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RESEARCH ARTICLE

Binding studies of ruthenium (II) complexes with DNA isolated from pea *(Pisumsativum)* extract

L.A. Gaflin Shelty, T. Sumitha Celin* and G. Allen Gnana Raj

Department of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamil Nadu 629003, India

Abstract- The increasing impact of group 8–10 metal organometallic complexes academically and industrially over the years demonstrates remarkable progress in harvesting useful reagents, catalysts, and chemotherapeutic agents. Recently, these complexes have been widely analyzed for their photophysical properties and medical applications as they efficiently treat different types of cancer through photodynamic therapy (PDT). Understanding the interaction of bipyridyl complexes with specific pea*(Pisumsativum)*genes could show how these metal complexes influence plant traits. Peas, mainly through their genetic modifications, show promise in cancer treatment by serving as bioreactors for producing therapeutic antibodies. The pea genome contains a large number of genes involved in various biological processes, including those responsible for plant growth, development, and stress responses. Ru (II) polypyridyl complexes have shown excellent DNA binding results. These complexes bind to DNA through the intercalative mode. The binding of Ru(II) polypyridyl complex $[RuL_3]^{2+}$ (where L= bpy, dmbpy) with DNA of pea extracts has been studied in an aqueous medium through absorption and emission spectral techniques. The binding constant (K_b) for these reactions is determined from the Benesi-Hildebrand equation using both the absorption intensity data and emission studies.

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1. Introduction

Organo ruthenium complexes are one of the most interesting species among other platinum group metals due to their flexibility, remarkable biological activity, unusual photophysical activity, catalytic properties, etc. These complexes exhibit intriguing photophysical properties, making them important in various applications. Organo ruthenium complexes are highlighted for their potential in photodynamic therapy [1][2], a treatment method for various cancers [3][4]. This is particularly relevant given the limitations of existing platinum-based drugs, such as severe side effects and drug resistance. Since there are several limitations in using platinum complexes in cancer treatment because of their toxic effects [5], there is a strong demand for the development of alternative solutions. The bipyridyl-based ruthenium complexes help design efficient dye-sensitized solar cells and efficient anticancer and antimalarial drugs. The increased bioactivity of these complexes is linked to their lipophilicity and the ability to release bioactive ligands from the Ru center. The shape of the metal complex also plays a crucial role in its effectiveness. The concentration of the complexes and the aryl units in the ligands significantly influence their interaction with DNA [6]. For evaluating the medicinal properties, biological assays would be necessary. This could involve testing the efficacy of the complexes in photodynamic therapy against cancer cells, assessing their cytotoxicity [7], and studying their mechanisms of action. Ruthenium complexes exhibit diverse binding properties with nucleic acids, particularly DNA and RNA, showcasing their potential in biomedical applications [8]. Studies reveal that ruthenium (II) polypyridyl complexes, such as [Ru(bpy)2(7-F-dppz)]2+, demonstrate strong intercalative binding to duplex RNA, with binding affinity influenced by ligand substituents and environmental conditions [9]. Similarly, ruthenium (III) hydroxamate complexes show groove binding to CT-DNA, confirmed by hypochromism and red shifts in UV-visible spectra [10][11]. Furthermore, new organo-ruthenium (II) complexes exhibit significant binding efficiency with CT-DNA and human serum albumin, indicating their potential for drug delivery [12]. The binding interactions are often characterized by spectroscopic techniques, revealing insights into the mechanisms of action and cytotoxicity against cancer cells. DNA binding is one of the key points for which ruthenium bipyridyl complexes are studied. As these complexes can intercalate or bind through electrostatic interaction to the DNA molecule, they act as potential agents in photodynamic therapy, which is an innovative treatment modality that utilizes photosensitizers activated by specific wavelengths of light to target and destroy cancerous cells and other pathological tissues. DNA is made of two linked strands that wind around each other to resemble a twisted ladder — a shape known as a double helix. Each strand has a backbone made of alternating sugar (deoxyribose) and phosphate groups. Attached to each sugar is one of four bases: adenine (A), cytosine (C), guanine (G), or thymine (T). Deoxyribonucleic acid (abbreviated DNA) is the molecule that carries genetic information for the development and functioning of an organism.

