AMINO ACID BINDING NATURE OF RUTHENIUM(II)-POLYPYRIDYL-PHENDIONE COMPLEXES IN AQUEOUS MEDIUM

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Abstract

Binding of amino acids(alanine, valine, histidine and tyrosine) with Ru(II)-polypyridyl-phendione complexes {$[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ (bpy = 2,2'-bipyridine, phen = 1,10-phenanthroline and phendione = 1,10-phenanthroline-5,6-dione)} in aqueous medium at pH 12.5 has been investigated by UV-Visible absorption spectral techniques. The complexes show a metal to ligand charge transfer (MLCT) absorption peak at 438 and 440nm in aqueous medium.The binding constant (K_b) of these complexes with each amino acidare determined from Benesi-Hildebrand plots. Among the four amino acids taken in the present study, histidine shows higher $K_b$value for both the complexes.The$K_b$values of histidinewith $[Ru(bpy)_2(phendione)]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complexesare 1.477x 10^4 M^{-1} and 1.926 x 10^6M^{-1}.Histidine shows better binding property withthese complexesbased on the factors of aromatic planarity and hydrophobicity. The K_b values of $[Ru(phen)_2(phendione)]^{2+}$ complex is higher than that of $[Ru(bpy)_2(phendione)]^{2+}$complex due to π - π interaction. The obtained results reveal that the nature of the ligand and the substituents present in the amino acids play a vital role in the binding of amino acids with these complexes.

Keywords: [Ru(II)-polypyridyl-phendione complexes;Aminoacids; Benesi-Hildebrand equation; Binding constant;

1. Introduction

The therapeutic applications of transition metal complexes have been developed since the discovery of cisplatin.The introduction of cisplatin in medical protocols for the treatment of cancers, have attracted significant attention on anticancer metallopharmaceuticals [1]. The cisplatin therapy has some drawbacks including the limited applicability of the medicine, the acquired resistance displayed by certain tumors and the serious side effects. Many researchers have proved that binding of a drug to

metalloelement enhances its activity and the complex possesses even more significant activity than the parent compound [2,3]. Among the transition metal complexes ruthenium(II)-polypyridyl complexes have particularly drawn significant interest for developing new diagnostic and therapeutic agents for recognize and cleave DNA [4,5]. Ru(II)-ploypyridyl complexes undergo binding with DNA, RNA and proteins and act as therapeutic agents [6]. The higher coordination number of ruthenium compared with platinum provides additional coordination sites, which can potentially be used to tune the properties of the complex, by influencing the way the complex interacts with DNA. The redox properties of ruthenium can also 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.

Ru(II)polypyridine complexes containingnovel aromatic bridging ligand subunits plays an important role for determining the photophysical and redox properties of the new species [7].Ruthenium complexes co-ordinated with polypyridyl ligands such as 2,2'-bypyridine(bpy),1,10-phenanthroline(phen),1,10-phenanthroline-5,6-dione (phendione) and dipyridophenazine (dppz) are well known.These ligands act as chelating agents for transition metal complexes which exhibit metal-to-ligand charge transfer (MLCT) and ligand-to-metal charge transfer (LMCT)transitions in the complex [8].1,10-phenanthroline-5,6-dione (Phendione) has the ability to form stable complexes with a wide variety of metal ions and carries an *ortho*-quinone moiety with pH Independent electroactivity [9]. Phendione an effective redox mediator and versatile chelating agent that forms both homo and heterocomplexes with metals and one such example for hetero complex is $[Ru(bpy)_2(phendione)^{2+}$ [10].The central metal ion of mixed ligand complex exerts an electronic effect on the coordinated molecules and serves as a template for intermolecular interactions between the coordinated ligands. These interactions provide spectroscopic and thermodynamic information on the coordination structures.

Amino acidsformchelates through the amino and carboxylato groups and acts as an excellent metal complexing agent. In additionto functional groups,a side chain with a metal binding group such as the imidazole group of histidine, the phenol ring of tyrosine, etc., shows weak interactions with the other interacting ligands.Metal ions coordinate with amino acids in a fixed geometry and are favourablefor its intermolecular interactions and electron density between the metal ions and the ligands.Thepossibilities regarding structures and bindingfunctions of metal–amino acid complexesare compared with alanine. Tyrosinehas a phenol moiety,abulky side chain group **a**nd the OH group which enhances aromatic ring stacking abilityand binds with the metal ions.The studies on metal-amino acids,related complexes and activities of amino acid side chain groups points out the emphasis on the metal binding and non-covalent interactions of imidazole, phenol and alkyl moieties contained in complexes and their

possible biological relevance.[11].Proteins, nucleic acids and amino acids are important cellular targets for metal complexes [12]. Metal centers are prone to participate in nucleophilic substitution reactions owing to their cationic nature. Since both amino acids and nucleotides are able to act as nucleophiles and undergo nucleophilic substitution reactions with metals.The alternative method todetermine the sequenceof amino acids is obtained from the binding residues of metal ions in proteins[13].

