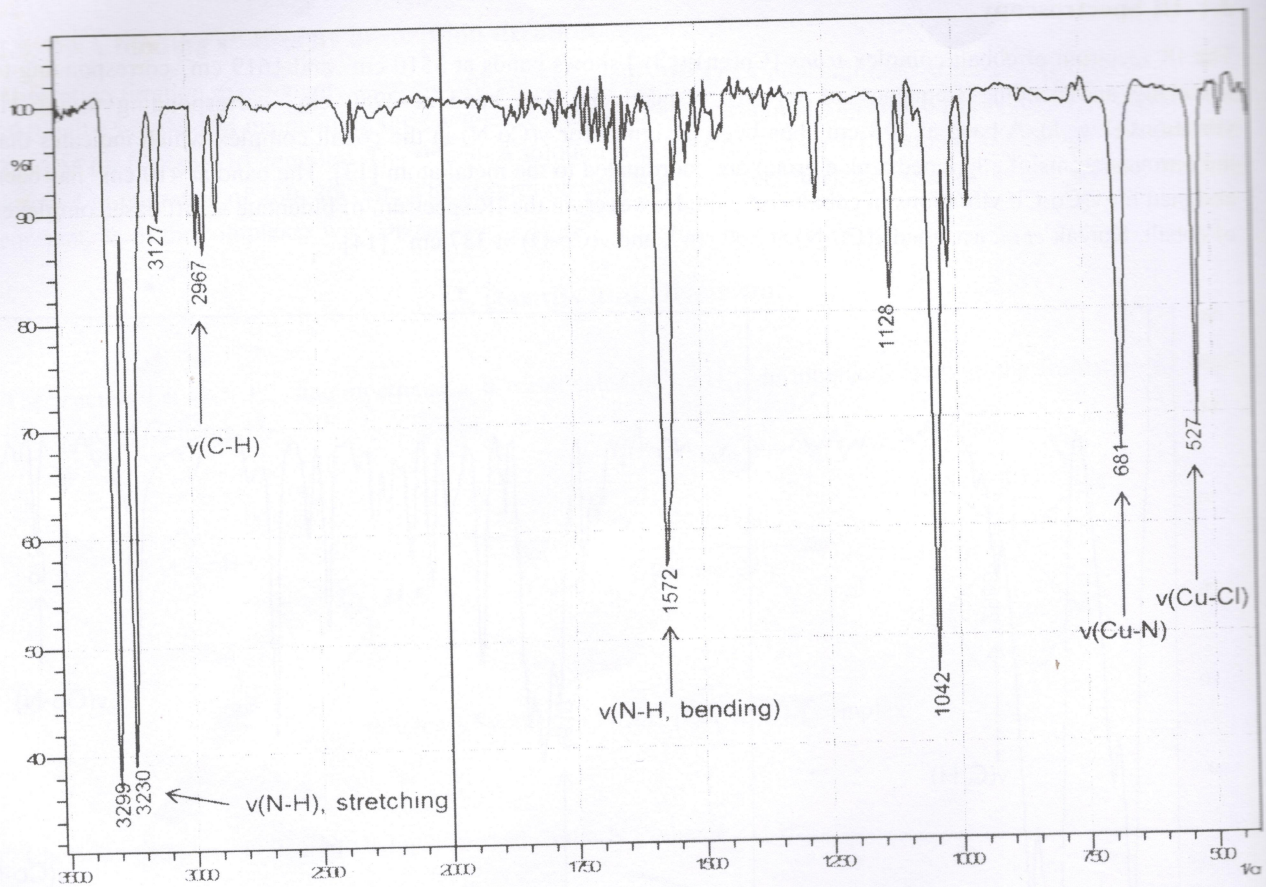


Figure 2 IR (KBr) spectrum of complex $[Cu(en)Cl_2]$

3.2. UV-Vis Spectroscopy

Complex $[Co(en)_2(Cl)_2]$ exhibits two absorption features within the visible spectrum at 338 nm and 466 nm (Fig. 3). This was due to both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of charge transfer transitions arising from π electron interactions between the metal and ligand.