The DNA of peas *(Pisum sativum*) has been studied extensively due to its historical significance in genetics, notably through Gregor Mendel's pioneering experiments that laid the foundation for the laws of inheritance. When it comes to the interaction of metal complexes with pea DNA, there are several potential research areas to explore, including genetic manipulation, gene expression studies, and stress response mechanisms. *Pisum sativum* L. seeds, recognized as a valuable agro-industrial by-product, are notably rich in polyphenolic compounds and DPPH radical scavenging activity, while maceration resulted in the greatest levels of flavonoids and tannins [13]. These findings reinforce the potential of pea seeds for applications in both the medicinal and food industries. Peas (Pisum sativum) exhibit significant anticancer

***CORRESPONDING AUTHOR | T. Sumitha Celin** | ✉ sumithaezhil77@gmail.com

properties attributed to their rich composition of bioactive compounds that have cytotoxic activity [14]. Research highlights various mechanisms through which these compounds exert their effects, making peas a promising candidate for cancer prevention and treatment [15][16]. Bipyridyl metal complexes may be used in the study of pea genetics, particularly in manipulating specific genes involved in the growth of disease resistance [17]. For example, complexes that bind with DNA could help in silencing undesirable traits or enhancing beneficial ones, such as those related to yield and stress tolerance. By targeting specific genes related to growth and yield, bipyridyl metal complexes could play an important role in pea productivity. This could be useful in breeding programs aimed at improving the nutritional content or resistance to environmental stresses. The binding affinity of metal complexes to DNA can be influenced by the size and structure of the ligands attached to the metal, with larger ligands generally increasing binding strength [17]. By binding to DNA regions involved in defense mechanisms, bipyridyl metal complexes could be employed in the development of pea varieties that are more resistant to pests and diseases. This could reduce the need for chemical pesticides, contributing to a more sustainable environment [17]. Metal complexes can be used to enhance genetic transformation in plants, as demonstrated by the use of bio-engineered nanoparticles in pea plants. The use of metal complexes in DNA binding can also lead to novel interactions that influence biological activity, potentially aiding in the development of crops with improved resistance to biotic and abiotic stresses [18][19]. Studies show that metal complexes can induce DNA cleavage, which may be harnessed for targeted genetic modifications or pathogen resistance in crops [20] [21]. The synthesized metal complexes demonstrate antimicrobial activity against various pathogens, suggesting potential applications in protecting crops from diseases [20]. While the binding of metal complexes to DNA presents promising applications in agriculture, concerns regarding the potential toxicity of heavy metals and their environmental impact must be addressed. The balance between beneficial effects and ecological safety remains a critical consideration in the development of these biotechnological applications.

2. Material and Methods

Sigma Aldrich supplied the ligands 2,2ˈ-bipyridine (≥ 99 % purity) and 4,4ˈ-dimethyl-2,2ˈ-bipyridine (≥ 98 % purity). The study's pea*(Pisum sativum*) was acquired locally, and double-distilled deionized water was used for the binding assays. All of the other chemicals and solvents were of reagent grade and employed precisely as directed.

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

The mixture was refluxed for 20 hours after 0.5g of $RuCl_3$ $3H_2O$ and 0.6g of 2, 2-bipyridine were dissolved in 25 mL of ethanol. The resulting orange-red complex was present in the ethanol solution. Using n-propanol as an eluent, the crude product was purified on a silica gel column.22 The pure complex was recovered after evaporation. The absorbance maximum (λ_{abs}^{max}) of the chemical in CH_3CN is 448 nm.

