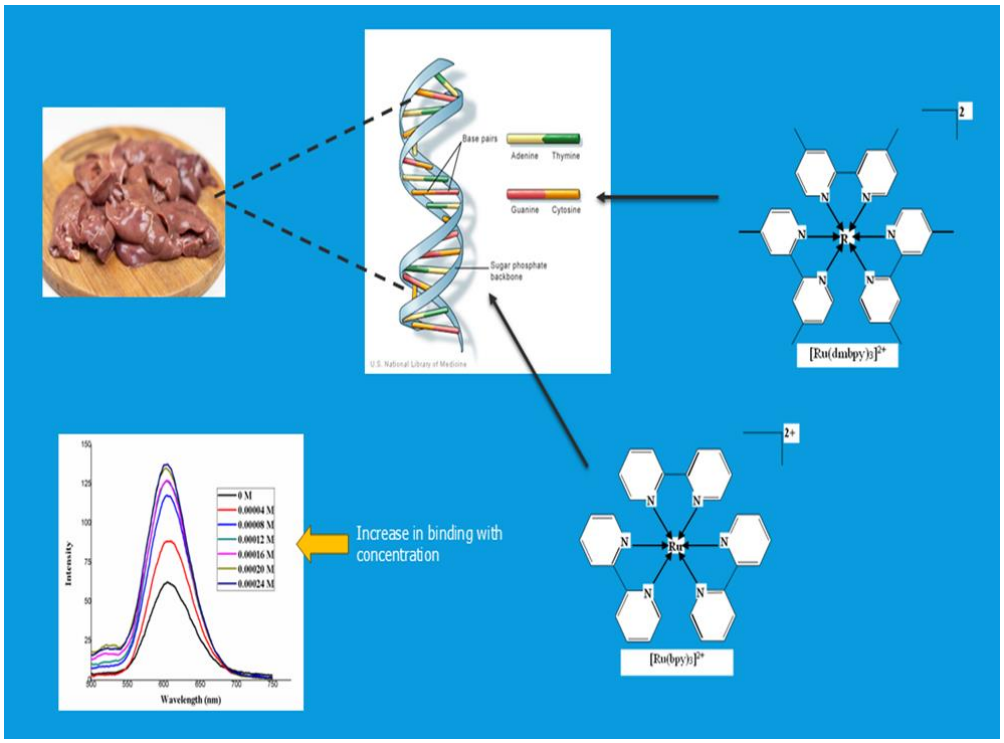
SciRad SCIENTIAE RADICES

Binding studies of Ru(II) complex with DNA isolated from chicken liver extract

Gaflin Shelty, [(1)]✉ Sumitha Celin, Allen Gnana Raj

[(1)] Department of Chemistry and Research Centre, Scott Christian College (Autonomous) Nagercoil, Affiliated to Manonmaniam Sundaranar University, Tamil Nādu, India

✉ Correspondence to: sumithaezhil77@gmail.com



Abstract: Ruthenium polypyridyl complexes have garnered attention due to their promising coordination chemistry and potential applications in biological and photophysical research. This study investigates the synthesis and binding properties of $[Ru(NN)_3]^{2+}$ complexes (where NN= bpy,dmbpy) with DNA models like chicken liver DNA. Using UV – Visible and emission spectroscopy, significant bathochromic shifts were observed, indicating strong interactions and possible intercalative binding. The results highlight the unique affinity and photophysical behavior of Ruthenium polypyridyl complexes, contributing to a deeper understanding of their potential probes as

therapeutic agents. The research opens avenues forfuture explorations in medical and agricultural chemistry.

Keywords: Chickenliverdna, Ruthenium complexes, Emission, Benesi-Hildebrand plot, Binding constant.

Received: 2024.12.15
Accepted: 2025.02.10
Published: 2025.02.13
DOI: 10.58332/ scirad2025v4i1a03

Introduction

Metal complexes are inherently endowed with extensive biological properties. Despite their scant implementation in the pharmaceutical industry as drugs, these compounds are widely used to unveil the structural behavior and functions of nucleic acids and promoters in various fascinating processes. The most investigated metal complexes to date entail platinum and ruthenium central metal ions. First-row transition metal complexes are considered promising candidates for the design of potential anticancer agents [1]. Organo ruthenium complexes are one of the most interesting species among the other platinum group metals due to their flexibility, remarkable biological activity and catalytic activity [2].

In bioinorganic chemistry, the interactions of metal complexes with DNA (deoxyribonucleic acid) have recently attracted attention. The ability of metal complexes to bind and cleave DNA is linked to its use in the synthesis of synthetic restriction enzymes, novel medications, DNA foot printing agents, and other products. Because of their ability to bind DNA through a variety of interactions and cleave the duplex due to their inherent chemical, electrochemical, and photochemical reactivities, metal complexes have been discovered to be very helpful for the aforementioned goals [3-5].

