BINDING STUDY OF RUTHENIUM(II)-PHENANTHROLINE COMPLEX ON DNAISOLATED FROM STRAWBERRY EXTRACT

Snow Havi Thev. A, Denshiya. P and Sheeba Daniel*

*Department of Chemistry, Holy Cross College (Autonomous), Nagercoil, Tamil Nadu, India.Email: sheebadaniel@holycrossngl.edu.in

Abstract

The binding study of $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) complex on DNA isolated from strawberry extract has been investigated by UV-Visible absorption spectral studies. The complex $[Ru(phen)_3]^{2+}$ shows a ligand centred (LC) and metal to ligand charge transfer (MLCT) absorption peak at 262 and 450 nm in aqueous medium. The absorption spectrum of DNA isolated from strawberry shows a high energy peak at 230 nm and a shoulder peak at 275 nm. The absorption spectrum of the DNA with the incremental addition of $[Ru(phen)_3]^{2+}$ complex display hyperchromic and bathochromic shifts this indicates that the complex interact with the DNA through intercalative and electrostatic modes of binding. The binding constant (K_b) of this complex with the DNA isolated from strawberry extract is determined from the Benesi-Hildebrand plot and is found to be 3.3 x 10^6 M^{-1} for LC region and 5.3 x 10^7 M^{-1} for MLCT region. The obtained results reveal that the complex bind strongly withthe DNA in the MLCT region than that of the LC region. The K_b depends on the purity of the DNA and the ligands present in the complex.

Keywords: $[Ru(phen)_3]^{2+}$ complex, Strawberry DNA, Binding Constant, Intercalative interaction, Electrostatic interaction.

1. Introduction

DNA has received attention in the interaction of transition metal polypyridyl complexesas DNA secondary structural probes and photocleavage reagents. Ruthenium(II) complexes due to the strong DNA-binding and potential anticancer activity currently focus on the DNA binding extensively as the material of inherence and control for the structure and functions of the cells [1-3]. Ru(II)-ploypyridyl complexes undergo binding with DNA,

RNA and proteins and act as therapeutic agents [4]. The higher coordination number of ruthenium and its redox properties play an important role in the transport mechanisms of the drug in the body, as well as in the interaction between the drug and several different biologically relevant proteins.

Ruthenium metal co-ordinated with polypyridyl ligand such as phen, acts as chelating agent for metal complexes which exhibit metal-to-ligand charge transfer (MLCT) and ligand- to-metal charge transfer (LMCT) transitions in the complex [5]. In order to understand the roleof Ru(II) complex with DNA, the present study focuses on the binding of $[Ru(phen)_3]^{2+}$ complex with the DNA isolated from strawberry extract. The binding of $[Ru(phen)_3]^{2+}$ complex with the DNA isolated from strawberry extract leads to better binding property which plays a way for applicability in various medicinal and biological field.

2. Materials and Methods

Materials

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

Synthesis of [Ru(phen)3]Cl2 Complex

$RuCl_3.\ 3H_2O$ (0.5 g) and phen (0.76 g) were treated with 25 mL of ethanol and was refluxed for 20 h. The red orange complex formed was remained in the ethanol solution. 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 n-propanol as eluent and on subsequent evaporation to recover the complex.

Extraction of DNA from Strawberry Extract

The strawberry fruit was mashed in a zipper bag. The extraction buffer was prepared inin a 100 mL beaker by adding liquid soap and salt in 50 mL of water. The extraction buffer was added into the strawberry 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 strawberry was separated as white strands. The isolated DNA from the extract was collected and used as such for the binding study.

Equipment

The absorption spectral measurements of the $[Ru(phen)_3]^{2+}$ complex and the binding studies of the synthesised complex with the DNA isolated from strawberry extract were carriedout using Shimadzu UV-1800 spectrophotometer. All the spectral measurements were carriedout at room temperature.

