Isolation of Dna from Guava Fruit and its Binding Interaction with Tris(2,2′-Bipyridine) Ruthenium (Ii) Complexes

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ABSTRACT

Isolation of DNA from guava and its binding interaction with three Ru(II)-bipyridine complexes$[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$and $[Ru(dtbpy)_3]^{2+}$(bpy = 2,2′-bipyridine, dmbpy = 4,4′-dimethyl-2,2′-bipyridine and dtbpy = 4,4′-di-ter-butyl-2,2′-bipyridine) have been investigated by UV-Visible absorption spectral studies. The complexes $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$show metal to ligand charge transfer absorption peaks in the region 455-465 nm. The absorption spectrum of DNA isolated from guava shows a shoulder peak at 255 nm. Theaddition of $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$complexes with the DNA isolated from guava exhibits hyperchromic and bathochromic shifts. This indicates that the complexes interact with the DNA through intercalative and electrostatic modes of binding. The binding constant (K_b) of these complexes with the DNA is determined from the Benesi-Hildebrand plot. The K_b value of $[Ru(dtbpy)_3]^{2+}$complex is higher than that of $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$complexes. This is due to the hydrophobic nature of $Ru(dtbpy)_3]^{2+}$ complex. The obtained results reveal that the complexes bind strongly with the DNA in the MLCT region and theK_b values depend on the nature of the ligands present in the complexes.

Keywords: *Guava DNA, Ru(II)-bipyridine complexes, Binding Constant, Intercalative interaction, Electrostatic interaction*

1. Introduction

DNA is the molecule that carries genetic information for the development and functioning of an organism. The DNA molecule directs the synthesis of protein and contains all the genetic information that is passed on to new cells. DNA can be easily extracted from fruits because they are soft and easy to pulverize. Guava is a tropical fruit that grows in dry or humid heat. Guavas are rich in dietary fiber, vitamin C, vitamin A, iron, calcium, and potassium.

Transition metal complexes containing heterocyclic ligands have been of considerable interest in terms of structural chemistry, catalysis and biological functions [1,2]. Metal complexes have been found to bind with DNA through multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities. The biological function of the metal complexes mainly depends on the interaction between the

ligand-binding residues and metal ions present in the complex [3,4]. The modification of the metal or ligands in the complexes leads to substantial changes in the binding properties.

Among the transition metal complexes, Ru(II)-polypyridine complexes undergo binding with DNA, RNA and proteins and act as therapeutic agents [5]. These complexes are reasonably stable to light, electricity and heat, because the bonds between the central metal and polypyridyl ligands are very strong. These complexes possess specific optical and electrochemical properties; moreover, these complexes exhibit strong absorption MLCT band in the visible region. The MLCT absorption band, emission wavelength, and lifetime can be easily varied by the introduction of various substituents in the polypyridine ligands. In order to understand the role of Ru(II) complexes with DNA, the present study focuses on the binding of $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$(bpy = 2,2′-bipyridine, dmbpy = 4,4′-dimethyl-2,2′-bipyridine and dtbpy = 4,4′-di-*ter*-butyl-2,2′-bipyridine) complexes with the DNA isolated from guava extract.

2. Materials and Methods

2.1 Materials

$RuCl_3.3H_2O$, bpy, dmbpy and dtbpywere purchased from Sigma-Aldrich. Analytical grade solvents were used for the synthesis of the complex. Double distilled deionized water was used as a solvent for the binding studies.

2.2 Synthesis of Ru(II)-bipyridine Complexes

2.2.1 Synthesis of $[Ru(bpy)_3](BF_4)_2$ complex

$RuCl_3.3H_2O$ (1 mM) and bpy (3 mM) were treated with 25 mL of ethanol and was refluxed for 20 h. The red orange complex formed was remained in the ethanolic solution. The product was filtered, washed with cold water and diethyl ether and dried in a vacuum desiccator.The tetrafluoroborate salt of $[Ru(bpy)_3]^{2+}$ complex was prepared from the corresponding chloride salt by adding a concentrated solution of sodiumtetrafluoroborate to the aqueous solution of the complex. The precipitate was filtered and washed with water, ethanol and diethyl ether. The complex was purified by column chromatography using silica gel as the adsorbent and n-propanol as eluent and on subsequent evaporation to recover the complex.

2.2.2 Synthesis of $[Ru(dmbpy)_3](BF_4)_2$ and $[Ru(dtbpy)_3](BF_4)_2$complexes

$RuCl_3.3H_2O$ (1 mM) and 4,4′-dimethyl-2,2′-bipyridine (3 mM) were dissolved in 20 mL of ethylene glycol and refluxed for 4 h. The solution was cooled and filtered to remove any insoluble impurities. A saturated solution of sodium tetrafluoroborate was then added dropwise into the filtrate until an orange precipitate 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. Similar procedure was adopted for the synthesis of $[Ru(dtbpy)_3]^{2+}$ complex.