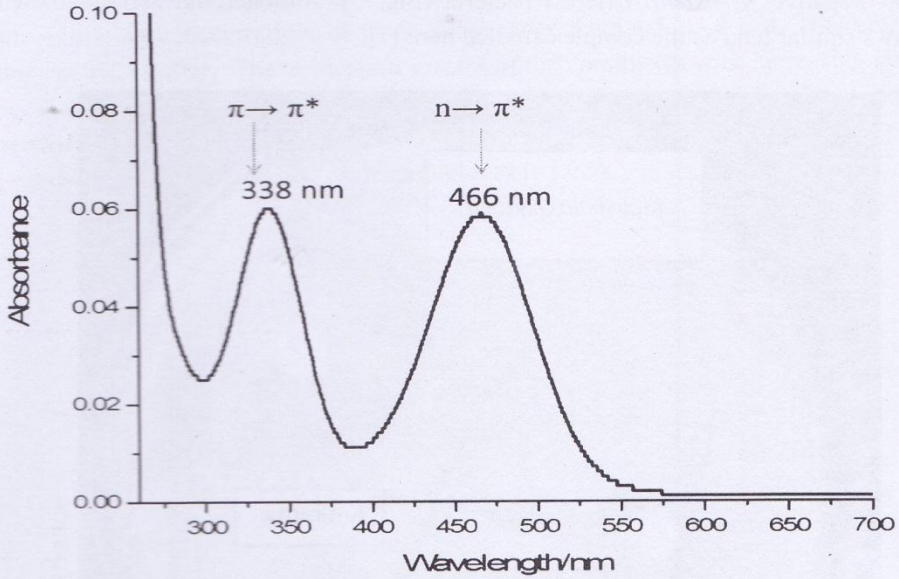


Fig. 3 UV-Vis spectrum of $[Co(en)_2Cl_2]$ complex

Complex $[Cu(en)Cl_2]$ exhibits a single absorption band in the UV region of spectrum at 229 nm (Fig. 4). The band is assigned to a charge transfer transition from the metal to the ligand which is a spin-allowed transition of the ligand.

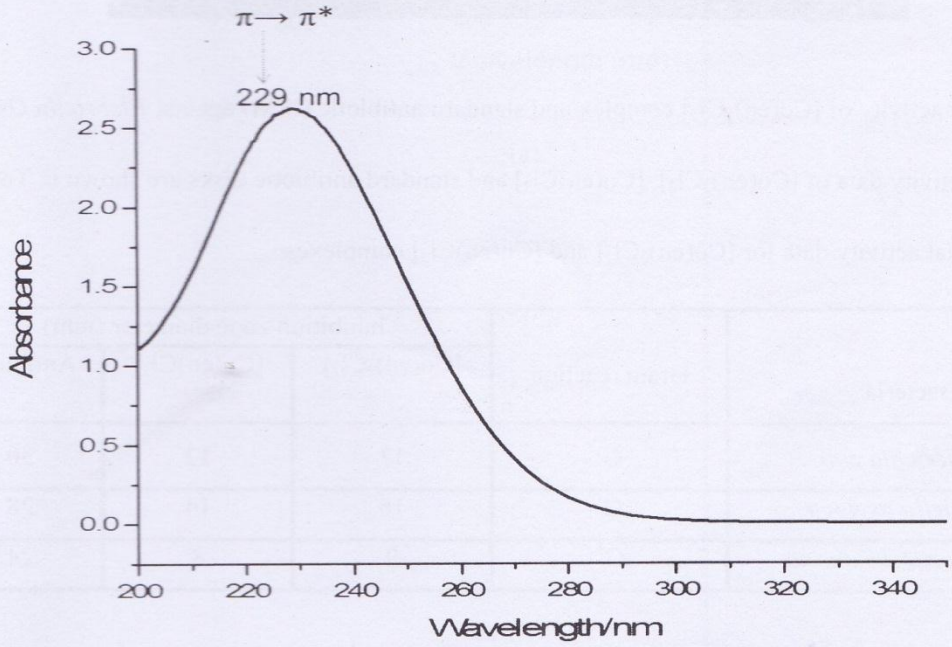


Fig. 4 UV-Vis spectrum of $[Cu(en)Cl_2]$ complex

3.5. Antibacterial Activity of $[Co(en)_2Cl_2]$ and $[Cu(en)Cl_2]$ Complexes

The complexes $[Co(en)_2Cl_2]$ and $[Cu(en)Cl_2]$ were tested for their in vitro antibacterial activities. It was observed that the complexes have inhibitor reactivity against the all tested bacteria and complexes show highest antibacterial activity against Gram- negative *Klebsiella oxytoca* bacteria (Fig. 5). Antibacterial activity of a metal complex containing copper shows similar tend as the complex studied here [7].