Determination of Purity and Quantity of Isolated DNA

The purity of the DNA isolated from strawberry 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 DNAconcentration was calculated as:

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

Determination of Binding Constant

The binding of $[Ru(phen)_3]^{2+}$ complex with the isolated DNA at various concentrations(5×10^{-5} - 3×10^{-4} M) in aqueous medium has been studied by absorption spectral technique. The solutions for the binding studies were prepared by dissolving the DNA and the complex indistilled water. The binding constant (K_b) of the $[Ru(phen)_3]^{2+}$ complex with the DNA isolatedfrom strawberry is determined from the Benesi-Hildebrand equation using absorption intensitydata [6].

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

where, [H] is the concentration of the host (sensitizer), [G] is the concentration of the guest (quencher), ΔA is the change in the absorbance of the [H] on the addition of [G]. Δε is the difference in the molar extinction coefficient between the free [H] and [H]-[G] complex. The plot of 1/ΔA *vs* 1/[G] gives a straight line. The K_b can be obtained from the ratio of Y-interceptto the slope of the straight line.

3. Results and Discussion

The structure of the synthesized complex used in the present study is shown in **Fig. 1.** The absorption spectrum of $[Ru(phen)_3]^{2+}$ complex in aqueous medium shows a high energy absorption in the region 262 nm corresponds to the ligand centered (LC) π - π* transition and the low energy absorption at 450 nm assigned to the dπ - π* metal to ligand charge transfer (MLCT) transition (**Fig. 2**).

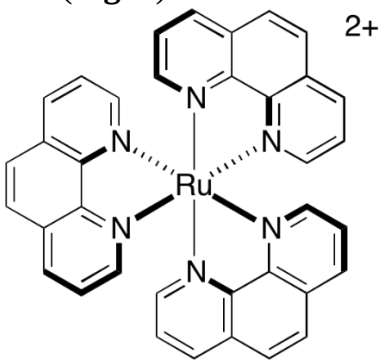

Fig. 1 Structure of $[Ru(phen)_3]^{2+}$ complex

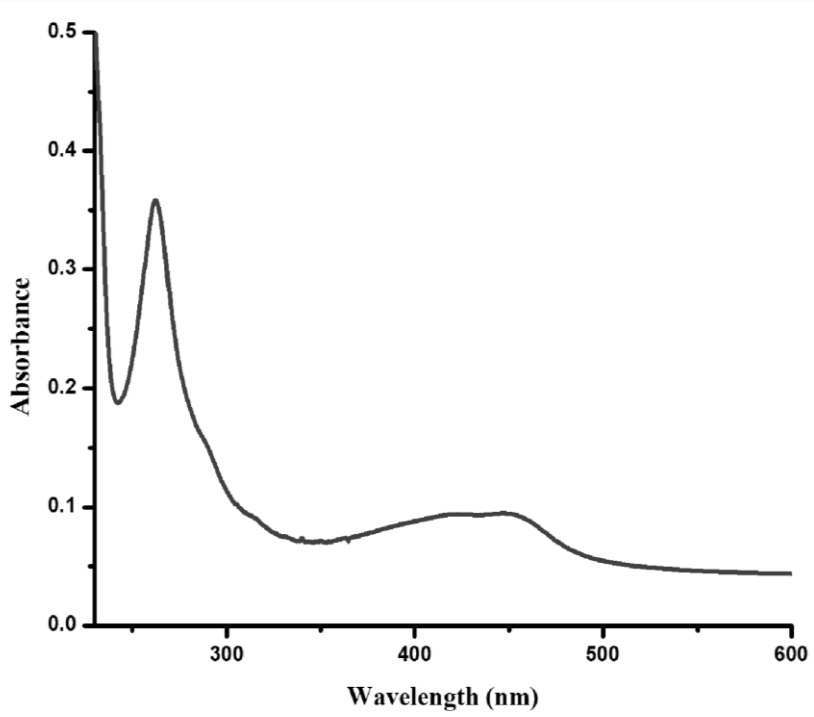

Fig. 2 UV spectrum of $[Ru(phen)_3]^{2+}$ complex

The DNA isolated from the strawberry extract is shown in **Fig. 3**. The absorption spectrum of the strawberry DNA shows a high energy absorption in the region at 230 nm

and shoulder peak at 275 nm and it does not show any peak in the MLCT region of the synthesisedcomplex (**Fig. 4**). The purity and the concentration of the DNA isolated from strawberry extractis found to be 1.5 and 450 ng/µL.