2.3 Extraction of DNA from Guava Extract

The guava fruit was mashed in a zipper bag. The extraction buffer was prepared by adding liquid soap and salt in 50 mL of water. The extraction buffer was added into the guava extracts and kept aside for 5 minutes. The content was transferred into a 100 mL beaker, 10 mL of ice-cold ethanol was added into it and allowed to stand for 5 minutes. The DNA of guava was separated as white strands. The isolated DNA from the extract was collected and used as such for the binding studies.

2.4 Equipment

The absorption spectral measurements of the $Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes and the binding studies of the synthesised complexes with the DNA isolated from guava extract were carried out using Shimadzu UV-1800 spectrophotometer. All the spectral measurements were carried out at room temperature.

2.5 Determination of Purity and Quantity of Isolated DNA

The purity of the DNA isolated from guava extract was measured by spectrophotometric methods. The absorbance of the isolated DNA was measured at 260 and 280 nm using UV-Visible spectrophotometer and its ratio (A_{260}/A_{280}) was calculated. The DNA concentration was calculated as:

$$\text{Total DNA concentration (ng/}\mu\text{L)} = A_{260} \times 50 \text{ ng/}\mu\text{L} \times 100$$

2.6 Determination of Binding Constant

The binding of $Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes with the isolated DNA at various concentrations (5×10^{-5} - 3×10^{-4} M) in aqueous medium has been studied separately by absorption spectral technique. The solutions for the binding studies were prepared by dissolving the DNA and the complex in distilled water. The binding constant (K_b) of the complexes with the DNA isolated from guavais determined from the Benesi-Hildebrand equation using absorption intensity data [6].

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

The plot of $1/\Delta A$ *vs* $1/[G]$ gives a straight line. The K_b can be obtained from the ratio of Y-intercept to the slope of the straight line.

3. Results and Discussion

The structure of the synthesized complexes used in the present study is shown in **Fig. 1.** The absorption spectrum of the $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes in aqueous medium shows a high energy absorption at 285-295 nm corresponding to the ligand

centered π - π* transition and the low energy absorption at 455-465 nm assigned to the dπ - π* metal to ligand charge transfer (MLCT) transition (**Fig. 2**).