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

After dissolving $RuCl_3.3H_2O$ (1 mM) and 4,4ˈ-dimethyl-2,2ˈ-bipyridine (3 mM) in 20 mL of ethylene glycol, the mixture was refluxed for four hours. Any insoluble contaminants were filtered out after allowing the solution to cool to ambient temperature. Next, dropwise additions of a saturated sodium tetrafluoroborate solution were made to the filtrate until an orange precipitate appeared. A vacuum desiccator was used to dry the product further after it had been filtered and cleaned with cold water and diethyl ether. The product was recrystallized from water to further purify it. The compound in CH_3CN has an absorption maximum (λ_{abs}^{max}) of 458 nm and an emission maximum (λ_{em}^{max}) of 601 nm.

2.3 Extraction of DNA from Pisum Sativum Extract

About 10 g of peas (*Pisum sativum*) 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 minutes to settle the insoluble protein. The supernatant is poured into a beaker, and 2-3 volumes of 95% cold ethanol are 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.

2.4 Equipment

In addition to the binding analyses of the generated complexes with the DNA sample, the SYSTRONICS Double Beam Spectrophotometer 2203 was used to record the absorption spectrum for both the complexes $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$. The JASCO/FP 8200 spectrofluorometer was used to capture the emission spectrum. To guarantee that the volume of the sample solutions used for emission measurements did not vary, they were always stored in cold water. At room temperature, all measurements were performed.

2.5 Determination of Association Constants Using Absorption and Emission Techniques

The Benesi-Hildebrand method (Eq.(1)) was used to determine the association constants (K_a^{abs}) of the $[Ru(NN)3]^{2+}$ complexes with DNA isolated from peas in a homogenous medium [15].

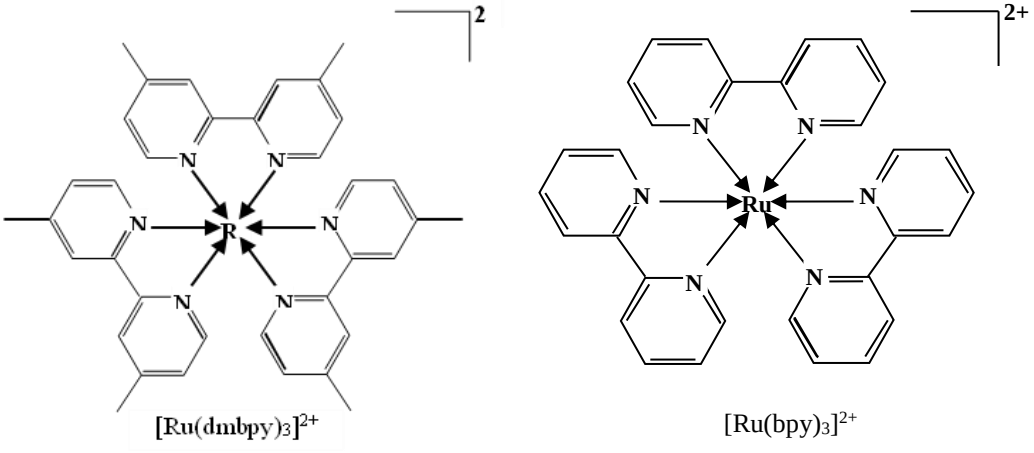
$$\frac{1}{\Delta A} = \frac{1}{K_a abs}\Delta\varepsilon[H] + \frac{1}{\Delta\varepsilon}[Q] \quad (1)$$

In this case, [H] represents the host's (sensitiser) concentration, [Q] represents the guest's (DNA) concentration, and /A represents the change in [H] absorbance upon the addition of [Q]. The molar extinction coefficient of the free [H] and [H]-[Q] complexes differs. The 1:1 complex formation is supported by the plot of 1/A values as a function of 1/[Q] values for each of the guest molecules that were studied. The ratio of the Y-intercept to the straight line's slope yields the association constant.

3. Results and Discussion

3.1 Structure of the Complexes

The molecular structure of the synthesised complexes is shown in Figure 1.