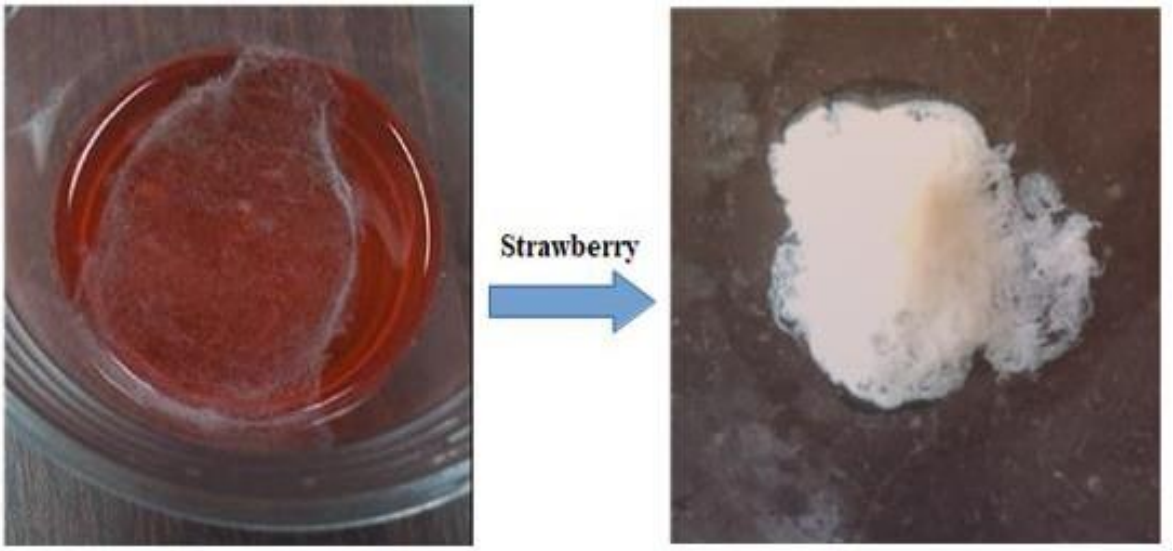

Fig. 3 DNA isolated from strawberry extract

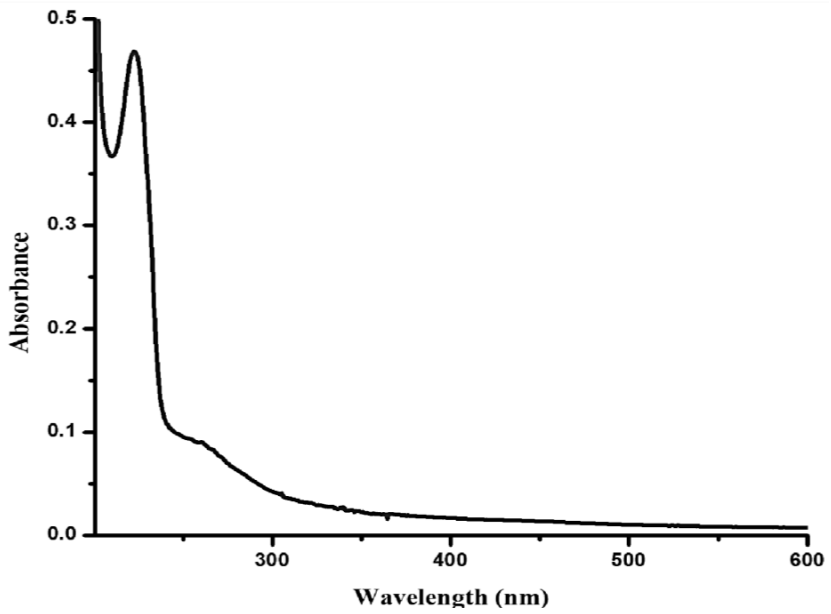

Fig. 4 UV spectrum of DNA isolated from strawberry extract

The binding affinity of the complex is determined by the change in the absorbance of the DNA with the incremental addition of the complex. The absorption spectrum of the