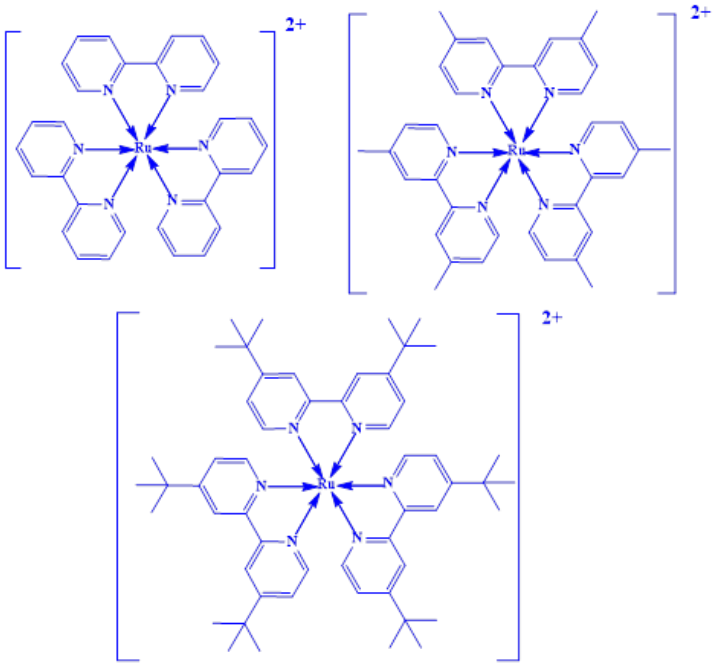

Fig 1. Structure of the synthesised complexes

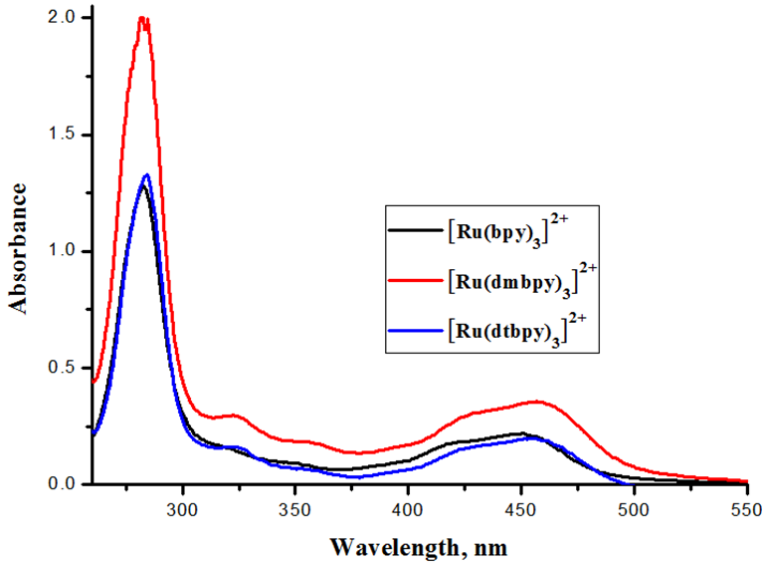

Fig 2. UV spectrum of the synthesised complexes

The DNA isolated from the guava extract is shown in **Fig 3**. The absorption spectrum of the guava DNA shows a shoulder peak at 255 nm and it does not show any peak in the MLCT region of the synthesised complexes. The purity and the concentration of the DNA isolated from guava extract is found to be 1.37 and 732 ng/µL.

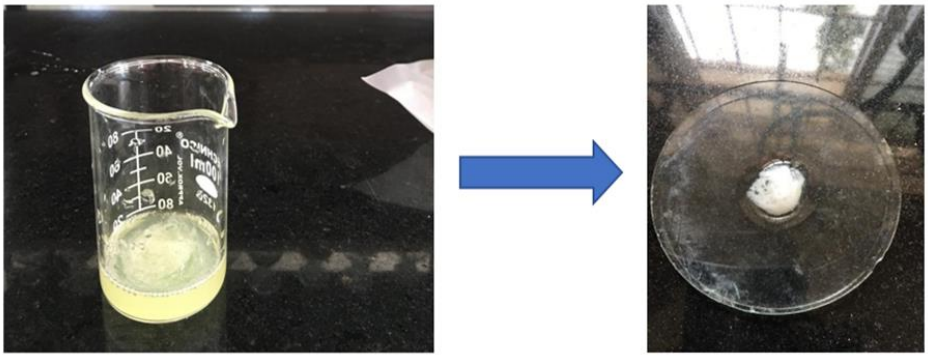

Fig 3. DNA isolated from guava extract

The absorption spectrum of the DNAwith the incremental addition of $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes in aqueous medium shows an increase in the MLCT absorption maximum, this indicates the formation of ground state complex (**Figs. 4-6**). The K_b values calculated from the Benesi-Hildebrand plots is represented in **Table 1**.

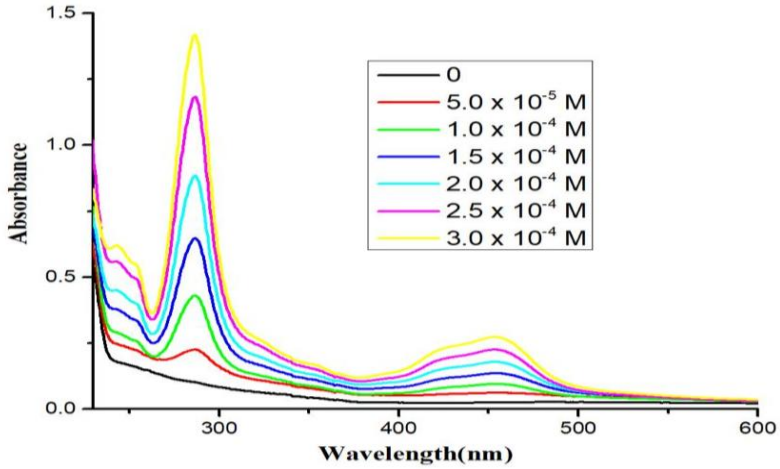

Fig 4. UV spectrum of guava DNA with the incremental addition of $[Ru(bpy)_3]^{2+}$ complex

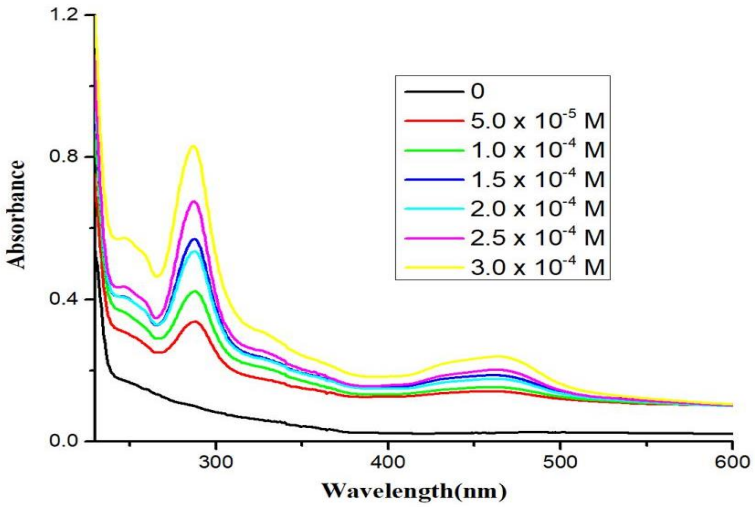

Fig 5. UV spectrum of guava DNA with the incremental addition of $[Ru(dmbpy)_3]^{2+}$ complex

