An Investigation Into how Onion DNA May Attach to Ruthenium (II) Polypyridyl Complexes

*Dr. Deepa Saxena

Abstract

Utilising absorption and emission spectrum techniques, it has been determined how the" Ru(II) polypyridyl complex $[RuL^3]^{2+}$ (where L=bpy, dmbpy)" binds to onion DNA in an aqueous solution. The range between 445 and 460 nm is where both compounds' MLCT absorption maxima are located. The Benesi-Hildebrand equation is used to compute the binding constant (Kb) for various activities using data from absorption intensity and emission research. At the ground state, a hydrophobic interaction occurs between the DNA molecule and luminophore. These complexes use intercalative mode to attach to DNA. The Kb value relies on the DNA's makeup as well as the ligand used.

Keywords: DNA, electrostatic interaction, binding constant, metal complex.

Introduction

DNA is the basic unit of genetic information in living things and is necessary for cells to operate properly. It has been shown that metals like ruthenium interact with DNA and change its structure, which may have a significant influence on biological functions. Due to their high selectivity and potency, ruthenium (II) polypyridyl complexes have received a lot of attention for their potential as DNA-targeting medications. But it is still unclear how they interact with DNA and what processes underlie such connections. This study looks on the possibility of onion DNA binding to ruthenium (II) polypyridyl complexes and the variables influencing this interaction.

Due to their high selectivity and potency, ruthenium (II) polypyridyl complexes have received a lot of attention for their potential as DNA-targeting medications. These complexes generally have two or more polypyridyl ligands bound to a ruthenium ion in the +2 oxidation state. The polypyridyl ligands provide a very stiff and stable structure that may interact with DNA through electrostatic interactions, intercalation, or groove binding (Kelland, 2007). The process of metal ion binding to DNA can occur via different modes, including electrostatic attraction between the positively charged metal ion and the negatively charged DNA backbone, as well as intercalation and groove binding modes where the ligand is inserted between the base pairs of DNA. The polypyridyl complex may interact with DNA to cause DNA damage, block DNA replication, and eventually cause cell death.

Materials and methods

The ligands 2, 2' bipyridine and 4,4'- dimethyl-2,2' -bipyridine were purchased from Sigma without being purified. Aldrich. The two $[Ru(NN)_3]^{2+}$ complexes [where NN=2,2' bipyridine (bpy), 4,4' -dimethyl-2, 2' bipyridine (dmbpy)] were produced in accordance with the previously disclosed process by reacting $RuCl_3.3H2O$ with the suitable ligands. Binding assays were carried out using water that had been doubly distilled. Onion powder, salt, ethanol, detergent, and liquid soap could be found at nearby shops. Ethanol was used for the DNA isolation at a 95% concentration.

Synthesis of Ru(II)-polypyridine complexes:

Ru(II) ions must normally be coordinated to two or more polypyridine ligands in order to create Ru(II)-polypyridine complexes. These complexes may be made in a variety of ways, but one process that is often used includes reacting ruthenium(III) precursor compounds with polypyridine ligands in the presence of reducing agents. The creation of stable Ru(II)-polypyridine complexes is made possible by the reduction of ruthenium(III) ions to ruthenium(II) ions.

A typical method for creating a Ru(II)-polypyridine complex is as follows:

Various techniques may be used to synthesise the polypyridine ligand, depending on the desired structure and functionality. One such technique includes the formation of a bipyridine or phenanthroline ligand by the interaction of a pyridine derivative with a halogenated pyridine derivative in the presence of a base.

How to make ruthenium(III) precursor compounds: Ruthenium(III) precursor compounds may be made by reacting a suitable ligand with a ruthenium salt, such ruthenium chloride, in the presence of a base. Normally, a complex with a coordination number of six is formed as a consequence of this reaction. This complex may then be further broken down into one with a coordination number of four.

Coordination of the polypyridine ligand to the ruthenium ion: The ruthenium(III) precursor solution is then added, and the mixture is agitated while being stirred in an inert environment. The ruthenium(III) ion is then reduced to a ruthenium(II) ion by the addition of a reducing agent, such as zinc or sodium borohydride, so that it can coordinate with the polypyridine ligand.

