Binding Study of Novel Ru(II)-Bipyridine-Benzoyl Picolinic Acid Complex on *E. Coli* Genomic DNA

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ABSTRACT

The binding efficiency of novel Ru(II)-bipyridine-benzoyl picolinic acid ($[Ru(bpy)_2(bzpic)_2]^{2+}$, bpy = 2,2′-bipyridine and bzpic = 3-benzoyl picolinic acid) complex on E. coli genomic DNA (gDNA) have been determined by UV-Visible spectral technique. The synthesised complex is characterized by elemental and spectral analysis. The changes in absorbance and wavelength upon incremental addition of the complex on gDNA clearly depict the binding nature of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on E. coli gDNA. The binding constant (K_b) values obtained for ligand centred (LC) and metal to ligand charge transfer (MLCT) transitions are found to be 1.206 x 10^4 and 6.049 x 10^4 M^{-1} respectively. The K_b values clearly explain that the complex get binds with the DNA more effectively in the MLCT region than that of the LC region. The binding interaction between the $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex and the E. coli gDNA is mainly due to electrostatic, intercalation and π-π stacking interactions between the aromatic ligands present in the complex and the DNA base pairs. Thus, the results reveal that the $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex get strongly binds with the gDNA of E. coli and the K_b values depends on the nature of the ligands present in the complex.

Keywords: $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex; DNA binding; *E. coli* genomic DNA; Binding constant

1. Introduction

Medicinal inorganic chemistry and translational medicine are the two emerging concepts in the fields of biomedical research and health care since 21st century. In molecular biology, the drug target often takes place on the DNA which acts as a carrier of genetic information and exhibits DNA-targeted pharmacological activity. Based on DNA binders, investigations of bioinorganic chemistry have only existed with the serendipitous discovery of the antitumor activity of cisplatin and its derivatives. In the search for drugs with improved clinical effectiveness, reduced toxicity and a broader spectrum of activity, ruthenium complexes are very promising, especially from the viewpoint DNA is identified as the primary molecular target of metal based anticancer drugs when compared to other metals. Due to their strong metal to ligand charge transfer (MLCT) transition and unique emission characteristics, Ru(II)-polypyridine complexes have received special attention to study their DNA binding properties. Recently, the bioinorganic research is focused on Ru(II) octahedral complexes containing at least one aromatic heterocyclic ligand for intercalation with the DNA base pairs [1,2]. Based on this concept, the present investigation mainly focuses on the synthesis, characterization and DNA binding study of novel $[Ru(bpy)_2(bzpic)_2]^{2+}$ (bpy = 2,2′-bipyridine and bzpic = 3-benzoyl picolinic acid) complex on *E. coli* genomic DNA (gDNA). The binding nature and the binding constant (K_b) of the synthesised complex on *E. coli* gDNA is analysed by UV-Visible spectral technique.

2. Materials and Methods

2.1 Materials

$RuCl_3.3H_2O$, ligands (bpy and bzpic) and ammonium hexafluorophosphate (NH_4PF_6) were procured from Sigma-Aldrich. HPLC grade solvents and double distilled deionized water were used for the synthesis and the binding study of the complex on *E. coli* gDNA.

2.2 Synthesis of $[Ru(bpy)_2Cl_2]$ Complex

$RuCl_3.3H_2O$, bpy and LiCl were refluxed in DMF for 8 h. The reaction mixture was cooled to room temperature. The mixture was then filtered, yielding a red-violet filtrate and a black crystalline substance. The solid was washed three times with water followed by diethyl ether and was subsequently dried by suction. This complex was then recrystallized from ethanol and used as the precursor for the synthesis of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex.