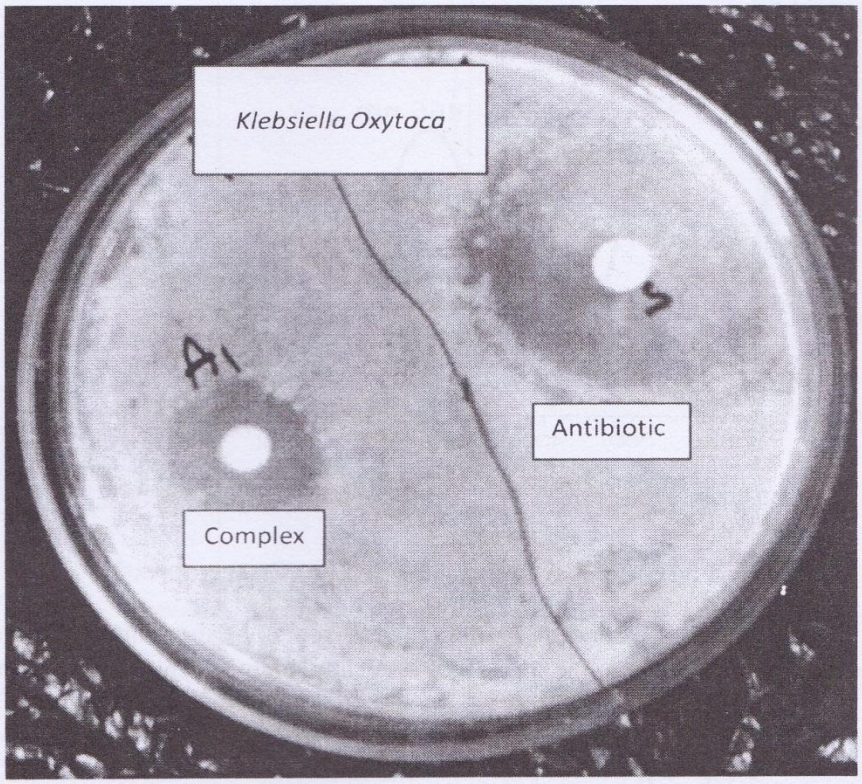


Fig. 5 Antibacterial activity of $[Co(en)_2Cl_2]$ complex and standard antibiotic (IPM) against *Klebsiella Oxytoca*,

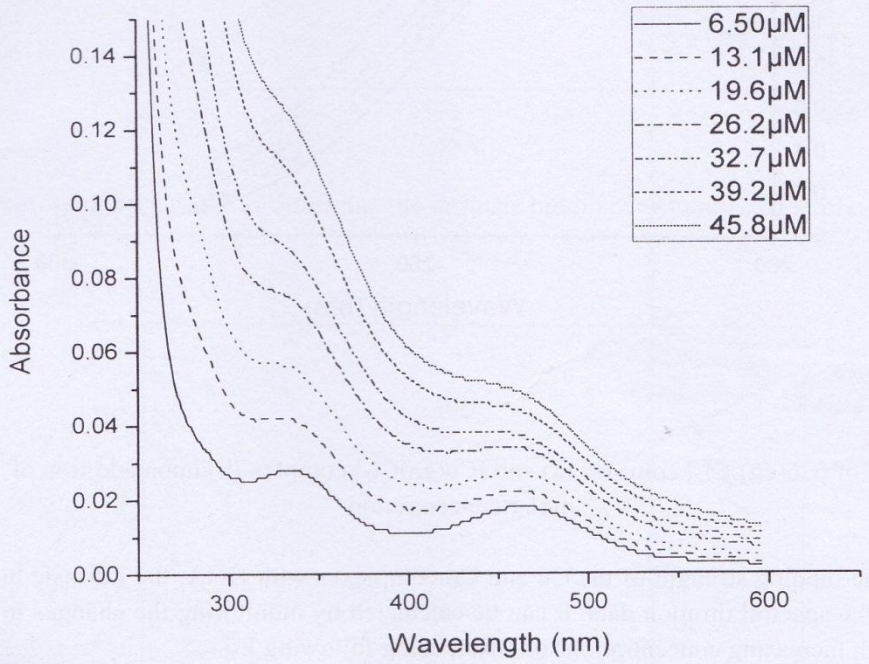
The antibacterial activity data of $[Co(en)_2Cl_2]$, $[Cu(en)Cl_2]$ and standard antibiotic disks are shown in Table 2.