isolatedDNA of strawberry with the incremental addition of $[Ru(phen)_3]^{2+}$ complex in aqueous medium shows an increase in the LC and MLCT absorption maximum, this indicates the formation of ground state complex (**Fig. 5**). The ground-state interactions between the complexwith the DNA are hydrophobic or π-stacking in nature [7]. To the extent that π - π stacking interactions exist between the ligands of Ru(II)-complexes and the DNA, the binding becomesstronger. The K_b of the $[Ru(phen)_3]^{2+}$ complex with the isolated DNA of strawberry is determined from the Benesi-Hildebrand plot (**Fig. 6**). The K_b of $[Ru(phen)_3]^{2+}$ complex with the DNA of strawberry at LC and MLCT region is found to be 3.3 $x10^6$ and 5.3 $x10^7$ M^{-1} respectively. The obtained results reveal that the complex bind strongly with the DNA in the MLCT region than that of LC region.

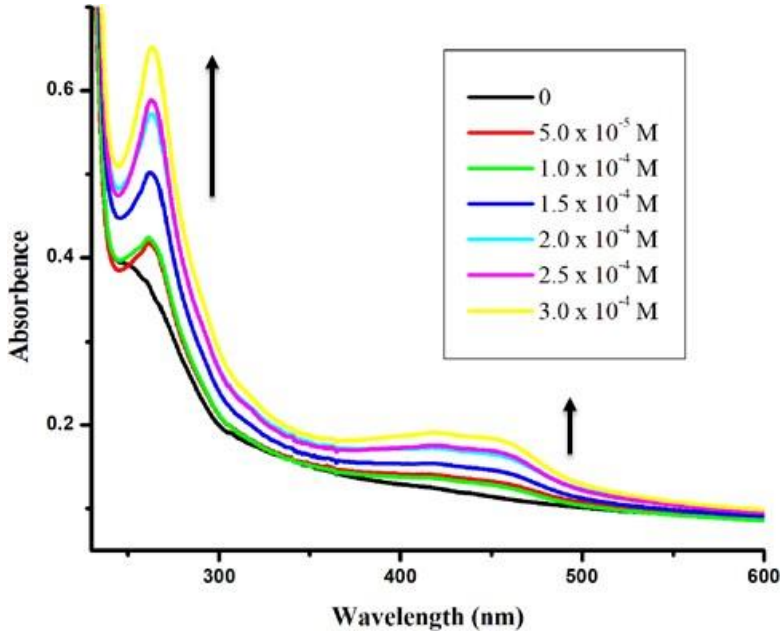

Fig. 5 UV spectrum of DNA isolated from strawberry extract with the incremental additionof $[Ru(phen)_3]^{2+}$ complex

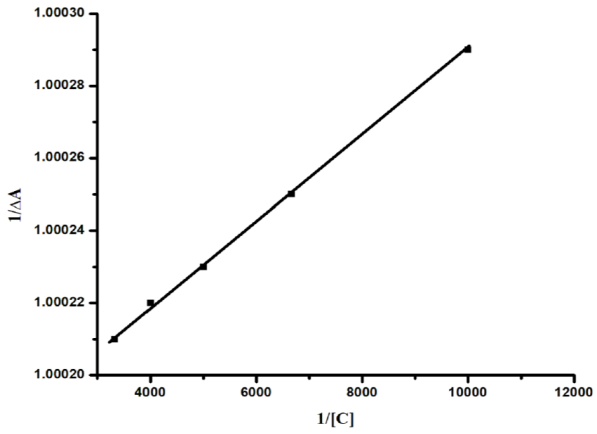

Fig. 6 Benesi-Hildebrand plot of DNA isolated from strawberry with $[Ru(phen)_3]^{2+}$

complex The absorption spectra of the synthesised complex at different concentrations on thestrawberry DNA display hyperchromic and bathochromic shifts and this clearly picturizes thatthe complex 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 complex binds with theDNA base pair through electrostatic interaction also. The hyperchromic shift is due to the partial uncoiling of the helical structure of the DNA. The existence of hyperchromic shift leadsto electrostatic binding between the positively charged complex and the negatively chargedspecies of the phosphate backbone of the DNA double helical structure at the peripheral region.