2.3 Synthesis of $[Ru(bpy)_2(bzpic)_2]^{2+}$ Complex

The precursor complex $[Ru(bpy)_2Cl_2]$ (1 mmol) and bzpic (2 mmol) were dissolved in 20 mL of methanol and refluxed for 4 h under nitrogen atmosphere. The solution was then allowed to cool at room temperature and filtered to remove any insoluble impurities. A saturated solution of NH_4PF_6 was then added dropwise into the filtrate until a red precipitate was formed. The product was filtered, washed with cold water and diethyl ether and further dried in a vacuum desiccator. The complex was purified by column chromatography using silica gel as the adsorbent and a mixture of methanol and dichloromethane (2:8 ratio) as an eluent and on subsequent evaporation gives $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex with a yield 0.421 g.

2.4 Characterization Techniques

The percentage compositions of the elements present in the synthesised $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex was determined by elemental analysis using Truspec Micro analyser. The absorption spectrum of the synthesized $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex was measured using SHIMADZU UV 1800 double beam spectrophotometer. The FT-IR spectrum of the complex was measured using FTIR SHIMADZU double beam spectrophotometer. The ^{1}H and ^{13}C NMR spectrum was measured using BRUCKER spectrometer. The Matrix Assisted Laser Desorption/Ionization -Time of Fight (MALDI-TOF) mass spectral analysis of the complex was carried out to determine the m/z peak using Bruker Daltonics Flex-PC microflex analyser.

2.5 Determination of Binding Constant of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA

The purity and the quantity of the diluted *E. coli* gDNA stock solution prepare from nuclease free water in 0.1 M sodium phosphate buffer at pH 7.4 (~250 ng/µL) is determined by UV-visible spectrophotometric method. The stock solution of the synthesised complex was prepared by dissolving 1 mg of the complex in 1 mL of DMSO at pH 7.0. A fixed concentration of *E. coli* gDNA (1.5 µg/mL) was incubated individually with different concentrations (25, 50,100 µg/mL) of the synthesised complex in phosphate buffer at pH 7.4 and incubated at 37 ^{o}C for 2 h. After incubation, the absorption spectrum of each samples were recorded and analysed with that of the untreated DNA control.

The binding study of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA was determined at various concentrations by Thermo Scientific UV spectrometer. The K_b of the synthesised complex on *E. coli* gDNA was calculated from the Benesi-Hildebrand equation:

$$\mathbf{1/\Delta A = 1/Kb\ \Delta\varepsilon\ [H]+1/\Delta\varepsilon[G]}$$

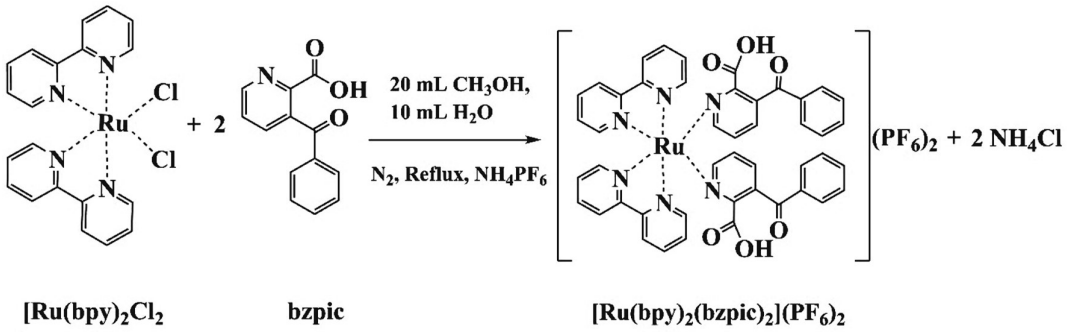
where, [H] = concentration of the host (DNA), [G] = concentration of the guest (complex), **Δ**A = change in the absorbance of the [DNA] on the addition of [complex] and **Δε** = difference in the molar extinction coefficient between the free [DNA] and [DNA]-[Ru] complex. The plot of 1/ **ΔA** *versus* 1/[complex] gave a straight line. The K_b value can be obtained from the ratio of Y-intercept to the slope of the straight line.