The genetic information necessary for every living organism's cellular efficiency and operation is contained in DNA, a biomacromolecule. Many anticancer medications target DNA as their main target molecule, and the way that DNA and metal complexes bind together has been used to understand how the drugs interact with DNA. Generally speaking, halting the reproduction of the abnormal DNA can destroy the tumor cells. There are three different ways by which metal complexes can cleave DNA: hydrolytic, oxidative, and photolytic cleavages. Since a significant portion of chemotherapeutic anticancer medications contain a substance that binds to DNA and alters DNA within cells, the DNA-metal complex interaction is crucial for the production of novel chemotherapy medications. Furthermore, bioactive metal complexes are practical biological instruments [6-9].

Actually, these complexes have interesting properties like being anti-inflammatory, and having anticancerand antitumor effects when they bind to DNA. Studies on transition

metal complexes with mixed ligands have shown that Ruthenium complexes are useful probes for examining DNA interactions because of their distinct electrical characteristics and stable coordination structures. These complexes frequently exhibit high intercalative binding affinities, which are impacted by the DNA's structural characteristics, including sequence specificity, helical conformation, and GC content. Assessing these interaction potential as therapeutic agents or diagnostic tools requires an understanding of cancer cells, evaluating their cytotoxicity, and researching how they work.

The promise of ruthenium complexes in biomedical applications is demonstrated by their varied binding characteristics with nucleic acids, especially DNA and RNA. Research indicates that ruthenium(II) polypyridyl complexes, like $[Ru(bpy)_2(7\text{-F-dppz})]^{2+}$, exhibit robust intercalative binding to duplex RNA, with binding affinity impacted by environmental factors and ligand substituents [10]. Spectroscopic methods are frequently used to analyze the binding interactions, providing information on the cytotoxicity and mechanisms of action against cancer cells. The capacity of ruthenium-bipyridyl complexes to bind DNA and produce radicals, which might result in cell death, is linked to their cytotoxic effects [11]. Ruthenium complexes have shown promising anticancer properties, with some complexes demonstrating the ability to inhibit DNA replication and induce cytotoxic effects in cancer cell lines. The unique binding properties of these complexes suggest their potential as therapeutic agents, particularly in targeting DNA in cancer cells.

Studies have shown that certain ruthenium complexes possess high binding constants (Kb), indicating strong interactions with DNA. For example, a specific Ru(II) complex exhibited a K_b of 2.2×10^4 M^{-1}, suggesting effective DNA targeting [12]. Ruthenium complexes have been designed to selectively target tumor cells, thus enhancing their therapeutic efficacy. The NBD-Ru complex was shown to penetrate the nucleus and interact with DNA, leading to significant tumor cell death [13]. Certain ruthenium complexes have the potential to be used in photodynamic treatment because they can produce reactive oxygen species when light is activated, which damages DNA and causes cell death [14]. Because of their special interaction with DNA, ruthenium-based medications like NAMI-A and KP1019, which are presently undergoing clinical trials, offer promise as successful cancer treatments.

In this present study binding studies were carried out using the DNA extracted from chicken liver. Chicken livers are high in protein and a rich store of folate, which is important for fertility and helps prevent certain birth defects. Livers are also loaded with iron to give energy and a treasure trove of certain B vitamins, most notably B_{12}. Chicken liver is known to contain a high concentration of nonhistone chromosomal proteins, which are important for studying DNA-binding activities. These proteins play a crucial role in gene regulation, making

chicken liver a valuable source for research in this area. The tightly-bound NHCP from chicken liver chromatin has been shown to exhibit specific DNA-binding activities. This characteristic allows researchers to investigate how these proteins interact with DNA, which is essential for understanding gene expression and regulation. In chicken liver chromatin, nonhistone chromosomal proteins exhibit high-affinity DNA binding. These proteins maintain their binding activity even under high ionic strength conditions, suggesting a robust and sequence-specific interaction with DNA.

Previous studies have indicated that NHCP from chicken liver may have different binding preferences compared to those from other sources, such as rat or mouse liver. The binding of the enantiomer Δ-[Ru(bpy)2MBIP]2+ intercalated into calf thymus DNA more deeply than Λ-[Ru(bpy)2MBIP]2+ exhibiting a better DNA photocleavage ability. This variability provides insights into the evolutionary differences in gene regulation among species**.**

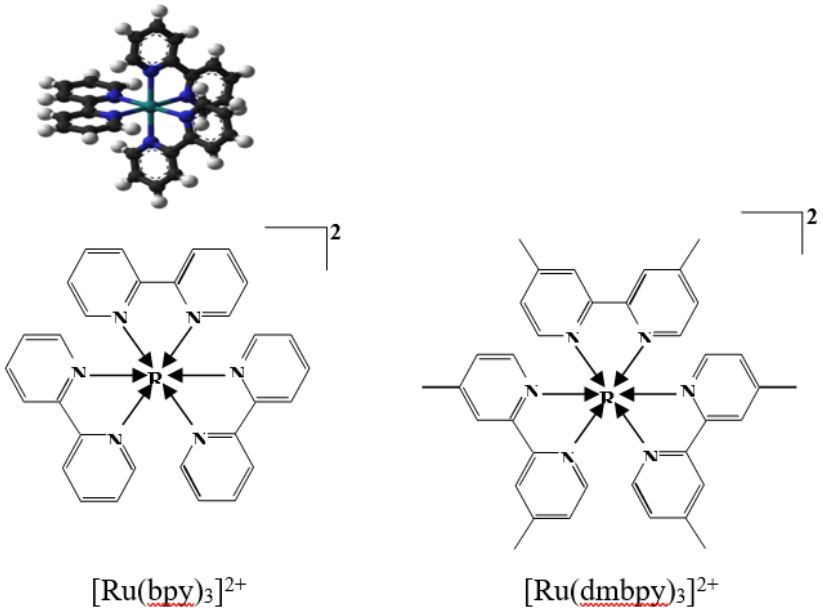
This work establishes that ruthenium complexes as potent tools for examining DNA interactions, creating new avenues for advancements in biotechnology and medicine [15-26].