The reaction typically occurs at room temperature or slightly higher.

The resultant complex may be purified using a variety of techniques, including column chromatography and recrystallization.

Overall, to assure the development of a stable and well defined complex, the reaction conditions for the synthesis of Ru(II)-polypyridine complexes must be carefully controlled. Due to their excellent selectivity and efficacy, the resultant complexes have been thoroughly investigated for their potential as DNA-targeting medications.

Extraction of DNA fragments from onion:

Onions were cut into small pieces. About 20 mL of water were used in the beaker. About 1.5 tablespoons of salt are added to the water, which is then thoroughly stirred to dissolve the salt. Two tablespoons of dish soap should be included. Onion mince, detergent solution, and table salt were mixed. The mixed mixture is then poured into a fresh beaker. The heated concoction was then whirled over a flame with the help of a tea strainer, and filtered out into a fresh beaker. The ethanol was kept in a petri plate. The filtered solution was pipette out, and drops of it were added to the petri plate. After a time, DNA pieces were visible. After a time, DNA pieces were visible. DNA is not made soluble by alcohol. When ethanol is added, all of the mixture's elements, except DNA, stay dissolved. In the ethanol layer, the DNA separates and condenses (Figure 1). The produced DNA is spooled onto a glass rod. The isolated DNA, which resembled white mucus, was used for the binding experiments.

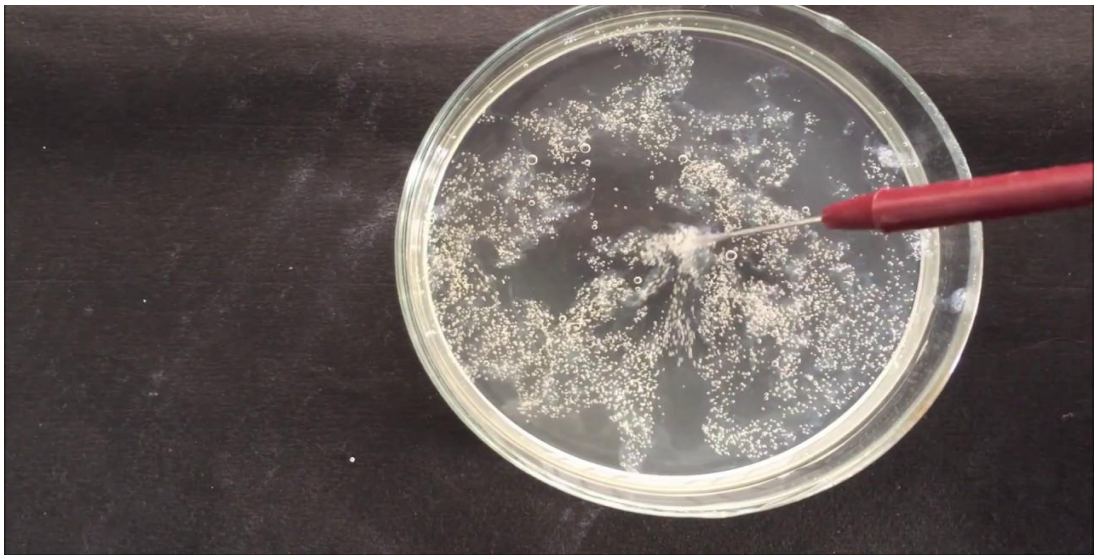


Figure 1 shows the DNA structure created from onions.

The Findings and Discussion

Measurements of the absorption spectrum were made using the SYSTRONICS 2203 Double Beam Spectrophotometer. Emission studies were recorded using a JASCO/FP 8200 Spectrofluorometer. The $[Ru(NN)_3]^{2+}$ concentration was established at $2x10^{-5}$M, whereas the DNA concentration varied from $4x10^{-6}$ to $2.8x10^{-7}$M. The same sample solutions were used for the emission and absorption tests. All sample solutions for the emission studies were kept in cold water to ensure that the volumes did not change. All tests were performed at room temperature. Figure 2 shows the two Ruthenium polypyridyl complexes' structures.