3. Results and Discussion

The synthesis, characterization and the binding nature of the novel $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex are discussed in this section. The formation of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex is confirmed from elemental analysis, UV-Visible, FT-IR, ^{1}H NMR, ^{13}C NMR and MALDI-TOF mass spectral analysis. The K_b value of the complex on *E. coli* gDNA is determined from the Benesi-Hildebrand plot.

3.1 Synthesis and Characterization of $[Ru(bpy)_2(bzpic)_2]^{2+}$ Complex

The schematic representation of the synthesis of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex is shown in **Scheme 1**. The synthesized cationic complex involves the coordination of Ru(II) atom with two bidentate bpy and two mono dentate bzpic ligands *via* nitrogen atom of pyridine moiety, forming an octahedral complex. The complex is a dark red coloured stable solid, soluble in water and organic solvents. The synthesized $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex is found to be thermally stable and soluble in water, acetonitrile, acetone, DMSO and DMF. The percentage composition of C, H, O and N of the complex determined by elemental analysis is in accordance with the theoretical value.



Scheme 1 Synthesis of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The absorption spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex in aqueous medium shows peaks at 246, 291.5, 340 and 476.5 nm respectively (**Fig. 1**). The peaks at 246 and 476.5 nm assigned to the dπ - π* MLCT transition. The MLCT transition involves electronic excitation from the metal orbital [dπ (Ru)] to the ligand centered acceptor π* orbitals. The peak at 291.5 nm corresponds to the LC π - π* transition and the shoulder peak at 340 nm is due to metal centered transition.

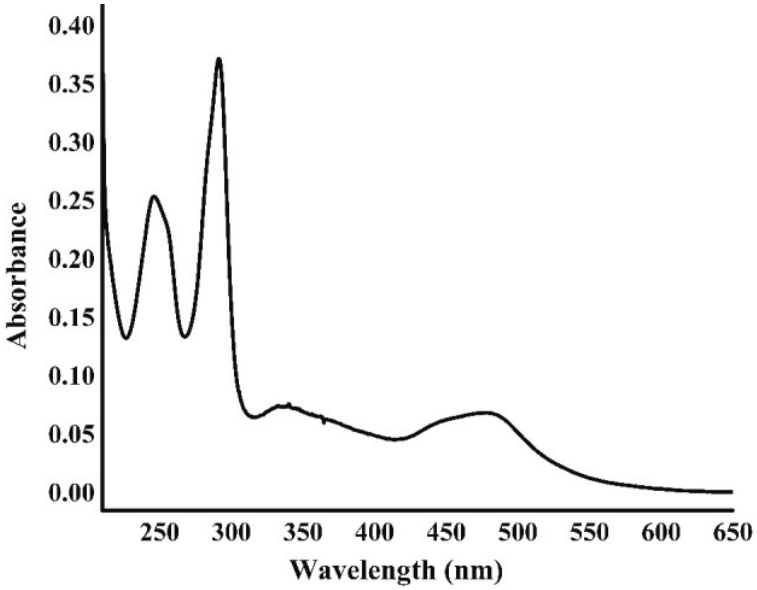


Fig. 1 Absorption spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The FTIR spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex **(Fig. 2)** shows absorption bands at 3623, 2920, 2852, 1788, 1764, 1681, 1662, 1653, 1585, 1558, 1473, 1411, 1379, 1344, 1336, 1321, 1286, 947, 844, 764, 554 and 489 cm^{-1}. The bands at 3623, 1788, 1286, 1411 and 947 cm^{-1} indicate the –OH stretching, C=O stretching and –OH bending frequencies of carboxylic acid group of bzpic ligands. The sharp band obtained at 3623 cm^{-1} designates the existence of free –OH group in bzpic ligand. The peak at 1764 cm^{-1} specifies the C=O stretching of the keto group present in the bzpic ligands. The peaks at 2920 and 2852 cm^{-1} are assigned for aromatic C-H stretching. The bands at 1681, 1662, 1653, 1585, 1558 and 1473 cm^{-1} describes the C=C stretching modes of the pyridine and benzene rings. The peaks at 1379, 1344, 1336, 1321, 1286, 947 and 844 cm^{-1} are assigned for the C-N and C-H stretching and bending vibrations of pyridine and benzene moiety. The weak band occurs at 764 cm^{-1} confirm the coordination of pyridine nitrogen atom of bzpic ligand to the Ru metal. Similarly, the weak absorption bands occur at 554 and 489 cm^{-1} validate the coordination of nitrogen atoms of the bpy ligands to ruthenium metal atom. Thus, the FT-IR spectral data verify the coordination of nitrogen atoms from pyridine and bpy ligands to the Ru metal and confirms the formation of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex.