Table 1. Constituents of chicken liver

Nutrients	Chiken liver (100 g)	Nutrient	Chiken liver (100 g)
Vitamin A	11077 IU	Selenium	54.6 mcg
Vitamin C	17.9 mg	Riboflavin	1.8 mg
VitaminB_{12}	16.6 mg	Folate	577 mg
Vitamin B6	0.9 mg	Iron	9.0 mg
Sodium	71 mg	Potassium	230 mg

Results and discussion

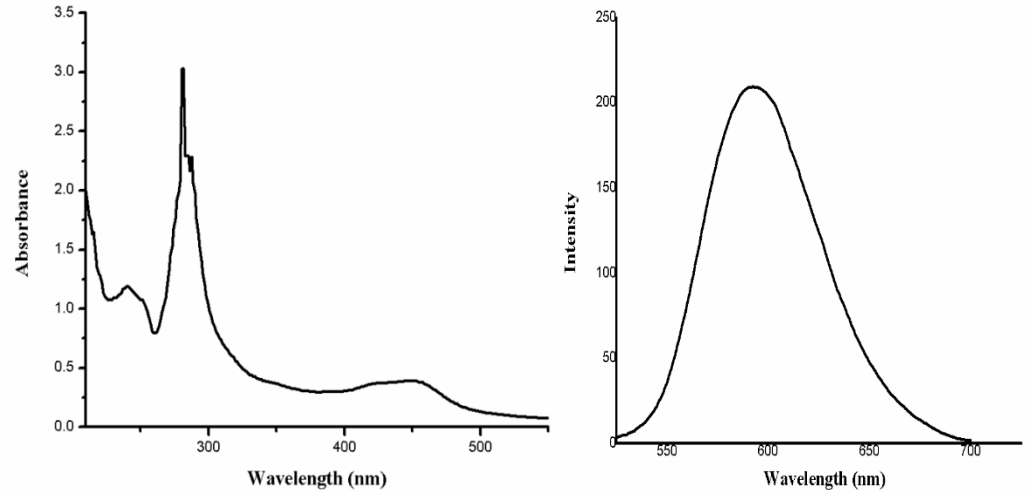
Ruthenium complexes are the most researched complexes because of their photophysical and excited state characteristics. In an aqueous solution, $[Ru(bpy)_3]^{2+}$ exhibits an absorption maximum at 453 nm and an emission maximum at 596 nm. Triplet metal to ligand charge transfer state (^{3}MLCT) is the lowest excited state of $[Ru(bpy)_3]^{2+}$. Three closely spaced, equilibrium excited states that are discernible at 5K but in equilibrium at and above 77K combine to form the lowest ^{3}MLCT. The emission maximum of Ru(II) complexes originates from $d\pi$-π^* ^{3}MLCT transition.



$[Ru(bpy)_3]^{2+}$ $[Ru(dmbpy)_3]^{2+}$

Figure 1. Structure of the chosen Ruthenium complexes.

Absorption and emission spectral measurement



(a) **(b)**

Figure 2. (a) Absorption, (b) emission spectrums of $[Ru(bpy)_3]^{2+}$ complexes in aqueous medium

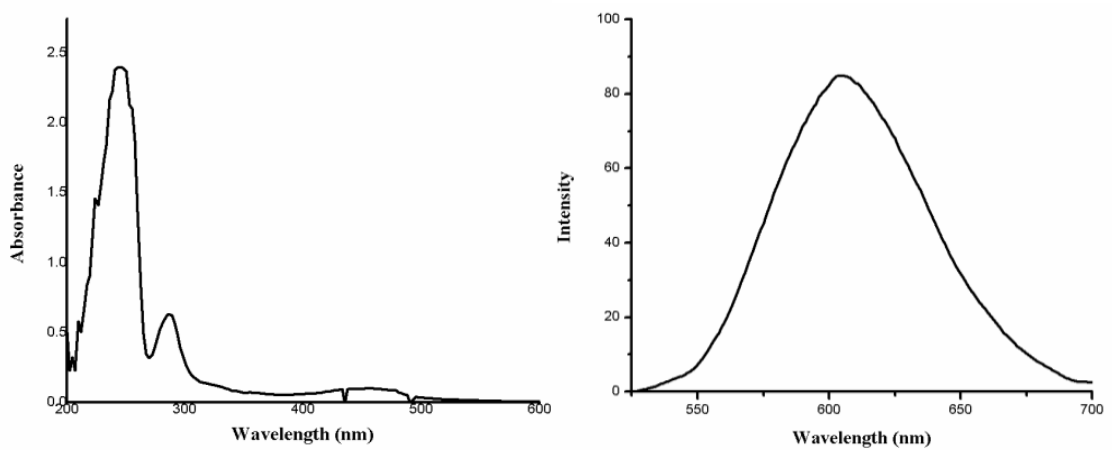


Figure 3. (a) Absorption, (b) emission spectrums of $[Ru(dmbpy)_3]^{2+}$ complexes in aqueous medium