The binding of the $[Ru(phen)_3]^{2+}$ complex to the DNA base pair leads to breakage in the DNA double helix and bind through intercalative and electrostatic modes which gives betterbinding property [8]. The hydrogen bonding formation between the DNA base pairs and also the van der 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 complexeswith hyperchromic shifts [9,10]. The results of the present investigation reveal that the $[Ru(phen)_3]^{2+}$ complex strongly binds with the strawberry DNA and the K_b depends on the purity of the DNA and the ligands present in the complex.

Conclusion

The DNA binding affinity of the synthesized $[Ru(phen)_3]^{2+}$ complex on the DNAisolated form strawberry extract has been investigated by absorption spectral techniques. TheK_b of $[Ru(phen)_3]^{2+}$ complex with the DNA of strawberry at LC and MLCT region is 3.3 $x10^6$and 5.3 $x10^7$ M^{-1} respectively. The K_b values clearly depict that the synthesized complex showsstrong binding affinity towards strawberry DNA through intercalative and electrostatic modesof binding. the K_b depends on the purity of the DNA and the ligands present in the complex.

References

1. Krishnaveni, V., Kumaraguru, N., Studies on DNA interaction, biological activities ofsurfactant-cobalt(III)-phendione complexes, J. Pharm. Med. Res., 3 (2017) 68-72.
2. Turel, I., Golobi, A., Kljun, J., Samastur, P., New synthetic routes for ruthenium-1,10-phenanthroline complexes. Tests of cytotoxic and antibacterial activity of selected ruthenium complexes, Acta Chimica Slovenica, 62 (2015) 337-345.
3. Bugarcic, T., Habtemariam, A., Deeth, R.J., Fabbiani, F.P., Parsons, S., Sadler, P.J. Ruthenium(II) arene anticancer complexes with redox-active diamine ligands, InorganicChemistry, 48 (2009) 9444-9453.
4. Zayat, L., Filevich, O., Baraldo, L.M., Etchenique, R., Ruthenium polypyridyl phototriggers: from beginnings to perspectives, Philosophical Transactions. Series A, Mathematical, Physical and Engineering Science, 371 (2013) 1-12.
5. Abdel-Shafi, A.A., Worrall, D.R., Ershov, A.Y., Photosensitized generation of singlet oxygen from ruthenium(II) and osmium(II) bipyridyl complexes, Dalton Transactions, 1(2004) 30-36.
6. Connors, K.A., Binding Constants: The Measurement of Stability. Chichester, U.K: JohnWiley & Sons, Ltd. 1987.
7. Li, C., Hoffman, M.Z., One-electron redox potentials of phenols in aqueous solution, Journal of Physical Chemistry B, 103 (1999) 6653-6656.
8. Sathiyaraj, S., Ayyannan, G., Jayabalakrishnan, C., Synthesis, spectral, DNA binding and cleavage properties of ruthenium(II) Schiff base complexes containing PPh_3/$AsPh_3$as co-ligands, Journal of the Serbian Chemical Society, 79 (2014) 151-165.
9. Dar, A.M., Khan, M.A., Mir, S., Gatoo, M.A., DNA binding, cleavage activity, molecular docking, cytotoxicity and genotoxicity studies of newly synthesized copperbased metal complexes, Pharmaceutica Analytica Acta, 7 (2016) 1-11.
10. Almarhoon,Z.M.,Al-Onazi,W.A., Alothman, A.A., Al-Mohaimeed, A.M. Al-Farraj, E.S., (2019). Synthesis, DNA binding, and molecular docking studies of dimethylaminobenzaldehyde-based bioactive Schiff bases, Journal of Chemistry,53.