

Effect of Substituent on the Binding of Ruthenium (II)-Polypyridyl Complexes with Amino Acids in Aqueous Medium

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ABSTRACT

Binding of 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)} with amino acids (histidine and tyrosine) 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 absorption peak at 438 and 440nm in aqueous medium. The binding constant (K_b) of these complexes with amino acid are determined from Benesi-Hildebrand plots. The K_b values of histidine with $[Ru(bpy)_2(phendione)]^{2+}$ and $[Ru(phen)_2(phendione)]^{2+}$ complexes are $1.477 \times 10^4 M^{-1}$ and $1.926 \times 10^6 M^{-1}$. Histidine shows better binding property with these complexes than that of tyrosine, due to aromatic planarity and hydrophobicity. The K_b value of $[Ru(phen)_2(phendione)]^{2+}$ complex is higher than that of $[Ru(bpy)_2(phendione)]^{2+}$ complex due to greater $\pi - \pi$ 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

Electronic absorption spectroscopy is an effective method to detect the mode and extent of binding of metal complexes with amino acids. The modification of the metal or ligands in the complexes leads to substantial changes in the binding properties. The biological function of the metal complexes mainly depends on the interaction between the ligand-binding residues and metal ions present in the complex [1]. 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 [2,3]. Ru(II)-polypyridyl 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 complexes co-ordinated with polypyridyl ligands such as 2,2'-bipyridine(bpy), 1,10-phenanthroline($phen$), 1,10-phenanthroline-5,6-dione ($phendione$) and dipyrrophenazine ($dppz$) are well known. These ligands act as chelating agents for metal complexes which exhibit metal-to-ligand charge transfer (MLCT) and ligand-to-metal charge transfer (LMCT) transitions in the complex [5]. 1,10-phenanthroline-5,6-dione ($phendione$) is an effective redox mediator and versatile chelating agent for both homo and hetero complexes with metals and has the ability to form stable complexes with a wide variety of metal ions, because of the presence of *ortho*-quinone moiety [6,7].

Proteins, nucleic acids and amino acids are important cellular targets for metal complexes [8]. 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 to determine the sequence of amino acids is obtained from the binding residues of metal ions in proteins [9]. Amino acids form chelates through the amino and carboxylato groups and acts as an excellent metal complexing agent. In addition to 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 favourable for its intermolecular interactions and electron density between the metal ions and the ligands. In order to understand the role of Ru(II) complexes with amino acids, the present study focuses on the binding of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with histidine and tyrosine. The binding constant (K_b) of the complexes with amino acids are determined from the Benesi-Hildebrand plots.

2. MATERIALS AND METHODS

2.1 Materials

$\text{RuCl}_3 \cdot 3\text{H}_2\text{O}$, ligands (bpy, phen and phendione) and ammonium hexafluorophosphate were procured from Sigma-Aldrich. Amino acids (histidine and tyrosine) and LiCl were purchased from Merck. HPLC grade solvents were used for the synthesis of the complexes. The complexes $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ were synthesized by reacting the corresponding complexes of $[\text{Ru}(\text{bpy})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$ or $[\text{Ru}(\text{phen})_2\text{Cl}_2] \cdot 2\text{H}_2\text{O}$ with phendione according to the procedure previously described [6]. The binding studies were carried out with double-distilled deionized water.

2.2 Equipment

Sample solutions of amino acids, $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes were freshly prepared for each measurement. The absorption spectral measurements were carried out using SHIMADZU UV1800 double beam spectrophotometer. All the spectral measurements were carried out at 293 K.

2.3 Binding studies

The binding of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ 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 $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$

and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with the corresponding amino acids histidine and tyrosine were determined from the Benesi-Hildebrand equation using the absorption intensity data [10].

3 Results and Discussion

The binding of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with histidine and tyrosine in aqueous medium at pH 12.5 has been studied separately and the obtained results are discussed in this section. The structure of the two Ru(II) complexes is shown in **Fig.1**.