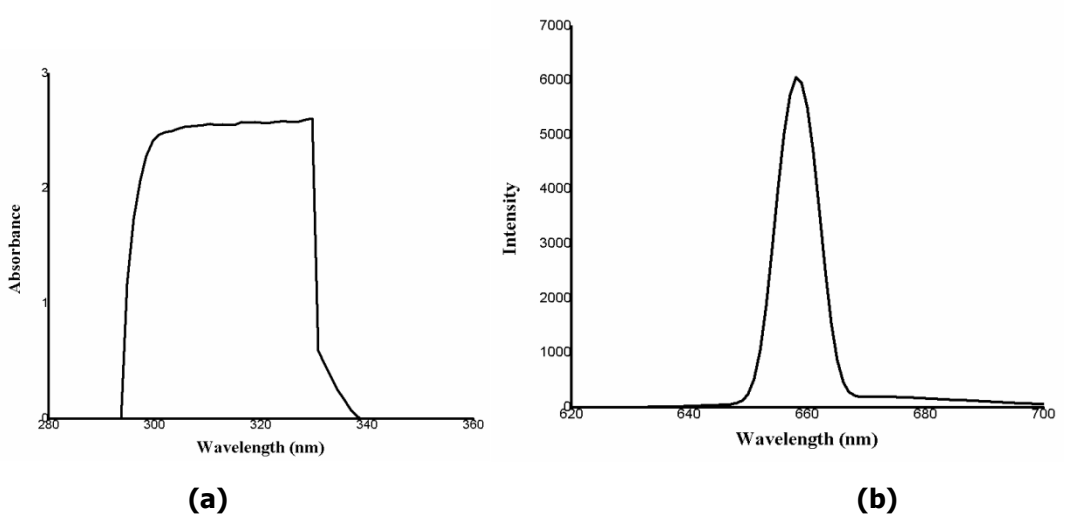
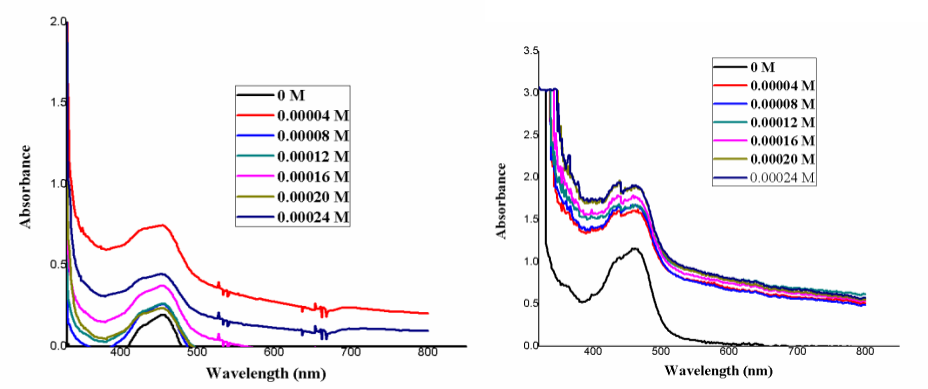


Figure 4. (a) Absorption, (b) emission spectral measurements of Chicken Liver DNA in aqueous medium

Figures 2, 3 and 4 represents the absorption and emission spectra of the two Ru complexes and the DNA extract. In an aqueous solution, the maximal absorption and emission wavelengths for the $[Ru(dmbpy)_3]^{2+}$ complex are 458 nm and 595 nm, respectively.[21] The highest absorption of the chicken liver DNA extract occurs at 329.9 nm.Its emission maximum is seen at 658.5 nm.

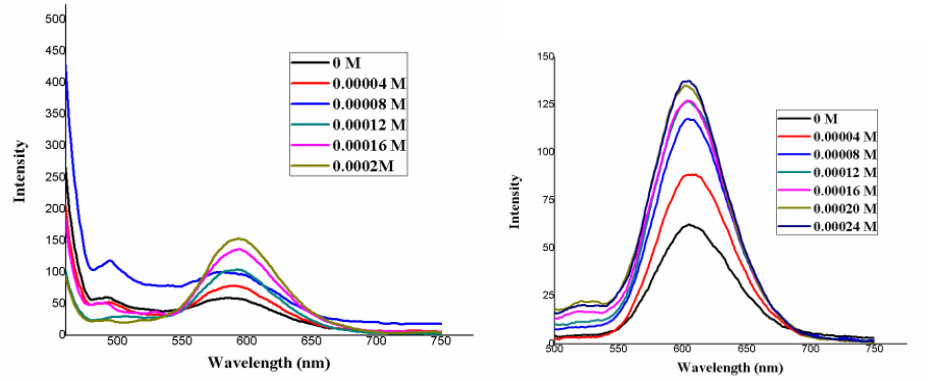
Table 2. Photophysical properties of $[Ru(NN)_3]^{2+}$ and Chicken Liver DNA in aqueous medium.