Table 2 Antibacterial activity data for $[Co(en)_2Cl_2]$ and $[Cu(en)Cl_2]$ complexes

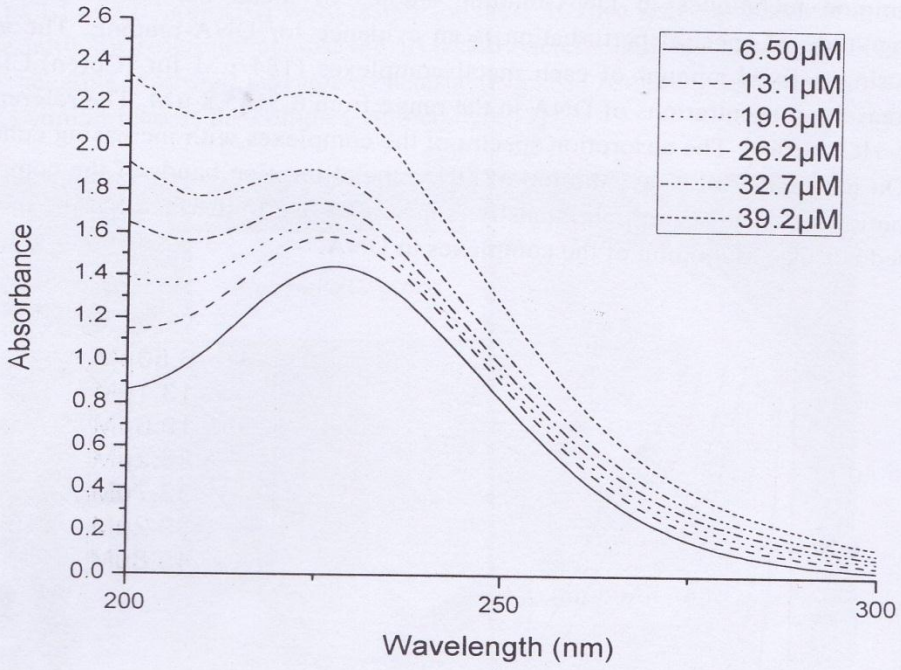
Bacteria	Gram reaction	Inhibition zone diameter (mm)		
		$[Co(en)_2Cl_2]$	$[Cu(en)Cl_2]$	Antibiotic
Escherichia coli	G^-	13	12	30
Klebsiella oxytoca	G^-	16	14	28
Staphylococcus aureus	G^+	9	8	24

3.6. DNA binding studies

One of the most common techniques in DNA-binding studies of metal complexes is electronic absorption spectroscopy. The magnitude of spectral perturbation is an evidence for DNA-binding. The absorption titrations were carried out by using a fixed amount of each metal complexes (184 μM for $[Co(en)_2Cl_2]$ and 288 μM for $[Cu(en)Cl_2]$) with increasing concentrations of DNA in the range from 6.5–45.8 μM. The reference solution used in this study was the *tris*–HCl buffer. The absorption spectra of the complexes with increasing concentrations of DNA are shown in Fig. 6. On increasing the concentration of DNA, the absorption bands of the complexes was affected, resulting in hyperchromic shift. The absorption intensity is increased due to the fact that the purine and pyrimidine DNA-bases are exposed because of binding of the complexes to DNA.