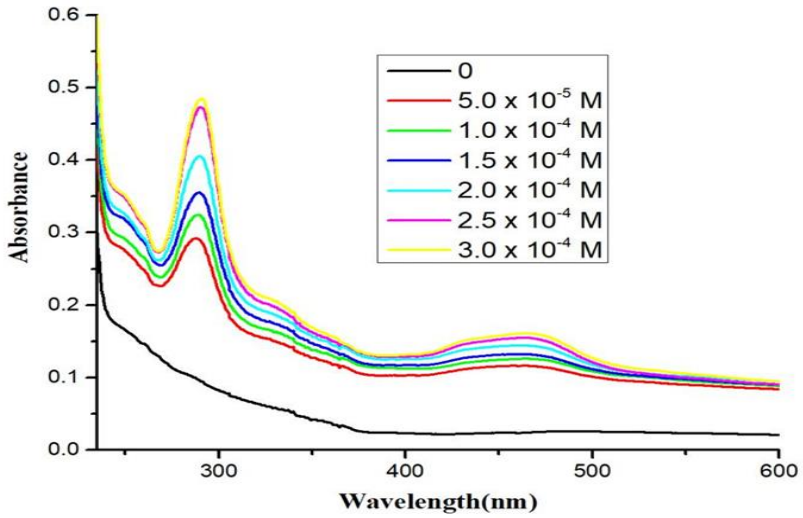

Fig 6. UV spectrum of guava DNA with the incremental addition of $[Ru(dtbpy)_3]^{2+}$ complex

Table 1. Binding constant, K_b (M^{-1}) of the synthesised complexes with guava DNA

Complex	Binding constant, K_b (M^{-1})
$[Ru(bpy)_3]^{2+}$	2.08×10^3
$[Ru(dmbpy)_3]^{2+}$	2.19×10^3
$[Ru(dtbpy)_3]^{2+}$	3.75×10^3

The results reveal that the $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$and $[Ru(dtbpy)_3]^{2+}$ complexes strongly bind with the guava DNA.The $K_b$value of $[Ru(dtbpy)_3]^{2+}$ complex with guava DNA is higher than that of $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ complexes. This is due to the hydrophobic nature of $[Ru(dtbpy)_3]^{2+}$ complex. As the hydrophobic nature of the complex increases binding also increases [7]. Thus the $[Ru(dtbpy)_3]^{2+}$ complex strongly binds with the base pair of the DNA.

Binding studies of all the three synthesised complexes on the DNA isolated from guava displays hyperchromic and bathochromic shifts and this clearly picturizes that the complexes interact with the DNA through intercalative and electrostatic modes of binding. The intercalative interaction is mainly due to the π-π stacking interaction between the aromatic ligands present in the complex and the DNA base pairs. The cationic complexes bind with the DNA base pair through electrostatic interaction. The hyperchromic shift is due to the partial uncoiling of the helical structure of the DNA. The existence of hyperchromic shift leads to electrostatic binding between the positively charged complexes and the negatively charged species of the phosphate backbone of the DNA double helical structure at the peripheral region.

The binding of $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes to the DNA base pair leads to breakage in the DNA double helix and bind through intercalative and electrostatic modes which gives better binding property [8]. The hydrogen bond formation between the DNA base pairs and the vander Waals interactions between the complex and the base pairs of the DNA leads to strong binding. This result is in accordance with the DNA binding studies of various complexes [9,10]. The obtained results of the present investigation reveals that the $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes strongly bind with the guava DNA and the $K_b$values depends on the nature of the ligands present in the complexes.

4. Conclusion

The DNA binding affinity of $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes on the DNA isolated form guava extract has been investigated by absorption spectral techniques. The $K_b$valuesof $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes clearly depictthestrong binding affinity towards guava DNA through intercalative and electrostatic modes of binding. The $K_b$of $[Ru(dtbpy)_3]^{2+}$ complex with guava DNA is higher than that of $[Ru(bpy)_3]^{2+}$ and $[Ru(dmbpy)_3]^{2+}$ complexes and the $K_b$values of the complexes depend on the nature of the ligands present in the complexes. The binding of Ru(II)-bipyridine complexes with the DNA isolated from guava extract leads to better binding property which paves a way for applicability in various medicinal and biological field.

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