Complexes	Absorption maximum (nm)	Emission Maximum(nm)
$[Ru(bpy)_3]^{2+}$	453	596
$[Ru(dmbpy)_3]^{2+}$	458	595
Chick Liver DNA	329.9	658.5



(a) **(b)**

Figure. 5 Absorption spectra of (a) $[Ru(bpy)_3]^{2+}$ and (b) $[Ru(dmbpy)_3]^{2+}$ complexes with incremental concentration of the Chicken liver DNA extract.



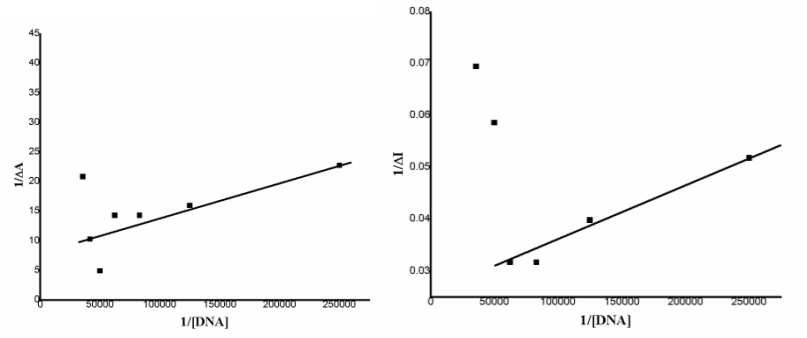
(a) **(b)**

Figure 6. Emission spectra of (a) $[Ru(bpy)_3]^{2+}$ and (b) $[Ru(dmbpy)_3]^{2+}$ complexes with incremental concentration of the Chicken liver DNA extract.

The binding of the DNA extracted fromthe chicken liver with various metal complexes are obtainedusing these data got from absorption as well as emission spectral data. The concentration of the metal complex was kept fixed and the DNA concentration was varied such that the total volume of the DNA-metal complex solution was 5 mL. The absorption and emission measurements were taken for various complex concentrations. The change in absorbance were calculated for the absorption measurements. For the emission spectral data, the change in emission intensity were also calculated. Figures 5 and 6 displays the absorption and emission spectral peaks of the complexes with incremental concentration of the DNA extract. Using these calculations, the binding constant for the drug-metal interaction were found out. This is done using the Benesi-Hildebrand plot.

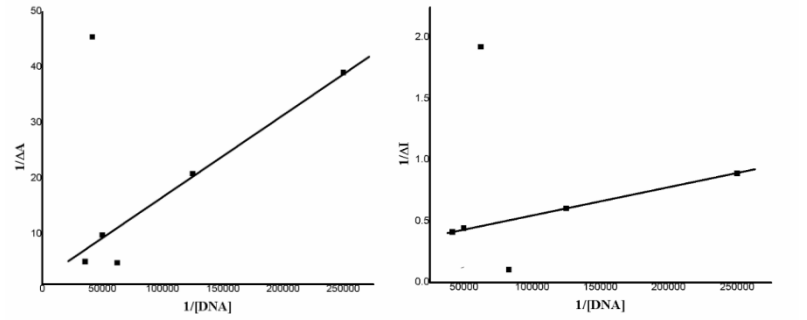
These data obtained from absorption and emission spectrum data are used to calculate the binding of the DNA with different metal complexes. The total volume of the

DNA metal complex solution was maintained at 5 mL by varying the DNA concentration while maintaining a constant metal complex concentration. Measurements of absorption and emission weretaken for different amounts of complex DNA. For the absorption measurements, the variations in absorbance were computed. The change in emission intensity was also computed using the emission spectrum data.



(a) **(b)**

Figure 7. Benesi-Hildebrand plot for the (a)absorption and (b) emission spectra data of $[Ru(bpy)_3]^{2+}$ on binding with the DNA extract.



(a) (**b**)

Figure 8. Benesi-Hildebrand plot for the (a) absorption and (b) emission spectra data of $[Ru(dmbpy)_3]^{2+}$ with incremental concentration of the DNA extract.

Table 3. Binding constant of the chicken liver DNA with $[Ru(NN)_3]^{2+}$ complexes

Complex	Binding type	Intercept	Slope	Binding constant (L/mol) K_b
$[Ru(bpy)_3]^{2+}$	UV bind	10.433	4.677×10^{-5}	2.23×10^{5}
$[Ru(bpy)_3]^{2+}$	Emission bind	0.06426	9.98×10^{-8}	2.23×10^{5}
$[Ru(dmbpy)_3]^{2+}$	UV bind	6.308	1.089×10^{-4}	5.792×10^{4}
$[Ru(dmbpy)_3]^{2+}$	Emission bind	0.6635	6.431×10^{-7}	5.792×10^{4}

Based on the absorption and emission spectral binding data, the binding constant for the samples for different concentration is given in Table 2. These data shows that the complex $[Ru(bpy)_3]^{2+}$ has the highest binding with the chicken liver DNA. Chick liver DNA, being from a complex eukaryotic organism, might have a more intricate secondary structure

(eg. More supercoiling or different level of methylation), which could impact binding. The more compact structure of chicken liver DNA, might explain the enhanced binding affinity of ruthenium complex, as reflected in increased bathochromic shift and stronger bathochromic effect. Chicken liver DNA has higher GC content, which can affect binding interactions.