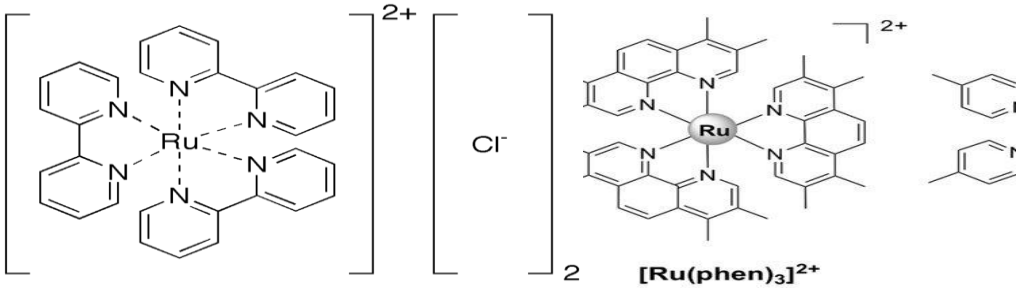
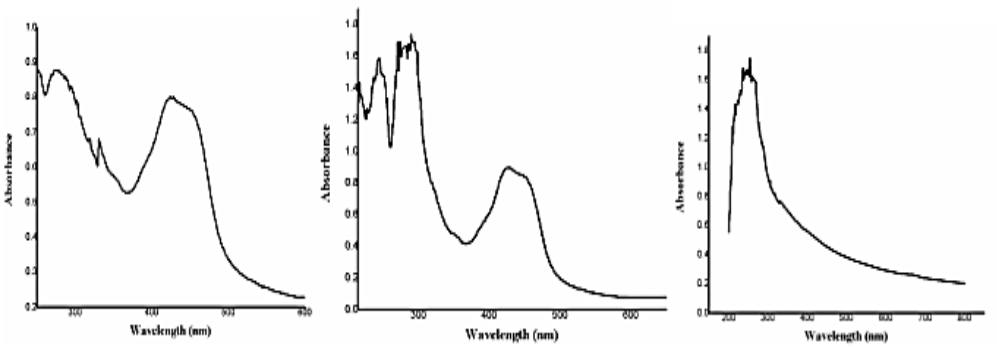
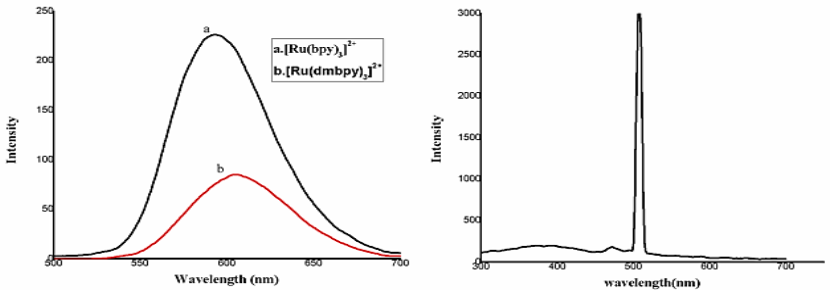


Figure-2: Structure of the Ruthenium polypy



(a) (b) (c)

Figure 3 displays the absorption spectra of (a) [Ru(bpy)3]2+, (b) [Ru(dmbpy)3]2+ complexes, and (c) DNA in an aqueous medium.



A B

Figure 4 displays the emission maximum of (a) Ruthenium polypyridyl complexes and (b) DNA in an aqueous medium.

Figure 3 displays the absorption spectra for [Ru(NN)3]2+ complexes, with [Ru(bpy)3]2+ being the most researched due to its photophysical and excited state properties. This complex has a maximum absorption of 453 nm and emission at 596 nm, as seen in Figure 4. Onion DNA has a maximum absorption at 263 nm, resulting in an emission maximum of 509 nm. The DNA concentration was determined using the absorbance ratio A260/A280, which was between 1.80-1.90, and the molarities were calculated using the equation DNA = 6600mol-1 cm-1 L. Table 1 shows the photophysical characteristics.

Table 1 presents the spectral properties of Ruthenium polypyridyl complexes and DNA in an aqueous medium.

