



e-ISSN: 2319-8753 | p-ISSN: 2347-6710

IJRSET

International Journal of Innovative Research in
SCIENCE | ENGINEERING | TECHNOLOGY

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

Volume 11, Issue 1, January 2022

ISSN INTERNATIONAL
STANDARD
SERIAL
NUMBER
INDIA

Impact Factor: 7.569

9940 572 462

6381 907 438

ijirset@gmail.com

www.ijirset.com



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

Santhiya. S¹, Sheeba Daniel^{2*}

Research Scholar, Department of Chemistry, Holy Cross College (Autonomous), Nagercoil. Affiliated to Manonmanium Sundaranar University, Tirunelveli, Tamilnadu, India¹

Department of Chemistry, Holy Cross College (Autonomous), Nagercoil, Tamilnadu, India^{2*}

ABSTRACT : The binding of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ (bpy- 2,2'-bipyridine, bzpic-3-benzoyl-picolinic acid) complex on *E. coli* genomic DNA (gDNA) has been carried out using UV-Visible spectral technique. The changes in the absorbance and wavelength upon incremental addition of the complex on gDNA clearly depicts the binding nature of complex. The binding constant (K_b) values of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex for ligand centred (LC) and metal to ligand charge transfer (MLCT) transitions obtained from the Benesi Hildebrand plots and are found to be 1.206×10^4 and $6.049 \times 10^4 \text{ M}^{-1}$. The K_b values indicate that the complex get binds with the DNA more effectively in the MLCT region than that of the LC region. The results reveal that the binding interaction between the $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex on *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.

KEYWORDS: $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex; *E. coli* genomic DNA, DNA binding

I. INTRODUCTION

Bacterial gDNA is a highly condensed and functionally organised nucleus-like structure without a nuclear membrane resides inside the cells. The first genome which gets completely sequenced and contributes major to recombinant DNA technology is *E. coli* [1]. It survives when released to the natural environment and gets colonizes in the lower gut of animals allowing widespread dissemination to new hosts [2]. Therefore, *E. coli* is frequently used as a model organism in microbiology and molecular biology studies [3]. Transition metal complexes, in particular, ruthenium polypyridyl complexes that bind to DNA with high affinity, sequence selectivity and induce single- or double-strand cleavage as potential chemotherapeutics [4]. Ruthenium(II) complexes with varying the nature of the substituent group in the intercalating ligands can create some differences in the spatial configuration and electron density distribution, resulting in differences in DNA binding behaviours and photocleavage properties [5]. The present investigation focuses on the binding interaction of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex on *E. coli* genomic DNA

II. EXPERIMENTAL METHODS

$\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$, bpy and bzpic were procured from Sigma-Aldrich. All the solvents and NH_4PF_6 were procured from Merck. HPLC grade solvents and double distilled deionised water were used for the synthesis and the binding study of the complex.

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

The $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex was synthesised by dissolving $[\text{Ru}(\text{bpy})_2\text{Cl}_2]$ (1 mmol) and bzpic (2 mmol) 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 to

recover the complex. Elemental analysis: C = 63.66 %, H = 3.95%, N = 9.68%, O = 11.06% (experimental) C = 65.52%, H = 4.45 %, N = 10.01 %, O = 11.97 % (calculated). The experimental value is in accordance with the theoretical value. The absorption maximum ($\lambda_{\text{abs}}^{\text{max}}$) of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex in CH_3CN was found to be at 476.5 nm. FT-IR (KBr pellet): 3583, 2920, 2852, 1788, 1764, 1653, 1662, 1681, 1585, 1558, 1473, 1411, 1286, 1321, 1336, 1344, 1379, 1321, 947 and 844 cm^{-1} . ^1H NMR (DMSO-d_6 , δ ppm): [bpy: 8.84 (1H, d), 8.68 (1H, d), 7.64 (1H, t), 7.23 (1H, t); bzpic: 9.99 (-OH), 8.86 (1H, d), 8.19 (2H, t), 8.23 (1H, d), 7.89 (1H, d), 7.61 (2H, t) and 7.64 (2H, t)]. ^{13}C NMR (DMSO-d_6 , δ ppm): 151.60 (-COOH), 137.54, 136.26, 129.06, 127.99, 127.53 and 124.35 ppm (aryl carbons). MALDI-TOF-MS: m/z of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2](\text{PF}_6)_2$ complex is found to be 1157.137 (M^+), 1012.345 ($\text{M}^+ - \text{PF}_6^-$) and 867.994 ($\text{M}^+ - 2\text{PF}_6^-$).

2.2 Equipment

The CHNSO Elemental analysis of the synthesized $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex was measured using Truspec Micro Analyser. The absorption spectrum of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex was measured using SHIMADZU UV 1800 double beam spectrophotometer. The FT-IR spectral analysis was measured using SHIMADZU FT-IR double beam spectrophotometer. The ^1H and ^{13}C NMR spectra of the synthesized complex were measured using BRUCKER spectrometer. The MALDI-TOF-MS analysis was carried out to determine the m/z peak using BrukerDaltonics Flex-PC microflex analyser.