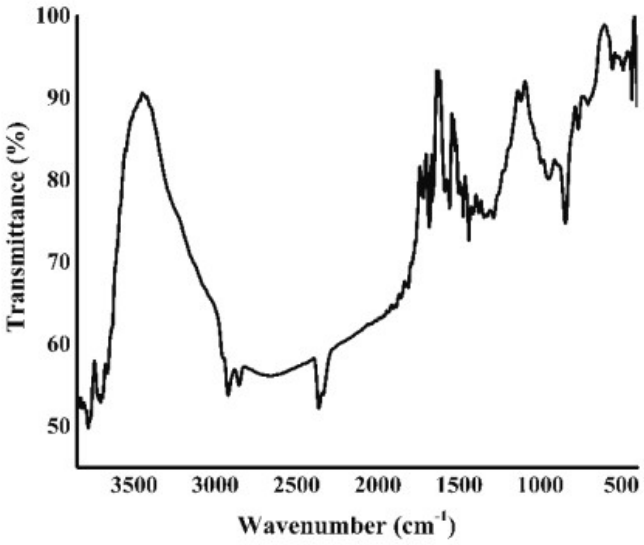


Fig. 2 FTIR spectrum of the $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The ^1H NMR spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$complex recorded in DMSO-d6 is associated with the bpy and bzpic ligands gets coordinated with the pyridine nitrogen atoms of bpy and bzpic ligands to form an octahedral geometry (**Fig. 3**). The H atoms in the bpy ligands coordinated to the Ru atom exhibit chemical shift signals at 8.84 δ, ppm for H^3 and $H^{3'}$ (1H, d); 7.64 ppm for H^4 and $H^{4'}$ (1H, t); 7.36 ppm for H^5 and $H^{5'}$ (1H, t); 8.68 ppm for H^6 and $H^{6'}$ (1H, d) respectively. The ancillary ligand bzpic containing picolinic acid group display pyridine ring proton signals of H^4 at 8.23 ppm (1H, d); H^5 at 8.19 ppm (2H, t) and H^6 at 8.86 δ, ppm (1H, d). The -COOH group in the *ortho*- position of the pyridine nitrogen of picolinic acid exhibit a signal due to O-H proton at 9.99 ppm. The benzoyl group substituted in the *meta*- position of the picolinic acid group shows the signals clearly belongs to the aromatic benzene protons. The five protons of the aromatic ring have the chemical shift values ranges between 7.6 - 7.9 ppm. On complexation with ruthenium metal, the characteristic signals except the protons adjacent to the coordinating nitrogen atoms are deshielded. Similar result is also predicted by Corral and co-workers for the NMR spectrum of $[(Ru(apy)(tpy))_2(l\text{-}H_2N\ (CH_2)6NH_2)]^{4+}$ complex [3].