Electronic absorption spectroscopy is an effective method to detect the mode and extent of binding of a metal complex with aminoacids. The modification of the metal or ligands in the complexes leads to substantial changes in the binding properties [14].The biological function of the metal complexes mainly depends on the interaction between the ligand-binding residues and metal ions present in the complex[15]. In order to understand the role of Ru(II) complexes with amino acids, the present study focuses on the binding of$[Ru(bpy)_2(phendione]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complex with various amino acids. The binding of ruthenium(II) complex with the aminoacids alanine, valine, histidine and tyrosinein aqueous medium at pH 12.5 has been investigated by UV-Visible absorptionspectral studies. The binding constant (K_b) of the complexes with amino acids are determined from the Benesi-Hildebrand plots

MATERIALS AND METHODS

Materials

$RuCl_3.3H_2O$, ligands (1,10-phenanthroline and 1,10-phenanthroline-5,6-dione)and ammonium hexafluorophosphate were procured from Sigma-Aldrich. Amino acids (alanine, valine, histidine and tyrosine) and LiCl were purchased from Merck. HPLC grade solvents were used for the synthesis of the complexes.The complexes$[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ were synthesized by reacting the corresponding complexes of $[Ru(bpy)_2Cl_2]2H_2O$ or $[Ru(phen)_2Cl_2]2H_2O$ with phendione according to the procedure previously described [9]. The binding studies were carried out with double-distilled deionized water.

Equipment

Sample solutions of amino acids,$[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$complexes were freshly prepared for each measurement. The absorption spectral measurements were carried out using SHIMADZUUV1800 double beam spectrophotometer.All the spectral measurements were carried out at 293 K.

Binding studies

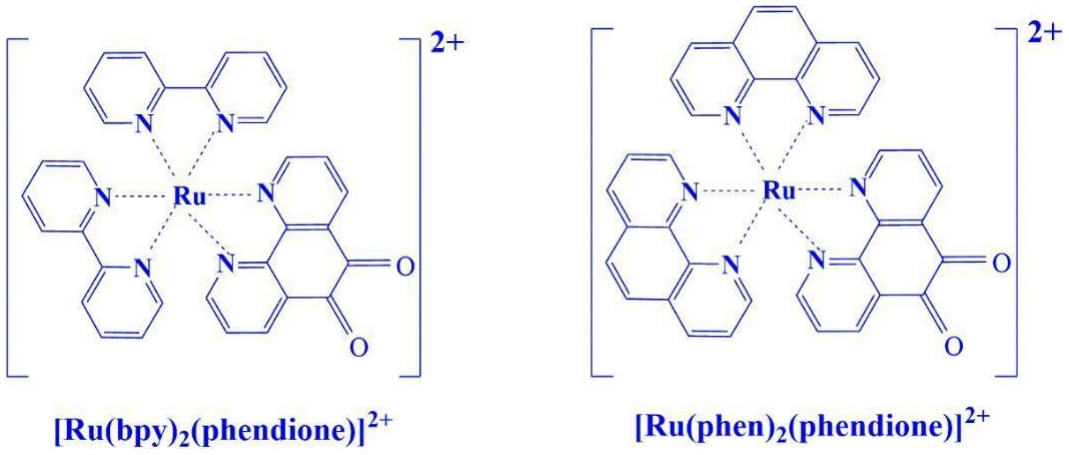
The binding of $[Ru(bpy)_2(phendione)]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complexes with the corresponding amino acids at various concentrations (5×10^{-5} - 3×10^{-4} M) in aqueous medium at pH 12.5 has been studied separately by absorption spectral technique. The solutions of amino acids for the binding studies were prepared by mixing the corresponding amino acids with NaOH and the pH of the solution was maintained at 12.5. The binding constant (K_b) of the $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with the corresponding amino acids alanine, valine, histidine and tyrosine were determined from the Benesi-Hildebrand equation using the absorption intensity data [16].

$$1/\Delta A = I/K_a \Delta\varepsilon [H] + 1/\Delta\varepsilon [G]$$

where ΔA is the change in absorption of the $[Ru(bpy)_2(phendione)]^{2+}$complex with different concentrations ([G]) of amino acids. The plots of 1/ΔA versus 1/ [G] give a straight line, K_b can be obtained from the ratio of Y-intercept to the slope of the straight line.