Material and methods

Sigma Aldrich was the supplier of the ligands 2,2ˈ-bipyridine and 4,4ˈ-dimethyl-2,2ˈ-bipyridine. The study's chick liver was bought locally, and double-distilled deionized water was used for the binding tests. The remaining solvents and chemicals were all of reagent grade and were used exactly as supplied.

Synthesis of Tris (2,2'- bipyridine) Ruthenium (II) Chloride, $[Ru(bpy)_3]Cl_2$

After dissolving 0.5g of $RuCl_3$ $3H_2O$ and 0.6g of 2, 2-bipyridine in 25 mL of ethanol, the mixture was refluxed for 20 hours. The ethanol solution contained the orange-red complex that was produced as a result. Using n-propanol as an eluent, the crude product was purified on a silica gel column.[22] Evaporation was followed by the recovery of the pure complex. The compound in CH_3CN has an absorption maximum (λ^{abs}_{max}) of 448 nm and an emission maximum (λ^{em}_{max}) of 596 nm.

Synthesis ofTris(4,4′-dimethyl-2,2′-bipyridine)ruthenium(II)tetrafluoroborate, $[Ru(dmbpy)_3](BF_4)_2$.

$RuCl_3.3H_2O$ (1 mM) and 4,4ˈ-dimethyl-2,2ˈ-bipyridine (3 mM) were dissolved in 20 mL of ethylene glycol and refluxed for 4 hours. The solution was then allowed to cool at room temperature and filtered to remove any insoluble impurities. A saturated solution of sodium tetrafluoroborate was then added dropwise into the filtrate until an orange precipitate is formed. The product was filtered, washed with cold water and diethyl ether and further dried in a vacuum desiccator. The product was further purified by recrystallisation from water. The absorption maximum (λ_{abs}^{max}) and emission maximum (λ_{em}^{max}) of the complex in CH_3CN are 458 nm and 601 nm respectively.

Synthesis of chicken liver DNA

About 5 g of chicken liver is weighed and added to a cold blender with 150 ml cold saline citrate buffer which is blended for 50-60 secs. The homogenate is centrifuged for 15 mins at 4 C and the supernatant is discarded. This step is repeated 3 times and the pellet is

then dissolved with 20 ml of 2.6 N NaOH and shaken vigorously. Then it is centrifuged for 20 mins to settle the insoluble protein. The supernatant is poured in a beaker and 2-3 volume of 95% of cold ethanol is added through the sides of the beaker. The genetic material floating on the surface is collected using a glass rod and washed with 70% ethanol.

Equipments

The absorption spectrum was recorded using SYSTRONICS Double Beam Spectrophotometer 2203 for both the complexes $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ as well as the binding studies of the produced complexes with the DNA sample. The emission spectrum were taken using JASCO/FP 8200 spectrofluorometer. All the sample solutions used for emission measurements were kept in cold water to ensure that there was no change in the volume of the solution. All measurements were carried out at room temperature.

Determination of association constants using absorption and emission techniques

The association constants (K_a^{abs}) of the $[Ru(NN)_3]^{2+}$ complexes with DNA extracted from Liver, peas and spinach in homogeneous medium were calculated using Benesi – Hildebrand method (eqn.1).[23]

$$\frac{1}{\Delta A} = \frac{1}{K_a^{abs}}\Delta\varepsilon[H] + \frac{1}{\Delta\varepsilon}[Q] \qquad (1)$$

Here, [H] - concentration of the host (sensitizer), [Q] - concentration of the guest (DNA), ΔA - the change in the absorbance of the [H] on the addition of [Q].$\Delta\varepsilon$- difference in the molar extinction coefficient between the free [H] and [H]-[Q]complex. For all the guest molecules examined, plot of $1/\Delta A$ values as a function of 1/[Q] values give good straight line, supporting the 1:1 complex formation. The association constant can be obtained from the ratio of Y-intercept to the slope of the straight line.

Conclusions

Ruthenium bipyridyl complexes have gained attention in medical field, particularly in cancer therapy, due to their unique physio-chemical properties. It is seen that ruthenium bipyridyl complex have shown selective toxicitytoward cancer cells while sparing healthy cells. Their ability to undergo ligand exchange and interact with biomolecules in cancer cells makes them promising for targeting tumor DNA.

Thus, the current endeavor has examined the binding of $[Ru(NN)_3]^{2+}$ (NN = 2,2ˈ-bipyridine,4,4ˈ-dimethyl-2,2ˈ-bipyridine.) with chicken liver DNA extract. In addition to the specifics of the electronic absorption and emission spectral measurements, the photophysical

and photochemical characteristics of these complexes are examined. It has been investigated how the DNA extract bind to the Ruthenium (II) complexes. The evaluation of binding constants were described in detail. According to our current research, the DNA extract has a strong affinity for $[Ru(bpy)_3]^{2+}$ complex. It has the highest binding with the chicken liver DNA with value of 2.23×10^5. This increase in binding constant shows good interaction which is crucial as it suggests that the complexes can effectively bind to DNA, which is a key factor in their potential therapeutic applications.