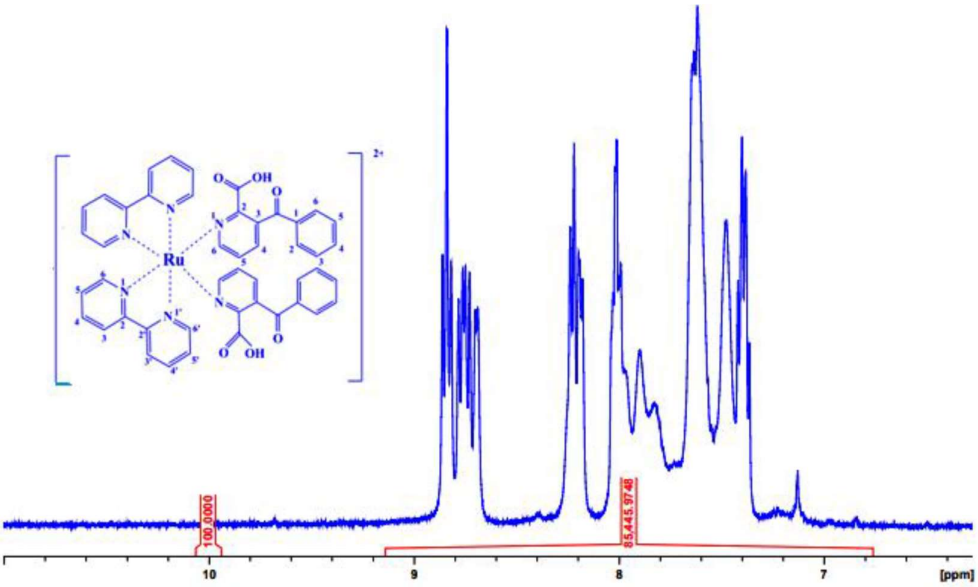


Fig. 3 ^1H NMR spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The ^{13}C NMR spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex in DMSO-d^6 solvent shows a solvent peak in the range from 39.40 - 40.65 ppm (**Fig. 4**). The complex exhibits chemical shift at 171.61 ppm indicate the presence of C=O group of carboxylic acid. The ^{13}C signals of bpy, pyridine and benzene rings exhibit in the same range between 124 - 152 ppm. The signals of benzene ring are predicted to be at 137.54 ppm for C^1, 127.99 ppm for $C^{2,6}$, 129.06 ppm for C^4, 127.53 ppm for $C^{3,5}$ and 124.35 ppm for C^5 respectively. The picolinic acid and the benzene ring are connected through a keto group, the ^{13}C NMR shift of C=O group exhibit at 187.54 ppm respectively. The ^{13}C NMR spectral data of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex is in accordance with its ^1H NMR spectral data and thus confirms the formation of complex A.

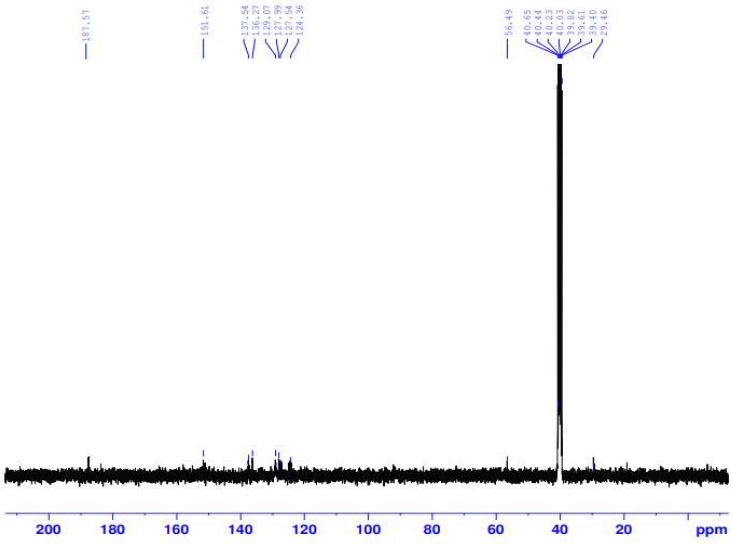


Fig. 4 ^{13}C NMR spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The MALDI-TOF mass spectral data of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex shows molecular ion peak at 1157.137 which corresponds to the ruthenium metal coordinated with two bpy and two bzpic ligands along with $(PF_6)_2$ counter ions (**Fig. 5)**. The molecular ion peak with loss of one PF_6^- and two PF_6^- counter ions show an m/z value of 1012.345 and 867.994 respectively. This obtained value is in accordance with the calculated value. The mass spectral data confirms the formation of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex. Thus, all the spectral data confirms the formation of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex with octahedral geometry.