2.3 DNA Binding Study

A fixed concentration of *E. coli* gDNA (1.5 $\mu\text{g}/\text{mL}$) in nuclease free water was incubated individually with different concentrations (25, 50, 100 $\mu\text{g}/\text{mL}$) of the synthesized complex in phosphate buffer at pH 7.4 and incubated at 37 °C for 2 h. After incubation, UV-Visible spectra were recorded. The change in absorbance with wavelength in treated samples were analysed and compared with that of untreated DNA control. The binding constant (K_b) of the synthesized complex on *E. coli* gDNA was calculated from the Benesi Hildebrand equation:

$$1/\Delta A = 1/K_b \Delta \epsilon [H] + 1/\Delta \epsilon [G]$$

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

III. RESULTS AND DISCUSSION

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

The structure of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex is shown in Figure 3.1. The synthesized $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex involves the coordination of Ru(II) atom individually with two bidentate bpy and two mono dentate bzpic ligands via nitrogen atom of pyridine moiety, forming an octahedral complex. The synthesized $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex is a dark red coloured stable solid soluble in water, acetonitrile, acetone, DMSO and DMF.

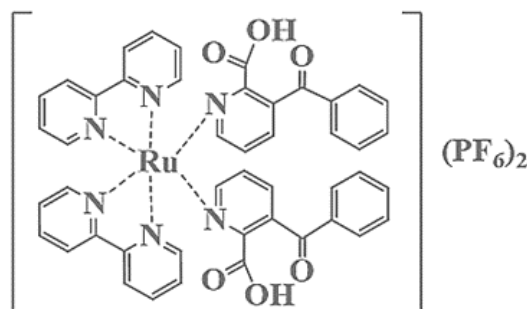


Figure.3.1 Structure of $[\text{Ru}(\text{bpy})_2(\text{bzpic})_2]^{2+}$ complex

3.2 Determination of DNA Purity

The purity and the quantity of the diluted *E. coli* gDNA stock solution prepared from nuclease free water in 0.1 M sodium phosphate buffer at pH 7.4 is determined by UV-visible spectrophotometric method. The absorbance value of *E. coli* gDNA at 260 and 280 nm is noted and the ratio of the two absorbance value is found to be 1.7 (Table 3.1).



This value indicates that the corresponding 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.

Table.3.1 Purity and quantity determination of *E. coligDNA*

DNA source	A ₂₆₀	A ₂₈₀	A ₂₆₀ /A ₂₈₀	DNA concentration (ng/μL)
<i>E. coli</i>	0.17	0.10	1.7	850

3.3 Binding Study of [Ru(bpy)₂(bzpic)₂]²⁺ complex on *E. coligDNA*

The binding study of [Ru(bpy)₂(bzpic)₂]²⁺ complex performed on *E. coligDNA* at neutral pH using UV-Visible spectrophotometric technique determine the mode of interaction and the binding affinity of the complex. The binding study of [Ru(bpy)₂(bzpic)₂]²⁺ complex on *E. coligDNA* are 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. coligDNA* (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. coligDNA* 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. coligDNA* displays bathochromic and hyperchromic shifts. The changes in the absorbance value and the wavelength by the addition of the synthesized 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)(PPh₃)₂] complex on ct-DNA [6].

Addition of [Ru(bpy)₂(bzpic)₂]²⁺ complex on gDNA exhibits shift in absorbance and wavelength, this indicates the interaction of the complex with the gDNA. 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 a slight shift in wavelength at 255 and 285 nm and does not show any marked absorbance, but as the concentration increases from 25 to 100 μg/mL it shows hypsochromic and hyperchromic shifts in the LC region. The incremental addition of [Ru(bpy)₂(bzpic)₂]²⁺ complex on *E. coligDNA*, shows shift in absorbance and wavelength at 425 nm. The MLCT peak of the complex shows bathochromic and hyperchromic shifts at 25, 50 and 100 μg/mL. The change in absorbance and wavelength of *E. coligDNA* by the addition of complex clearly depicts the binding nature of complex on *E. coligDNA* (**Figure 3.2**). 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 [7].

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 gDNA. The complex interacts with *E. coligDNA* through intercalative and electrostatic modes of binding. The cationic complex binds with the DNA base pair through electrostatic interaction. The binding interaction between the [Ru(bpy)₂(bzpic)₂]²⁺ complex and the *E. coligDNA* is mainly due to the π-π stacking interaction between the aromatic ligands present in the complex and the DNA base pairs. Hence, this binding efficiency of the synthesized complex is due to the planar bpy ligand and the presence of the hydrogen atoms in the DNA base pairs get coordinated with the carbonyl moiety present in the bzpic ligand of the complex.