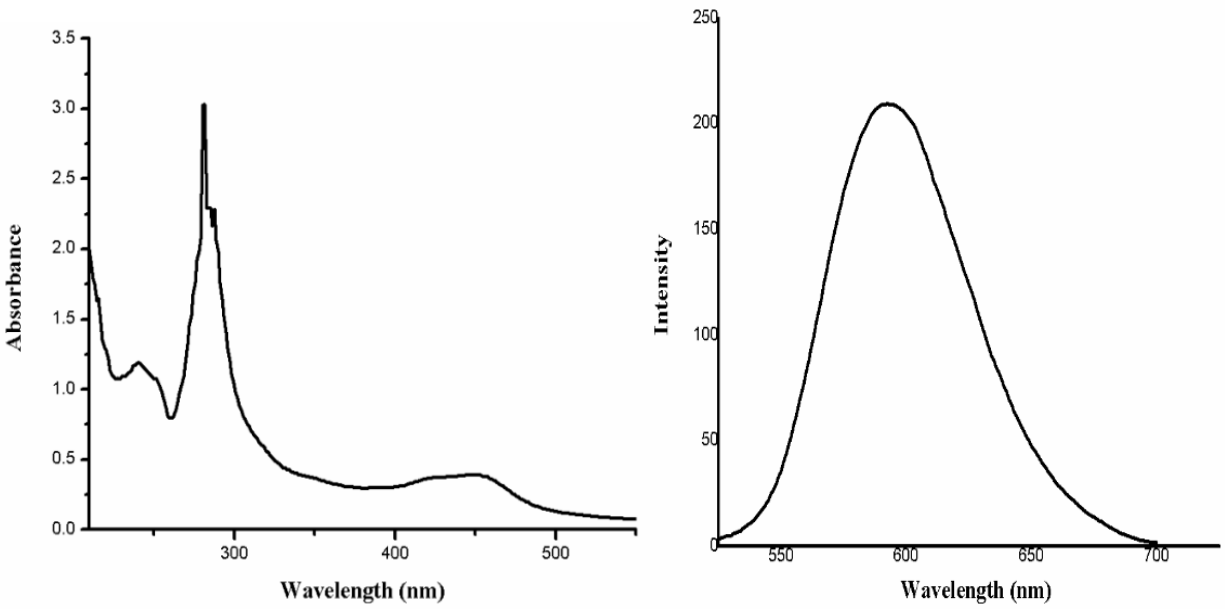


(a) (b)

Figure 1. Structure of the chosen Ruthenium complexes (a) $[Ru(dmbpy)_3]^{2+}$ (b) $[Ru(bpy)_3]^{2+}$

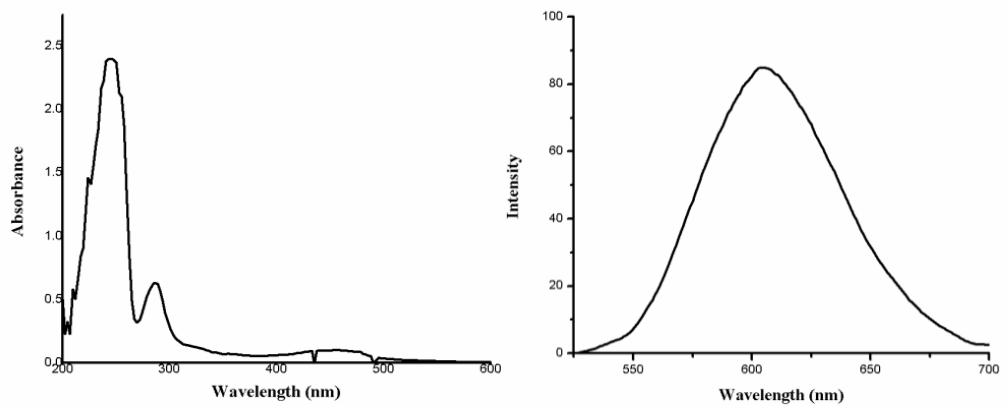
3.2 Absorption and Emission Spectral Measurement

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. The 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 the dπ-π* ^{3}MLCT transition. Figures 2, 3 and 4 represent 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.The highest absorption of the Pea DNA extract occurs at 431.6 nm. Photophysical properties of $[Ru(NN)_3]^{2+}$ and pea DNA in aqueous medium are summarized in Table 1.