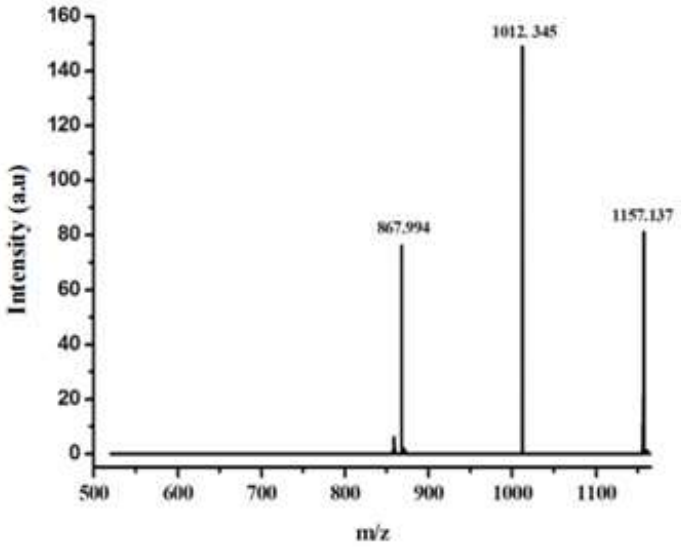


Fig. 5 Mass spectrum of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

3.2. Binding Study of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA

The binding study of the synthesised $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex performed on *E. coli* gDNA at neutral pH determines the mode of interaction and the binding affinity of the complex. The purity of *E. coli* gDNA is determined from the ratio of the absorbance value at 260 and 280 nm and it is found to be 1.7, this value indicates that the DNA is good for downstream studies. The obtained result suggests that the gDNA isolated from *E. coli* is of good quality and is used for DNA binding experiment.

Binding study of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA is carried out by keeping the gDNA concentration (1.5 µg/mL) as constant and varying the complex concentration from 25, 50 and 100 µg/mL. Absorption spectrum of *E. coli* gDNA (1.5 µg/mL) in nuclease free water shows high energy absorptions at 215 and 260 nm and a least minimal absorption at 425 nm respectively. The absorption of E. coli gDNA is mainly due to the DNA base pairs and the phosphate linkages. The incremental addition of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA displays bathochromic and hyperchromic shifts. The changes in the absorbance value and the wavelength by the addition of the synthesised complex indicate that the complex gets intercalated with the secondary structure of gDNA and breaks its double helix structure leading to DNA denaturation. This result is in accordance with the DNA binding study of $[RuCl(CO)(PPh_3)\ L_2]$ complex on ct-DNA [4].

At neutral pH, the DNA bases, guanosine and cytidine get bind with the complex and as a result, broad bands are formed near 255 and 285 nm. At 25 µg/mL, the complex exhibits a slight shift in wavelength at 255 and 285 nm and does not show any marked absorbance, but as the concentration of complex increases from 25 to 100 µg/mL it shows bathochromic and hyperchromic shifts in the LC and MLCT regions. The change in absorbance and wavelength of *E. coli* gDNA by the addition of complex clearly depicts the binding nature of complex on *E. coli* gDNA (**Fig. 6**). This result is in accordance with the previously reported binding study of copper(II) complex with pyridine-2,6-dicarboxylic acid in the presence of 3-amino-1H-1,2,4-triazole and sodium hydroxide on gDNA of *E. coli*. The interactions between the Cu(II) complex and the DNA takes place directly by forming a

novel complex with the DNA double-helical strand and represents an intercalative mode of binding due to interactions of aromatic chromophore of the complex with the DNA base pairs [5].