Acknowledgements

The authors thank the management for providing the DST-FIST lab facilities and the Chemistry Department of Scott Christian College(Autonomous), Nagercoil for carrying out the project work.

References

[1] Akasaka, H.; Mizushina, Y.; Yoshida, K.; Ejima, Y.; Mukumoto, N.; Wang, T.; Inubushi, S.; Nakayama, M.; Wakahara, Y.; Sasaki, R. MGDG Extracted from Spinach Enhances the Cytotoxicity of Radiation in Pancreatic Cancer Cells. *Radiat. Oncol.*; BioMed Central 2016, *11* (1), 153–153. DOI: 10.1186/s13014-016-0729-0

[2] Baskaran, S.; Murali Krishnan, M.; Arumugham, M. N. Photochemical and DFT/TD-DFT Study of Trifluor Ethoxy Substituted Asymmetric Metal Free and Copper(II) Phthalocyanines. *Inorg. Nano Met. Chem.* 2017, *47* (2), 269–277. DOI: 10.1080/15533174.2016.1186039

[3] Baskaran, S.; Murali Krishnan, M.; Arumugham, M. N.; Kumar, R. Synthesis, DFT Analysis and DNA Studies, Cytotoxicity and Luminescence Properties of a DinuclearCopper(II) Complex with 1,10-Phenanthroline and 4-Aminobenzoate. *J. Coord. Chem.* 2019, *72* (5–7), 941–961. DOI: 10.1080/00958972.2019.1584295

[4] Berlin Dinosha, D. S.; Sumitha Celin, T.; Allen Gnana Raj, G. Binding Studies of Ruthenium Complexes with Antidiabetic Drug. *Acta Chim. Asiana* 2024, *7* (2), 509–519.

[5] Chen, Y.; Li, W.; Yang, Y.; Zhong, R.-X.; Hu, H.; Huang, C.-Y.; Chen, J. M.; Liang, L. N.; Liu, Y. Significant Increase of Anticancer Efficacy *In Vitro* and *In Vivo* of Liposome Entrapped Ruthenium(II) Polypyridyl Complexes. *Eur. J. Med. Chem.* 2023, *257*, 115541–115541. DOI: 10.1016/j.ejmech.2023.115541

[6] Ezhilarasan, D.; Saravanan, P. C.; Arumugham, M. N. Synthesis, Characterization and DNA Binding of Copper(II) Complexes with Mixed Ligands of Heterocyclic Bases, L-valine

and Imidazole: Studies on Anti-microbial and Anticancer Activities, *JCBPS*, *10* (1) (A), 033–044.

[7] Geierstanger, B. H.; Mrksich, M.; Dervan, P. B.; Wemmer, D. E. Design of a G,C-Specific DNA Minor Groove –binding Peptide. *Science* 1994, *266* (5185), 646–650. DOI: 10.1126/science.7939719

[8] Hafez, E.; Shati, A. The Potential Anticancer Action of Lectin Extracted from *Pisum sativum* Against Human Hepatocellular Carcinoma Cell Lines. *Egypt. J. Hosp. Med.* 2016, *65* (28), 683–692.

[9] Hu, H.; Zhang, H.; Zhong, R.; Yang, Y.; Huang, C.; Chen, J.; Liang, L.; Chen, Y.; Liu, Y. Synthesis, RNA-Sequence and Evaluation of Anticancer Efficacy of Ruthenium(II) Polypyridyl Complexes Toward HepG2 Cells. *J. Inorg. Biochem.* 2023, *244*, 112230. DOI: 10.1016/j.jinorgbio.2023.112230

[10] Huang, H.; Zhang, P.; Chen, Y.; Qiu, K.; Jin, C.; Ji, L.-N.; Chao, H. Synthesis, Characterization and Biological Evaluation of Labile Intercalative Ruthenium(II) Complexes for Anticancer Drug Screening. *Dalton Trans.*; 2016, *45* (33), 13135–13145. DOI: 10.1039/c6dt01270a

[11] Iqbal, J.; Abbasi, B. A.; Mahmood, T.; Kanwal, S.; Ali, B.; Shah, S. A.; Khalil, A. T. Plant-Derived Anticancer Agents: A Green Anticancer Approach. *Asian Pac. J. Trop. Biomed.* 2017, *7* (12), 1129–1150. DOI: 10.1016/j.apjtb.2017.10.016

[12] Jiang, X. Binding Properties of Ruthenium(II) Polypyridyl Complexes [Ru(bpy)2(L)]2+ (L = 7-F-dppz and 7-OCH3-dppz) with Poly(A)-Poly(U) Under Dilute and Molecular Crowding Conditions. *J. Mol. Struct.* 2024, *20*, 138585. DOI: 10.1016/j.molstruc.2024.138585

[13] Mahmood, K.; Akhter, Z.; Fouzia Perveen, A.; Bibi, M.; Ismail, H.; Tabassum, N.; Yousuf, S.; Ashraf, A. R.; Qayyum, M. A. Synthesis, DNA Binding and Biological Evaluation of Benzimidazole Schiff Base Ligands and Their Metal (II) complexes. *RSC Adv.* 2023, *13* (18), 11982–11999.