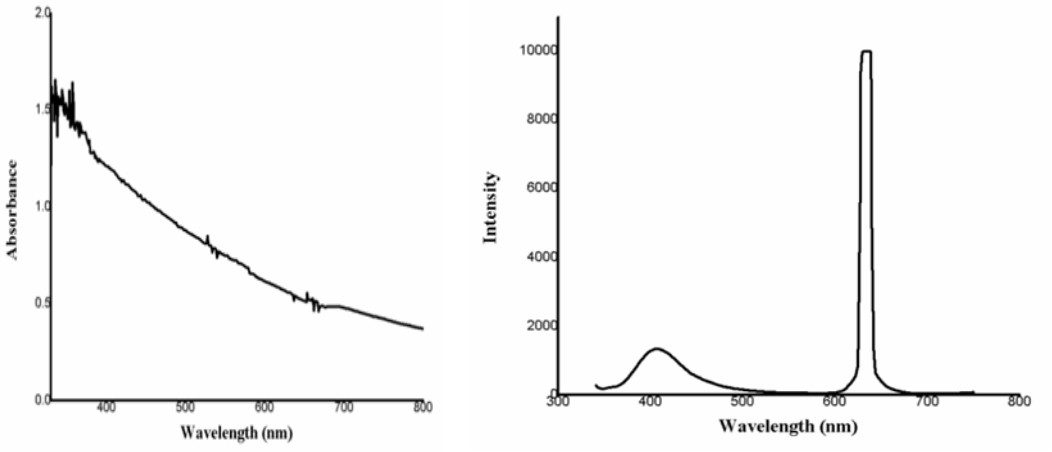
(a) (b)

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



(a) (b)

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

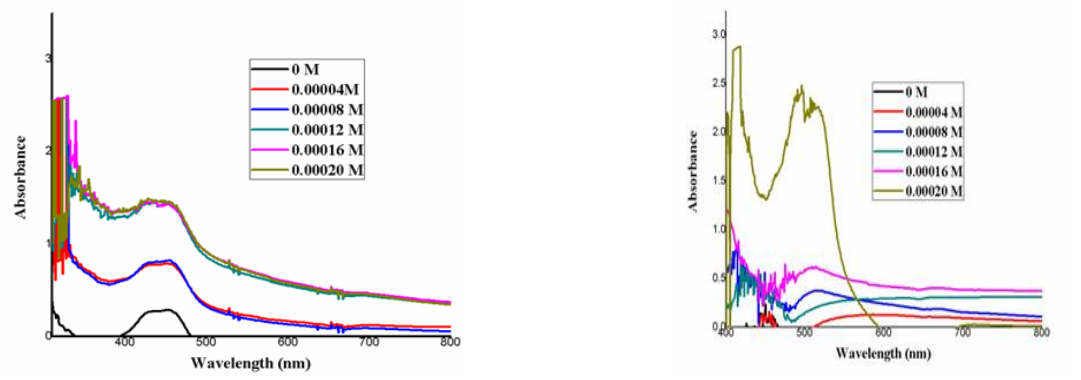


(a) (b)

Figure 4. (a) Absorption (b) emission spectrum of pea DNA in aqueous medium

Table 1. Photophysical properties of $[Ru(NN)_3]^{2+}$ and pea DNA in aqueous medium

Complexes	Absorptionmaximum (nm)	Emission Maximum(nm)
$[Ru(bpy)_3]^{2+}$	453	596
$[Ru(dmbpy)_3]^{2+}$	458	595
Pea DNA	431.6	-



(a) (b)