3 Results and Discussion

The binding of $[Ru(bpy)_2(phendione)]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complexes with the four amino acids in aqueous medium at pH 12.5 has been studied separately and the obtained results are discussed in this section. The structure of the complexes and the amino acids used in the present study are shown in **Fig.1**and **Fig. 2**.



$[Ru(bpy)_2(phendione)]^{2+}$ $[Ru(phen)_2(phendione)]^{2+}$

Fig. 1 Structure of the complexes.

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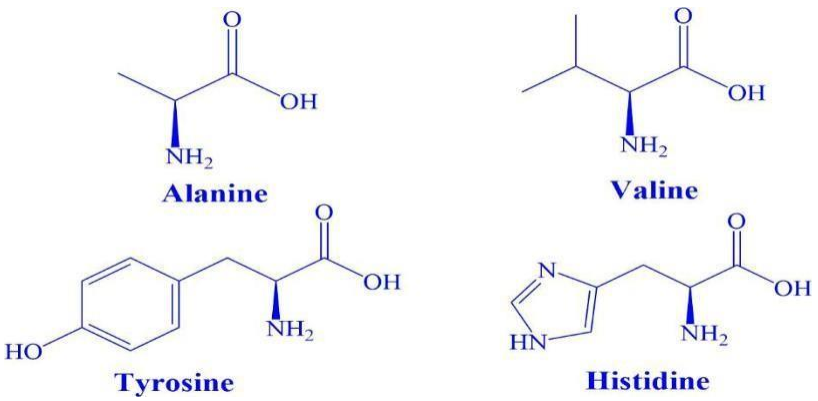

Fig. 2 Structure of amino acids

The absorption spectra of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$complexes in aqueous medium shows a high energy absorption in the region 284-286 nm corresponding to the ligand centered π - π* transition and the low energy absorption at 438 and 440nm assigned to the dπ - π* MLCT transition (**Fig.3**). The MLCT transition involves electronic excitation from the metal orbital [dπ (Ru)] to the ligand centered acceptor π* orbitals.These values are in accordance with the reported values [9].