[14] Nie, Y.; Dai, Z.; Fozia, G. Z.; Zhao, G.; Jiang, J.; Xu, X.; Ying, M.; Wang, Y.; Hu, Z.; Xu, H. Comparative Studies on DNA-Binding Mechanisms Between Enantiomers of a Polypyridyl Ruthenium(II) Complex. *J. Phys. Chem. B* 2022, *126* (26), 4787–4798. DOI: 10.1021/acs.jpcb.2c02104

[15] Pandya, C.; Sivaramakrishna, A. Organoruthenium-Bipyridyl Complexes – A Platform for Diverse Chemistry and Applications. *Coord. Chem. Rev.* 2024, *504*, 215655. DOI: 10.1016/j.ccr.2024.215655

[16] Sreekantha.B., Krishnamurthy G., BhojyaNaikc H.S., Vishnuvardh Fe(II) and Zn(II) complexes containing mixed ligand of 1,10-phenanthroline and 2-hydroxy-4-methyl1,8-naphthyridine *J. Chem. Pharm. Res*. 3(5):407-419.

[17] Pratviel, G.; Bernadou, J.; Meunier, B. A Hydroxyl-Bridged Dinuclear Copper Complex Having Planar Structure Shows Efficient DNA Cleavage Activity in Aqueous Solution. *Angew. Chem. Int. Ed. Engl.* 1995, *34* (7), 746–769. DOI: 10.1002/anie.199507461

[18] Puttaswamy, N. Y.; Mahanta, P.; Sarma, P.; Medhi, C.; Kaid, S. M. A.; Kullaiah, B.; Basumatary, D.; Manjasetty, B. A. Structure-Based Biological Investigations on Ruthenium Complexes Containing 2,2′-Bipyridine Ligands and Their Applications in Photodynamic Therapy as a Potential Photosensitizer. *Chem. Biol. Drug Des.* 2023, *102* (6), 1506–1520. DOI: 10.1111/cbdd.14341

[19] Saravanan, P. C.; Murali Krishnan, M.; Arumugham, M. N(2019) DNA Binding and Cytotoxicity Studies of Ternary Copper(II) Complexes Containing Heterocyclic Bases and Thiourea. *Int. J. Pharm. Sci. Res.* 2019, *10* (1), 148–156.

[20] Zhao, Z.; Zhang, X.; Li, C.-E.; Chen, T. Designing Luminescent Ruthenium Prodrug for Precise Cancer Therapy and Rapid Clinical Diagnosis. *Biomaterials* 2019, *192*, 579–589. DOI: 10.1016/j.biomaterials.2018.12.002

[21] Sumitha Celin, T.; Allen Gnana Raj, G. Micellar Effect on the Photoinduced Electron Transfer Reactions of Ruthenium(II)-Polypyridyl Complexes with Quinones. *J. Chem. Mater. Res.* 2017, *6* (2, 3), 31–36.

[22] Balou, S.; Zarkadoulas, A.; Koukouvitaki, M.; Marchiò, L.; Efthimiadou, E. K.; Mitsopoulou, C. A. Synthesis, DNA-Binding, Anticancer Evaluation, and Molecular Docking Studies of Bishomoleptic and Trisheteroleptic Ru-Diimine Complexes Bearing 2-(2-Pyridyl)-Quinoxaline. *Bioinorg. Chem. Appl.* 2021, 2021, 5599773.

[23] Elgar, C. E.; Yusoh, N. A.; Tiley, P. R.; Kolozsvári, N.; Bennett, L. G.; Gamble, A.; Péan, E. V.; Davies, M. L.; Staples, C. J.; Ahmad, H.; Martin, R. Gill. Ruthenium(II) Polypyridyl Complexes as FRET Donors: Structure- and Sequence-Selective DNA-Binding and Anticancer Properties-Christopher. *J. Am. Chem. Soc.* 145 (2), 1236–1246. DOI: 10.1021/jacs.2c11111

[24] Gates, D. M.; Bekhor, I. DNA-Binding Activity of Tightly-Bound Nonhistone Chromosomal Proteins in Chicken Liver Chromatin. *Nucleic Acids Res.* 1979, 6 (10), 3411–3426.

[25] Navale, G.; Singh, S.; Agrawal, S.; Ghosh, C.; Roy Choudhury, A.; Roy, P.; Sarkar, D.; Ghosh, K. DNA Binding, Antitubercular, Antibacterial and Anticancer Studies of Newly

Designed Piano-Stool Ruthenium(II) Complexes. *Dalton Trans.* 2022, 51 (42), 16371–16382.

[26] Nie, Y.; Dai, Z.; Fozia; Zhao, G.; Jiang, J.; Xu, X.; Ying, M.; Wang, Y.; Hu, Z.; Xu, H. Comparative Studies on DNA-Binding Mechanisms Between Enantiomers of a Polypyridyl Ruthenium(II) Complex. *J. Phys. Chem. B* 2022, 126 (26), 4787–4798.