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

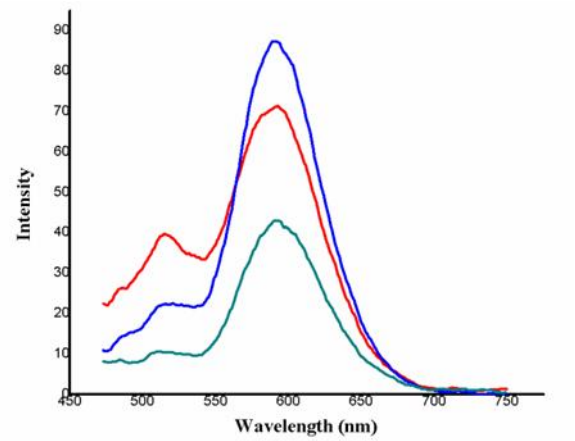
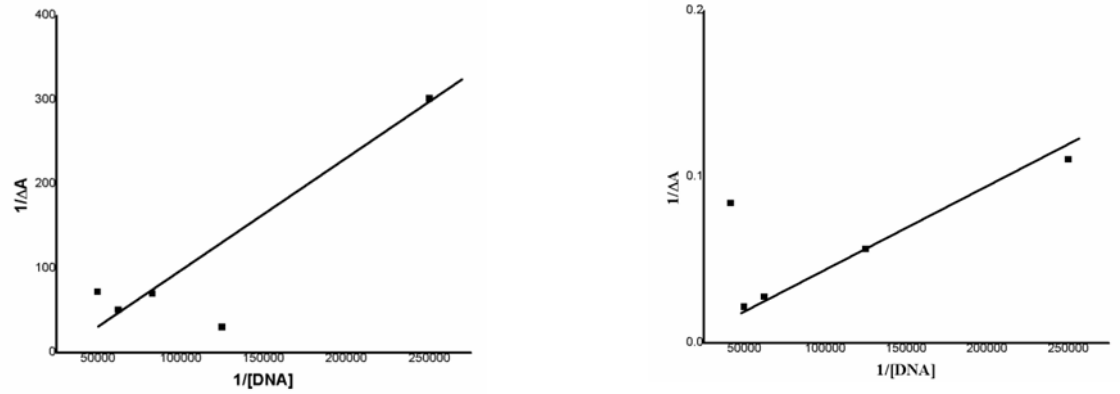


Figure 6. Emission spectra of $[Ru(bpy)_3]^{2+}$ complex with incremental concentration of the Pea DNA extract

These data obtained from absorption and emission spectrum data are used to calculate the binding of the DNA isolated from peas 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. For different complex concentrations, measurements of absorption and emission were made. For the absorption measurements, the change in absorbance was computed. The change in emission intensity was also computed using the emission spectrum data. The absorption and emission spectrum peaks of the complexes with increasing DNA extract concentration are shown in Figures 5 and 6. These computations were used to determine the drug-metal interaction's binding constant. The Benesi-Hildebrand plot is used for this. These absorption and emission spectra measurements are used to determine how well DNA binds to various metal complexes. By adjusting the DNA concentration while keeping the metal complex concentration constant, the overall volume of the DNA metal complex solution was kept at 5 mL. Absorption and emission measurements were performed for varying concentrations of complex DNA. Variations in absorbance were calculated for the absorption data. The emission spectrum data was also used to calculate the change in emission intensity. Figures 7 and 8 shos the Benesi - Hildebrand plot for the absorption and emission spectral data of the complexes. Based on the absorption and emission spectral binding data, the binding constant for the samples for different concentrations is given in Table 2. These data show that the complex $[Ru(dmbpy)_3]^{2+}$ has the highest binding with the pea DNA. In the interaction of pea DNA with ruthenium complex, the UV-visible spectroscopy intercalation is indicated by a bathochromic shift with the increase in concentration of the DNA sample. This reflects strong interaction as the π-π* transitions of the DNA bases are affected. Pea DNA's specific base composition, particularly the ratio of guanine-cytosine (GC) to adenine-thymine (AT), can influence how well the ruthenium complex binds. GC–rich regions tend to form stronger π-π stacking interactions with metal complexes, which enhances binding.