Complex	Absorption maximum(nm)	Emission maximum(nm)
$[Ru(bpy)_3]^{2+}$	448	593
$[Ru(dmbpy)_3]^{2+}$	458	605
DNA	263	509

Electronic absorption spectroscopy is a crucial method for investigating the interactions between metal complexes and DNA. By measuring the absorption and emission spectra of various metal complexes with DNA at varying concentrations, researchers can determine the extent of DNA binding. To conduct this experiment, the metal complex concentration is held constant while the DNA concentration is varied, and the volume of the solution is kept constant at 5 mL. The absorbance change and change in emission intensity are calculated from the data. The Benesi-Hildebrand plot is used to determine the binding constant for the DNA-metal interaction, allowing for the quantitative computation of the binding constant K and the stoichiometry of non-bonding interactions.

The Benesi-Hildebrand method is commonly used for chemical equilibria that produce one-to-one complexes such as charge-transfer complexes and guest-host molecule complexation. The method's theoretical basis is that the electronic absorption spectra of one of the reactants are transparent in the overall absorption/emission range of the reactant system when either one of the reactants is present in excess amounts over the other reactant. This method enables the determination of the association constant of the reaction by comparing the reaction's absorption spectra before and after the creation of the product and its equilibrium.

In the case of complexes containing [Ru(NN)3]2+ and DNA, the Benesi-Hildebrand equation is used to calculate the binding constant (Kb) using the absorption intensity ratio. This approach is useful for evaluating the DNA-binding ability of metal complexes and determining the potential of these complexes as therapeutic agents. The findings from this research have the potential to assist in creating and advancing metal-containing medications that can selectively target DNA and be utilized to cure various ailments.

$$\frac{1}{\Delta A} = \frac{1}{K_b}\Delta\varepsilon\,[H] + \frac{1}{\Delta\epsilon}[Q] \qquad (1)$$

Where A stands for the difference in complex absorbance at different DNA quantity levels ([Q]). The concentration of the luminophore is [H]. the proportion of the y-intercept to the

The slope of the straight line may be calculated from the plot of 1/A of versus 1 / ([Q]) [12] to find -K_b. The Benesi-Hildebrand plot for the metal DNA complex is shown using both the adsorption and emission data.

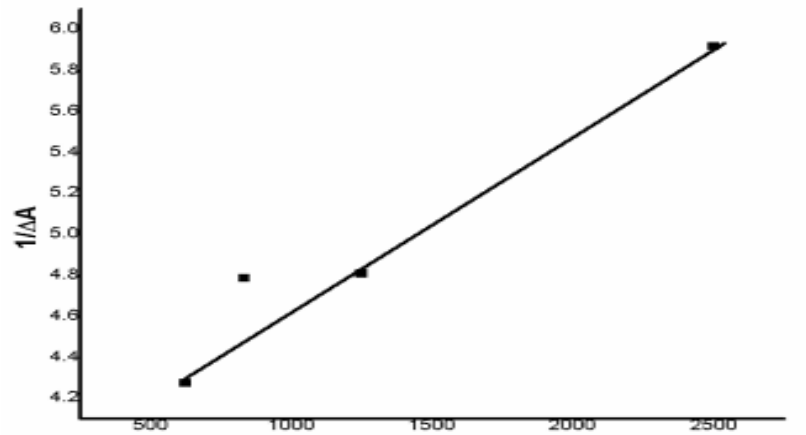


Figure 5 shows the Benesi-Hildebrand plots for the binding of [Ru(dmbpy)3]2+ with increasing amounts of Onion DNA in an aqueous medium.

Complex	Binding Constant(M^{-1})	
	Absorption measurement	Emission measurement
$[Ru(bpy)_3]^{2+}$	2.58×10^3	4.59×10^3
$[Ru(dmbpy)_3]^{2+}$	3.23×10^3	4.55×10^3

Table 2 displays the binding constant Kb (M-1) for [Ru(NN)3]2+ complexes with DNA in an aqueous environment.