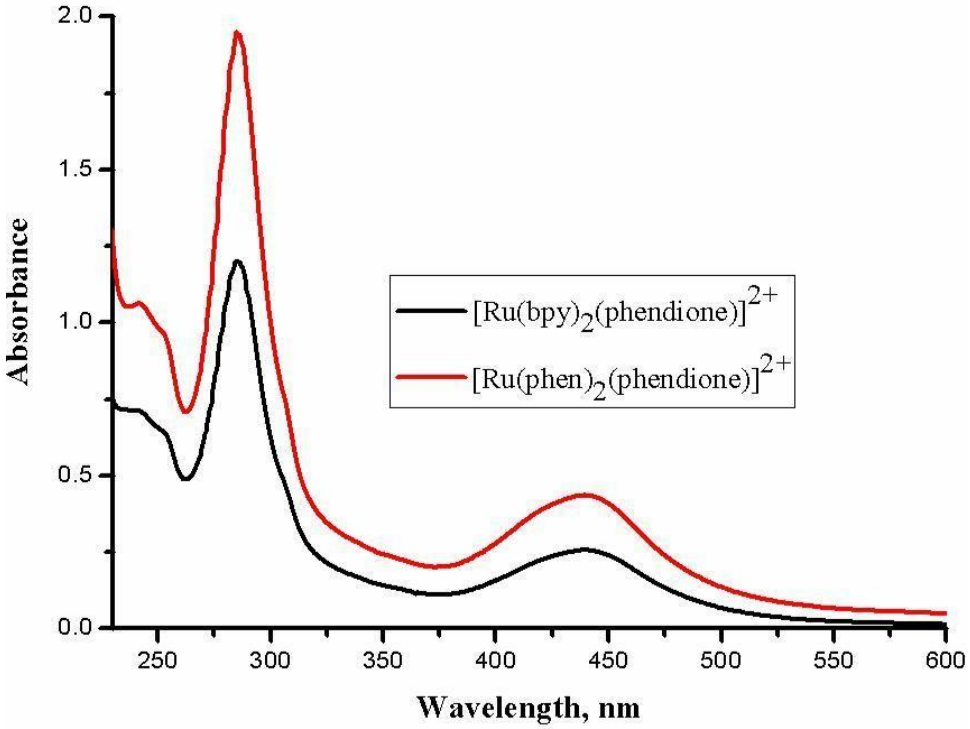

Fig.3 Absorption spectrum of Ru(II) complexes in aqueous medium

The absorption spectral studies of $[Ru(bpy)_2(phendione)]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complexes in aqueous medium with the incremental addition of amino acids alanine, valine, histidine and tyrosine shows a slight increase in the MLCT absorption maxima, indicates the formation of ground state complexes (**Fig. 4**). The cationic complexes interact with amino acids. The binding constant (K_b) of this complexes with the corresponding amino acids are determined from the Benesi-Hildebrand plot (**Fig.5**). The K_b calculated from the Benesi-Hildebrand plots is represented in **Table 1**. The ground-state interactions between the amino acids and the ligands of $[Ru(bpy)_2(phendione)]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complexes are hydrophobic or π-stacking in nature [17]. As the extent of π - π stacking interactions between the ligands of Ru(II)-complexes and the amino acids, the binding becomes stronger.

Table 1 Binding constant, K_b (M^{-1}) of $[Ru(bpy)_2(phendione)]^{2+}$and $[Ru(phen)_2(phendione)]^{2+}$complexes with amino acids

Amino acids	**Binding Constant (K_b) M^{-1}**	
	$[Ru(bpy)_2(phendione)]^{2+}$	**$[Ru(phen)_2(phendione)]^{2+}$**
Alanine	$1.105x10^3$	$7.402\ x10^3$
Valine	$2.549\ x10^3$	$2.084\ x\ 10^4$
Tyrosine	$3.365\ x\ 10^3$	$5.660\ x\ 10^4$

Histidine	1.477×10^4	1.926×10^6

Among the amino acids taken in the present study, histidine shows higher K_b values with the two Ru(II) complexes. The K_b values of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with histidine are 1.477×10^4 and 1.926×10^6 M^{-1}. The K_b values of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with tyrosine are 3.360×10^3 and 5.660×10^4 M^{-1}. The results reveal that histidine binds strongly with $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes. Size, structure and the stereospecificity of the aliphatic alkyl groups of the aminoacids plays a major role in the binding of aminoacids with ruthenium metal complexes.