(a)



(b)

Fig. 6 UV-Vis spectra of $[Co(en)_2Cl_2]$ complex (a) and $[Cu(en)Cl_2]$ complex (b) upon addition of DNA in different molar concentration

In order to illustrate the binding strength of the Cu and Co complexes with DNA, the intrinsic binding constant K_b was determined from the spectral titration data. It can be calculated by monitoring the changes in absorbance at the corresponding λ_{max} with increasing concentrations of DNA, using following Eq.:

$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f)+1/K_b(\varepsilon_b-\varepsilon_f)$

Where [DNA] is the concentration of DNA in base pairs, ε_f, ε_a, and ε_b correspond to the extinction coefficients, respectively, for the free complexes, for each addition of DNA to the complexes and for the complexes in fully bound form [7,8,11]. A plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA] gives K_b, for $[Co(en)_2Cl_2]$ (Fig. 7) and for $[Cu(en)Cl_2]$ (Fig. 8) as the ratio of the slope to the intercept. From the $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA] plots, the intrinsic binding constant K_b for $[Co(en)_2Cl_2]$ was 1.18×10^3 and that for $[Cu(en)Cl_2]$ was 6.3×10^6 , which reveals a strong binding to lemon DNA. The observed DNA binding constant of $[Cu(en)Cl_2]$ complex was significantly higher than that of $[Co(en)_2Cl_2]$ complex for their geometrical structures [17]. Nagababu *et al.* reported that binding constant K_b for similar complex $[Co(en)_2(pyz)_2]^{3+}$ was 4.8×10^3 with protein free CT-DNA [11].

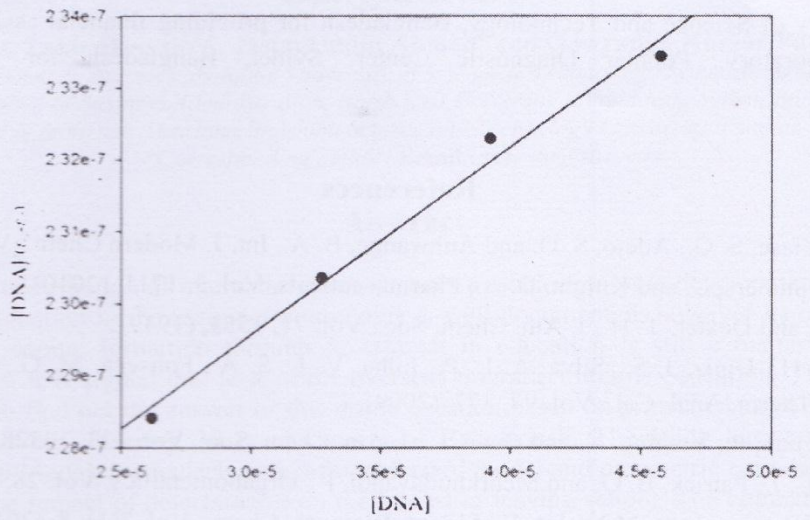


Fig. 7 Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ Vs [DNA] to determine the intrinsic binding constant K_b of $[Co(en)_2Cl_2]$ with DNA

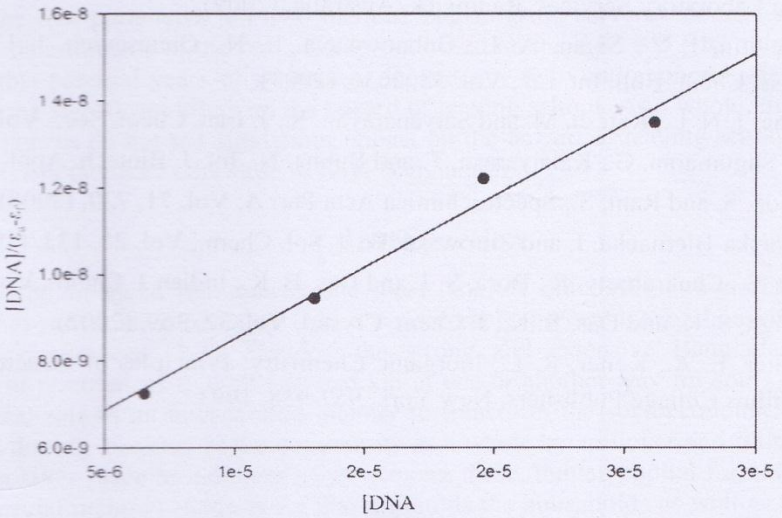


Fig. 8 Plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ Vs [DNA] to determine the intrinsic binding constant K_b of $[Cu(en)Cl_2]$ with DNA