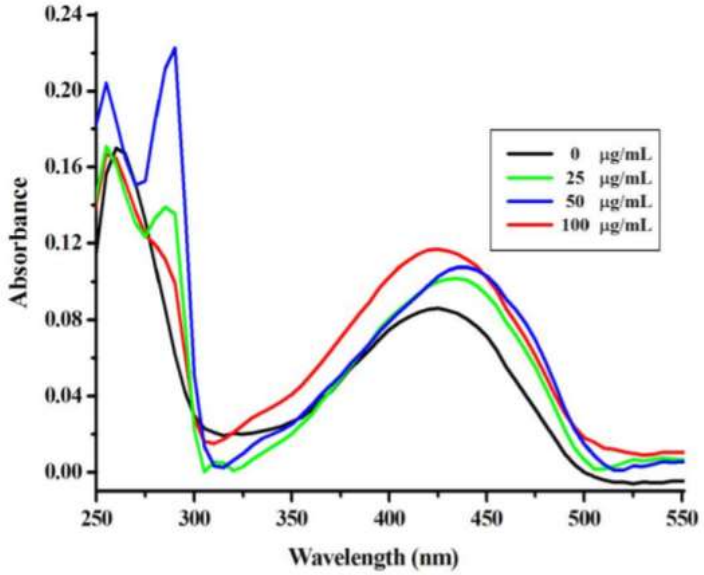


Fig. 6 Absorption spectra of gDNA with incremental addition of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The K_b values of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA obtained from the Benesi Hildebrand plots **(Fig. 7**) are 1.206 x 10^4 M^{-1} and 6.049 x 10^4 M^{-1} for LC and MLCT regions. The K_b values clearly explain that the complex get binds with the DNA more effectively in the MLCT region than that of the LC region.

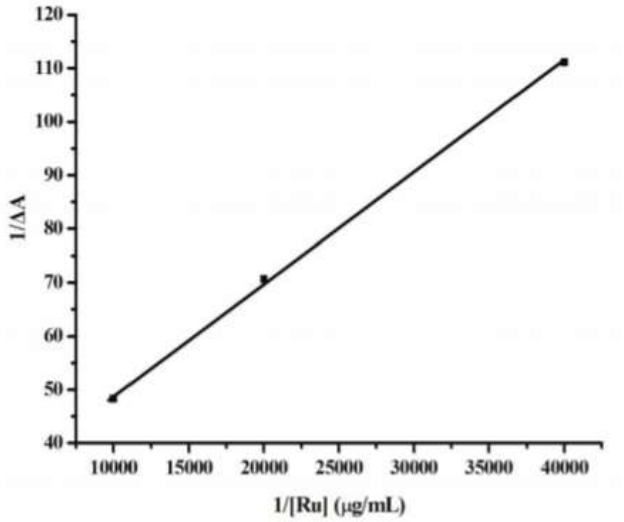


Fig. 7 Benesi-Hildebrand plot of gDNA with incremental addition of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The $[Ru(bpy)_2(bzpic)_2]^{2+}$ contains two bpy and two bzpic ligands, these ligands play a major role for the binding of the complex on *E. coli* gDNA. Therefore, it clearly picturizes that the complex interact with the *E. coli* gDNA through intercalative and electrostatic modes of binding. The cationic complex binds with the DNA base pair through electrostatic interaction. The binding interaction

between the complex and the *E. coli* gDNA is mainly due to the π-π stacking interaction between the aromatic ligands present in the complex and the DNA base pairs. The binding efficiency of the synthesised complex is due to the planar bpy ligand and the presence of the hydrogen atoms in the DNA base pairs which gets coordinated with the carbonyl moiety present in the bzpic ligand of the complex.

Conclusion

The binding nature and binding constant of novel $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA is determine from UV-Visible spectral technique. The synthesised complex binds with the gDNA of *E. coli* through electrostatic and intercalative modes and this can be clearly describing from the absorption spectra of *E. coli* gDNA with the complex at different concentrations. The K_b values of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coli* gDNA validate the binding property of the complex. The results reveal that the complex gets bind with the gDNA more effectively in the MLCT region than that of the LC region and the binding ability depends on the nature of the ligands present in the complex.

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