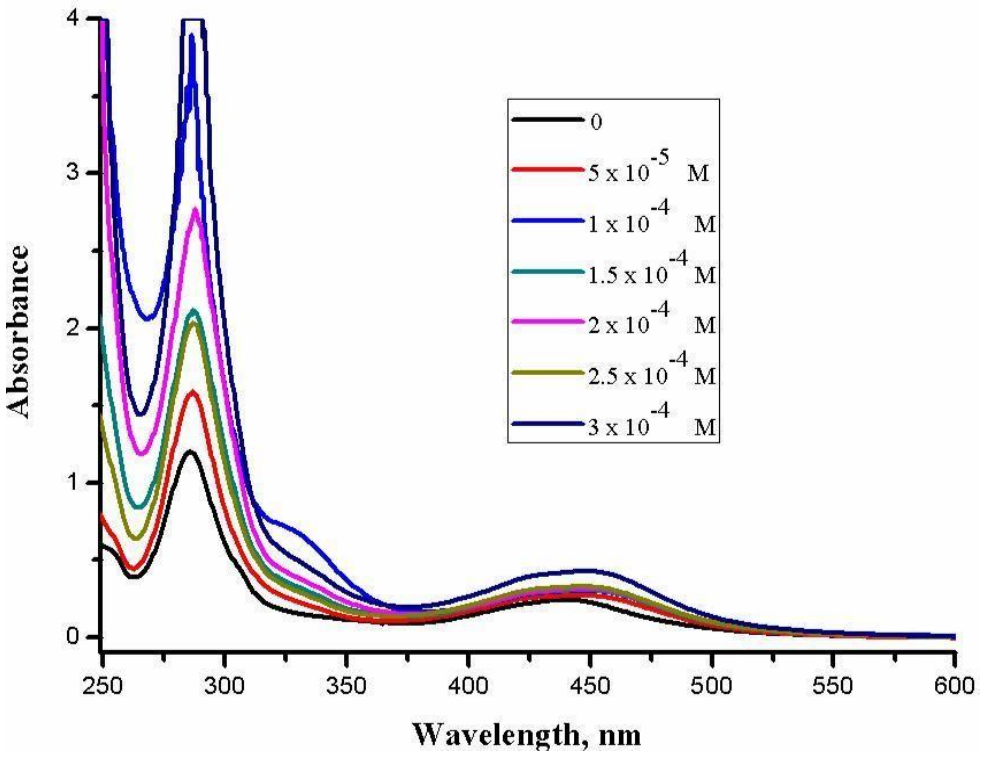

Fig. 4 Absorption spectra of $[Ru(bpy)_2(phendione)]^{2+}$complex with histidine

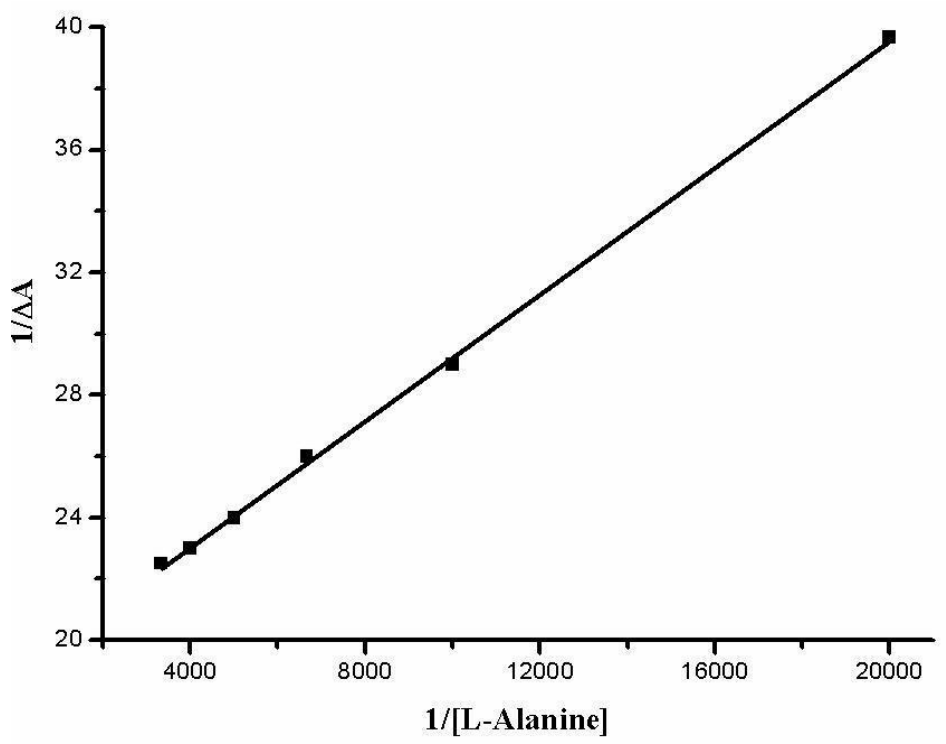

Fig. 5 Benesi-Hildebrand plot of $[Ru(phen)_2(phendione)]^{2+}$complex with incremental addition of alanine

Alanine and valine are non-polar aliphatic α-aminoacidsconsist of a sidechain of non-reactive methyl group and an iso-propyl group. Alanine and valine are therefore considered to be hydrophobic in nature [18]. The hydrophobic aminoacids are incompetent to associate with hydrogen bonding in an aqueous environment.Alanine and valine forms Zwitter ions by protonation of amine groupas ($-NH_3^+$) and deprotonation of carboxylic acid group as($-CO_2^-$). In strongly basic solutions (pH > 9), the predominant form is the fully deprotonated amino carboxylate anion. The presence of non-reactive side chain methyl group of alanine seems to be neutral in nature and shows less binding with Ru(II)-polypyridyl-phendione complexes when compared to valine [19].The distance between the hydrophobic isopropyl group and carboxyl group in valine permits the binding to a higher affinity in which the carboxyl groups exerts a steric effect thus leading to a higher binding constant valuewhen compared to Alanine [20].

Thus, the K_b values of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with alanine are 1.105×10^3 and $7.402 \times 10^3 M^{-1}$.The K_b values of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with valine are 2.549×10^3 and $2.084 \times 10^4 M^{-1}$. These results indicate that valine show higher K_b values than that of alanine and it binds strongly with the two Ru(II) complexes compared to that of alanine.