(a) (b)

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

Table 2. 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	33.36	0.00121	2.757×10^{4}
	Emission bind	0.1156	6.77×10^{-8}	-
$[Ru(dmbpy)_3]^{2+}$	UV bind	117.94	9.66×10^{-5}	12.20×10^{5}
	Emission bind	1.032	2.83×10^{-6}	3.6×10^{5}

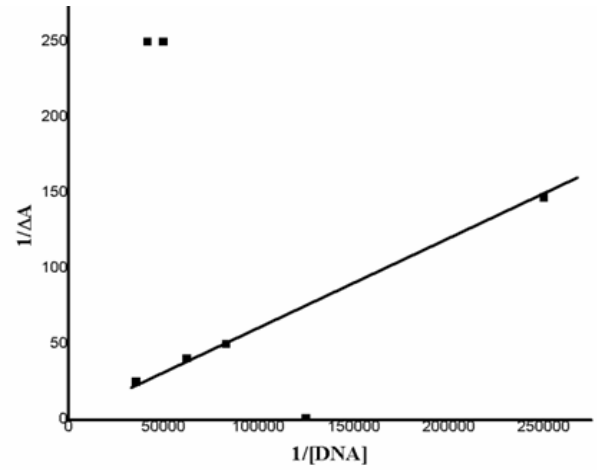


Figure 8. Benesi-Hildebrand plot for the absorption spectra data of $[Ru(dmbpy)_3]^{2+}$

4. Conclusions

The ability of these Ru complexes to undergo ligand exchange and interact with biomolecules in cancer cells makes them promising for targeting tumor DNA. Some ruthenium bipyridyl complexes are photosensitive and can be activated by light, generating reactive oxygen species that damage cancer cells. This makes them attractive in photodynamic therapy (PDT). The complexes $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ will be bound to the DNA by the intercalation method, and as seen from the absorption and emission graph, the peak rises with the increase in concentration in the DNA sample. The lift in the peak indicates the increase in the binding of the complex with the DNA sample. The complex $[Ru(dmbpy)_3]^{2+}$ shows the highest binding with pea extract DNA with a binding constant of 12.20×10^5 L/mol. This type of binding of ruthenium complex to the plant DNA could be harnessed for precise gene editing, enabling the insertion, deletion, or modification of target genes in plant genomes. Binding constants were evaluated by plotting the Benesi-Hildebrand plot. These studies reveal that poorly soluble drugs can be complexed with metal polypyridyl complexes and can be administered so that their solubility is quite enhanced. Thus, the current endeavor has examined the binding of $[Ru(NN)_3]^{2+}$ (NN = 2,2'-bipyridine, 4,4'-dimethyl-2,2'-bipyridine) with pea 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 binds to the Ruthenium (II) complexes. The evaluation of binding constants was described in detail. According to our current research, the DNA extract has a strong affinity for $[Ru(dmbpy)_3]^{2+}$ complex. It has the highest binding with the pea DNA, with a value of 12.20×10^5 L/mol. 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.

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Declaration of Competing Interest

The author declares no conflicts of interest.

CRediT Authorship Contribution Statement

L.A.Gaflin Shelty (Methodology; Investigation; Data Curation; Writing- Original draft.)
T.Sumitha Celin (Conceptualization; Supervision; Validation; Writing- review and editing.)
G.Allen Gnana Raj (Project administration; Formal Analysis).

Availability of Data and Materials

The data supporting this study's findings are available on request from the corresponding author.

Ethics Declarations

This study did not involve human participants or animals. Ethical approval was therefore not required.

Generative Artificial Intelligence Declarations

The authors claim that artificially intelligent-assisted technologies, such as generative AI, were not used to generate content, ideas, or theories. We have just utilised AI to enhance readability and refine the language. This was used with extreme human control and oversight. The authors take full responsibility for reviewing and approving the content.

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