4. Conclusions

The present work describes the preparation, characterization, DNA interaction and antibacterial activity of $[Cu(en)Cl_2]$ and $[Co(en)_2Cl_2]$ complexes. The intrinsic binding strength K_b of these metal complexes with DNA was determined by UV-Vis spectrophotometry and antibacterial activity of these metal complexes studied by disk diffusion method. These complexes exhibit strong binding ability to DNA and good antibacterial activity.

Acknowledgements

We thank the Ministry of Science and Technology, Bangladesh for providing financial assistance. We also thank Microbiological Laboratory, Popular Diagnostic Center, Sylhet, Bangladesh for providing pathogenic microorganism.

References

[1] Asemave, K., Yiase, S. G., Adejo, S. O. and Anhwange, B. A., Int. J. Modern Chem., Vol. 1(2), 93, (2012).

[2] Chang, E. L., Simmers, C. and Knight, D. A., Pharmaceuticals, Vol. 3, 1711, (2010).

[3] Jonassen, H. B. and Dexter, T. H., J. Am. Chem. Soc., Vol. 71, 1553, (1949).

[4] Arakaki, L. N. H., Diniz, J. S., Silva, A. L. P., Filha, V. L. S. A., Fonseca, M. G., Espinola, J. G. P. and Arakaki, T., J. Therm. Anal. Cal., Vol. 97, 377, (2009).

[5] Liang, S., Congjian, P., Yeming, X. and Quan, L., J. Am. Chem. Soc., Vol. 133, 10328, (2011).

[6] Yu, I., Wallis, C. J., Patrick, B. O. and Mehrkhodavandi, P., Organometallics, Vol. 28, 6370, (2009).

[7] Kannan, D. and Arumugham, M.N., Int. J. of Inorg. Bioinorg. Chem., Vol. 3(1), 8, (2013).

[8] Nagababua, P., Latha, J. N. L., Prashanthi, Y. and Satyanarayan, S., J. Chem. Pharm. Research, Vol. 1(1), 238, (2009).

[9] Bell, S. M., Pham, J. N. and Carter, I. W., Antibiotic Susceptibility Testing by the CDS Method, 5th Ed., South Eastern Area Laboratory Services, Randwick, Australia, (2009).

[10] Osinsky, S. P., Levitin, I. Y., Sigan, A. L., Bubnovskaya, L. N., Ganusevich, I. I., Campanella, L. and Wardmand, P., Russ. Chem. Bull. Int. Ed., Vol. 52, 2636, (2003).

[11] Nagababu, P., Latha, J. N. L., Rajesh, M. and Satyanarayan, S., J. Iran. Chem. Soc., Vol. 6, 145, (2009).

[12] Madhan, S. S. R., Sugumaran, G., Kalaiyarasu, T. and Bubna, N., Int. J. Biotech. Appl., Vol. 1, 16, (2009).

[13] Chandra, S., Raizada, S. and Rani, S., Spectrochimica Acta Part A, Vol. 71, 720, (2008).

[14] Kurzak, K., Kuzniarska-Biernacka, I. and Zurowska, B., J. Sol. Chem., Vol. 28, 133, (1999).

[15] Sarmah, P., Singha, S., Chakrabarty, R., Bora, S. J. and Das, B. K., Indian J. Chem., Vol. 46A, 1929, (2007).

[16] Barman, R. K., Singh, S. K. and Das, B. K., J. Chem. Crystal. Vol. 32, 369, (2002).

[17] Huheey, J. E., Keiter, E. A., Keiter, R. L., Inorganic Chemistry: Principles of Structure and Reactivity, 4th Edition, Harper Collins College Publishers, New York, 957-958, 1993.