Histidine is a basic,polar aromatic amino acid containing a partially protonated imidazole group having two -NH bonds as a side chain which carries a positive charge equally distributed between the two nitrogens at physiological pH and also serves as a buffer for chemical reactions and are hydrophilic in nature [21]. One of the two nitrogen atoms present in the imidazole ring gets protonated and shows a basic nature and acts as a nucleophile. The protonated form shows hydrophilic naturewhereas the non-protonated form shows aromatic character and hydrophobic in nature. As the pH increase the imidazolate ion formation also increase and at pH greater than 7, the non-protonated form gets dominated and prefers hydrophobic environment. Histidine has three binding sites namely, carboxylate oxygen, imidazole nitrogen and amino nitrogen [22]. The imidazole ring of L-histidine is aromatic in all pH [23].The increase in binding nature of amino acids on Ru(II) complexes is based on the factors of aromatic planarity and hydrophobicity. Hence histidine binds strongly with these complexes and shows higher K_b values in both the complexes.

Tyrosine is an aromatic, hydrophobic and non-polar amino acid.The phenolic hydroxyl of tyrosine is more acidic than aliphatic hydroxyl group. Tyrosine shows both hydrophobic and hydrophilic features and can exhibit both behaviours depending on the circumstances [24]. The ring is aromatic and hydrophobic, but the hydroxyl substituent is hydrophilic. Tyrosine –OH group exist as phenoxide ion at pH12.5. This phenoxide ion binds with the Ru(II) complexes

and the strength of binding is less than that of histidine, hence the K_b values of the two Ru(II) complexes with tyrosine is less than that of histidine. Thus, in the present investigation histidine binds strongly with $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes than that of alanine, valine and tyrosine. The K_b values of the two Ru(II) complexes depends on the nature and the substituents present in the amino acids taken in the present study.Hence the aromatic amino acids (histidine and tyrosine) has unique binding capabilities and shows greater binding property when compared with the aliphatic amino acids (alanine and valine) [25].

The $K_b$values of $[Ru(phen)_2(phendione)]^{2+}$ complex with the four amino acids taken in the present study is higher than that of $[Ru(bpy)_2(phendione)]^{2+}$ complex. The ground-state interactions between the $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexeswith the amino acids are π -πstacking in nature. As the extent of π - π stacking interactions exist between the ligands of Ru(II) complexes and the amino acids increases, the binding also increases [19]. The π -π interactions of $[Ru(phen)_2(phendione)]^{2+}$ complex is more than of $[Ru(bpy)_2(phendione)]^{2+}$ complex, therefore, it binds strongly with the amino acids. Hence, the K_b values of $[Ru(phen)_2(phendione)]^{2+}$ complex is higher compared to that of $[Ru(bpy)_2(phendione)]^{2+}$ complex. This can be explained from the K_b values, the K_b of $[Ru(phen)_2(phendione)]^{2+}$ complex with histidine is 1.926×10^6 M^{-1} whereas for $[Ru(bpy)_2(phendione)]^{2+}$ complex is $1.477 \times 10^4 M^{-1}$. Thus, the $K_b$of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with amino acids not only depends on the nature and the substituents present in the amino acids but also depends on the nature of the ligands present in the complexes.

Conclusion

The binding of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with aminoacids(Alanine, Valine, Histidine andTyrosine) in aqueous medium at pH 12.5 has been investigated by UV-Visible absorption spectral techniques.The K_b Valuesof the two Ru(II) complexes with amino acids are determined from Benesi-Hildebrand plots. The K_b values of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with histidine are 1.477×10^4 and 1.926×10^6 M^{-1}. Among the four amino acids taken in the present investigation, histidine shows higher K_b Values in both the complexes and indicates that it binds strongly with the complexes. The increase in binding nature of histidine on these complexes is based on the factors of aromatic planarity and hydrophobicity.The K_b values of $[Ru(phen)_2(phendione)]^{2+}$ complex with the amino acids is higher than that of $[Ru(bpy)_2(phendione)]^{2+}$ complex because of more π - interactions. The K_b of $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes with amino acids depends on the nature and the substituents present in the amino acids and the ligands.This study confirms the structural effects on the binding of amino acids with the Ru(II) complexes in the ground state.

Conflict of Interest.

The authors declare no conflict of interest.

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