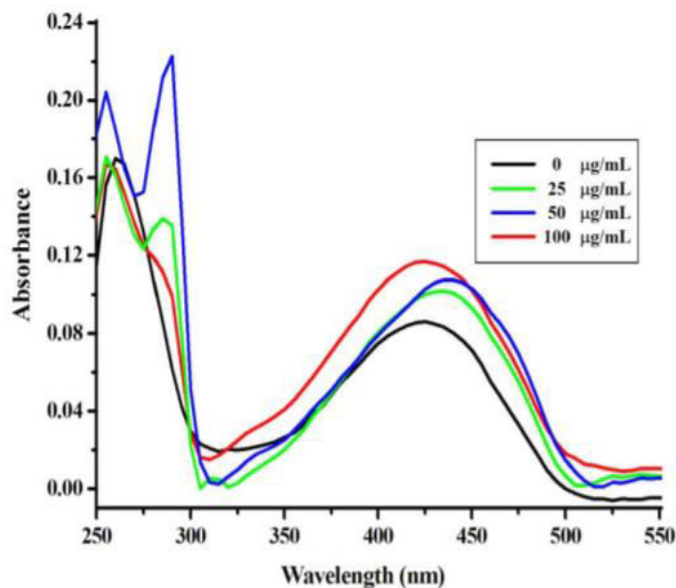


Figure 3.2 Absorption spectra of *E. coligDNA* with incremental addition of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

The K_b values of $[Ru(bpy)_2(bzpic)_2]$ complex on *E. coligDNA* obtained from the Benesi Hildebrand plots (Figure 3.3) are $1.206 \times 10^4 M^{-1}$ for LC region and $6.049 \times 10^4 M^{-1}$ for MLCT region. The K_b values clearly explain that the complex get binds with the DNA more effectively in the MLCT region than the LC region.

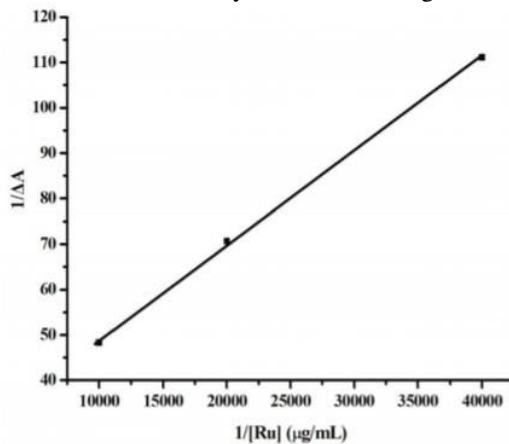


Figure.3.3 Benesi-Hildebrand plot of *E. coligDNA* with incremental addition of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex

IV. CONCLUSION

The present investigation deals with the binding of $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex on *E. coligDNA*. The synthesized $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex binds with *E. coligDNA* through electrostatic and intercalative modes. The binding interaction between the $[Ru(bpy)_2(bzpic)_2]^{2+}$ complex and the *E. coligDNA* is mainly due to the π - π stacking interaction between the aromatic ligands present in the complex and the DNA base pairs.

REFERENCES

- [1]. Launay, A 2016. Study of the emergence of the diversity of Escherichia coli in vivo by whole genome sequencing. Ph.D. Thesis, Université Pierre et Marie Curie-Paris VI.
- [2]. Blattner, F.R., Plunkett, G., Bloch, C.A., Perna, N.T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J.D., Rode, C.K., Mayhew, G.F. and Gregor, J., 1997. The complete genome sequence of Escherichia coli K-12. science, 277(5331), pp.1453-1462.



- [3]. Kurylo, C.M., Alexander, N., Dass, R.A., Parks, M.M., Altman, R.A., Vincent, C.T., Mason, C.E. and Blanchard, S.C., 2016. Genome sequence and analysis of *Escherichia coli* MRE600, a colicinogenic, nonmotile strain that lacks RNase I and the type I methyltransferase, EcoKI. *Genome biology and evolution*, 8(3), pp.742-752.
- [4]. Gao, F., Chao, H. and Ji, L.N., 2008. DNA Binding, Photocleavage, and Topoisomerase Inhibition of Functionalized Ruthenium (II)-Polypyridine Complexes. *Chemistry & biodiversity*, 5(10), pp.1962-1979.
- [5]. İncesu, Z., Blnkli, K., Akalin, G. And Gundogdukaraburu, N., 2013. The Effects Of Some Phenanthroline Ruthenium (Ii) Complexes On A549 Cell Proliferation. *Turkish Journal of Pharmaceutical Sciences*, 10(2).
- [6]. Sathiyaraj, S., Ayyannan, G. and Jayabalakrishnan, C., 2014. Synthesis, spectral, DNA binding and cleavage properties of ruthenium (II) Schiff base complexes containing PPh₃/AsPh₃ as co-ligands. *Journal of the Serbian Chemical Society*, 79(2), pp.151-165.
- [7]. Tabatabaee, M., Bordbar, M., Ghassemzadeh, M., Tahriri, M., Tahrir, M., Lighvan, Z.M. and Neumüller, B., 2013. Two new neutral copper (II) complexes with dipicolinic acid and 3-amino-1H-1, 2, 4-triazole formed under different reaction conditions: Synthesis, characterization, molecular structures and DNA-binding studies. *European journal of medicinal chemistry*, 70, pp.364-371.



Impact Factor:
7.569



INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH

IN SCIENCE | ENGINEERING | TECHNOLOGY

 9940 572 462  6381 907 438  ijirset@gmail.com



www.ijirset.com

Scan to save the contact details