When onion DNA is added to the Ru-polypyridine complexes, a red shift occurs indicating stronger binding. The binding between the luminophore and DNA is through hydrophobic or stacking interactions. The hydrophobicity of the ligands affects the binding constant (Kb) value, which increases as hydrophobicity increases. The purity of DNA and ligands also affects Kb. The Kb values for [Ru(bpy)3]2+ and [Ru(dmbpy)3]2+ complexes with onion DNA are 2.584 x 103 and 3.236 x 103 M-1, respectively. The Kb values at the MLCT region are higher than the LC region, indicating stronger binding in the MLCT region. Onion DNA binds more effectively to [Ru(dmbpy)3]2+ than [Ru(bpy)3]2+. This interaction offers a potential way to treat cancer cells due to the antitumor properties of onions.

Conclusion

The interaction of Ruthenium polypyridyl complexes with DNA purified from onions has been studied using absorption and emission spectrum techniques. The Kb values for the $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ complexes with onion DNA are 2.584×10^3 M^{-1} and 3.236×10^3 M^{-1}, respectively. Both electrostatic and intercalative ways of onion DNA binding are shown by the provided data.

***Lecturer**
Department of Chemistry
Government College
Tonk (Raj.)

References

1. Gomathi, R., Ramu, A., and Murugan, A. (2013). Synthesis, spectral characterization of N-benzyl isatin schiff base Cu(II), Co(II) and Ni(II) complexes and their effect on cancer cell lines. International Journal of Innovative Research in Science, Engineering and Technology, 10, 5156 - 5166.
2. Gomathi, R., and Ramu, A. (2013). Synthesis, DNA binding, cleavage, antibacterial and cytotoxic activity of Novel Schiff base Co(II) Complexes of substituted isatin. International Journal of Advanced Research, 1(8), 556– 567.
3. Rosenberg, B., Van Camp, L., and Krigas, T. (1965). Inhibiton of cell division in Escherichia coli by electrolysis products from a platinum electrode. Nature, 205, 698-699. https://doi.org/10.1038/205698a0
4. Barton, J. K. and Lolis E. (1985). Chiral discrimination in the covalent binding of bis(phenanthroline)dichloro ruthenium(II) to B-DNA. Journal of the American Chemical Society, 107(3), 708-709. https://doi.org/10. 1021/ja00289a035
5. Guo, Z., and Sadler, P. J. (2000). Medicinal Inorganic Chemistry. Advances in Inoranic Chemistry, 49, 183-184. https://doi.org/10.1016/S0898-8838(08)60271-8

6. Erkkila, K.E., Odom, D.T. and Barton, J.K. (1999). Recognition and reaction of metallo intercalators with DNA. Chemical Reviews, 99(9), 2777-2796. https://doi.org/10.1021/cr980434

7. Lippert, B. (2000). Multiplicity of metal ion binding patterns to nucleo bases. Coordination Chemistry Reviews, 200, 487-516.

8. Li,C., Liu, S. L., Guo, L. H., and Chen, D. P. (2005). A new chemically amplified electrochemical system for DNA detection in solution Electrochem. Commuications Chemistry, 7(1), 23-28.

9. Swarnalatha, K., Rajkumar, E., Rajagopal, S., Ramaraj, R., Banu, I. S. & Ramamurthy, P. (2011). Proton coupled electron transfer reaction of phenols with excited state ruthenium (II)–polypyridyl complexes. Journal of Physical Organic Chemistry, 24(1), 14-21.

10. Sumitha Celin, T. and Allen Gnana Raj, G. (2020). Luminescence quenching of tris(4,4'-dimethyl-2,2'bipyridyl)ruthenium(II) complex with quinones in aprotic polar medium. Indian Journal of Chemistry, 59A, 923- 928.

11. Nordell, K. J., Jackelen, A. L., Condren, S. M., Lisensky, G. C., and Ellis, A. B. (1999). Liver and Onions: DNA Extraction from Animals and Plant Tissues. Journal of Chemical Education,76(3), 400A, https://doi.org/10.1021/ ed076p400

12. Saha, B. and Stanbury, D. M. (2000). Thermal and Photochemical Reduction of Aqueous Chlorine by Ruthenium(II) Polypyridyl Complexes. Inorganic Chemistry, 39(6), 1294-1300. https://doi.org/10.1021/ic 9910920