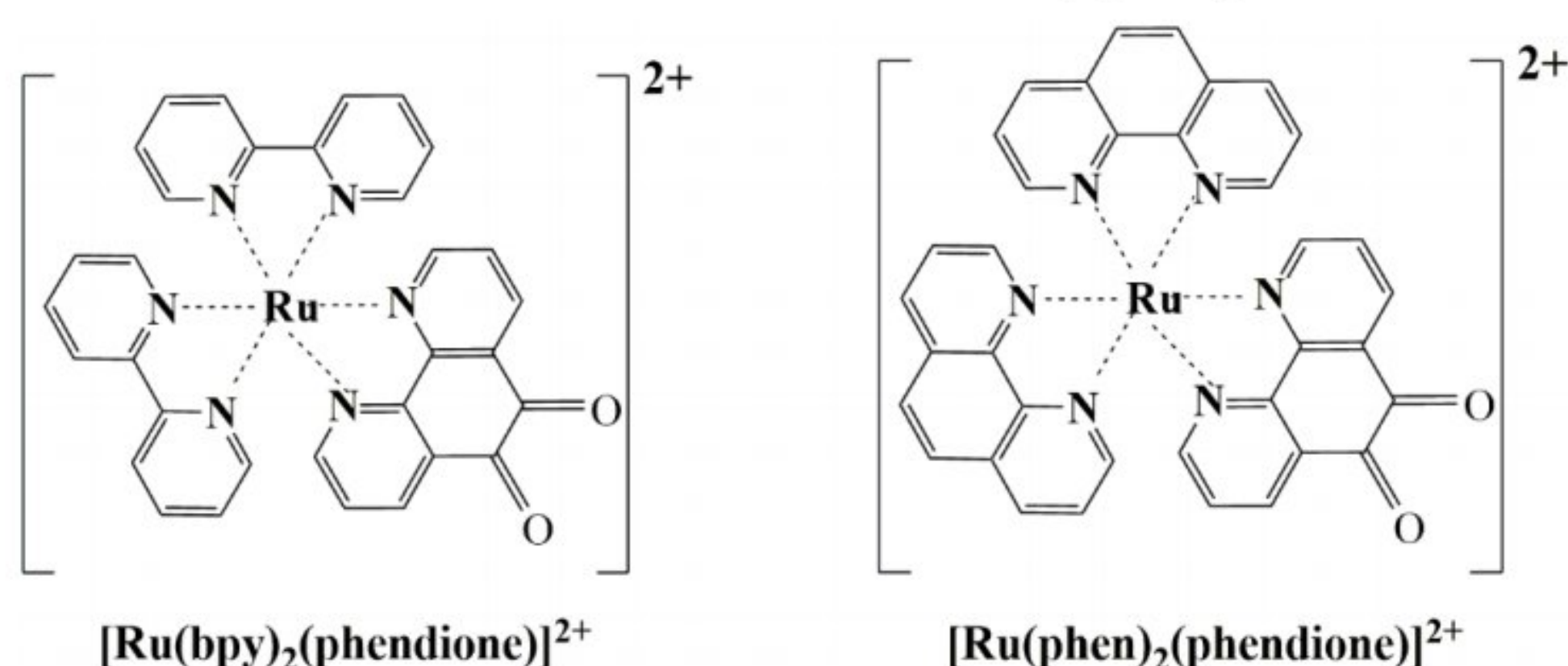


Fig. 1 Structure of the Ru(II) complexes

The absorption spectra of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes in aqueous medium shows a high energy absorption in the region 284-286 nm corresponding to the ligand centered $\pi - \pi^*$ transition and the low energy absorption at 438 and 440nm assigned to the $d\pi - \pi^*$ MLCT transition (**Fig.2**). The MLCT transition involves electronic excitation from the metal orbital [$d\pi$ (Ru)] to the ligand centered acceptor π^* orbitals. These values are in accordance with the reported values [6].

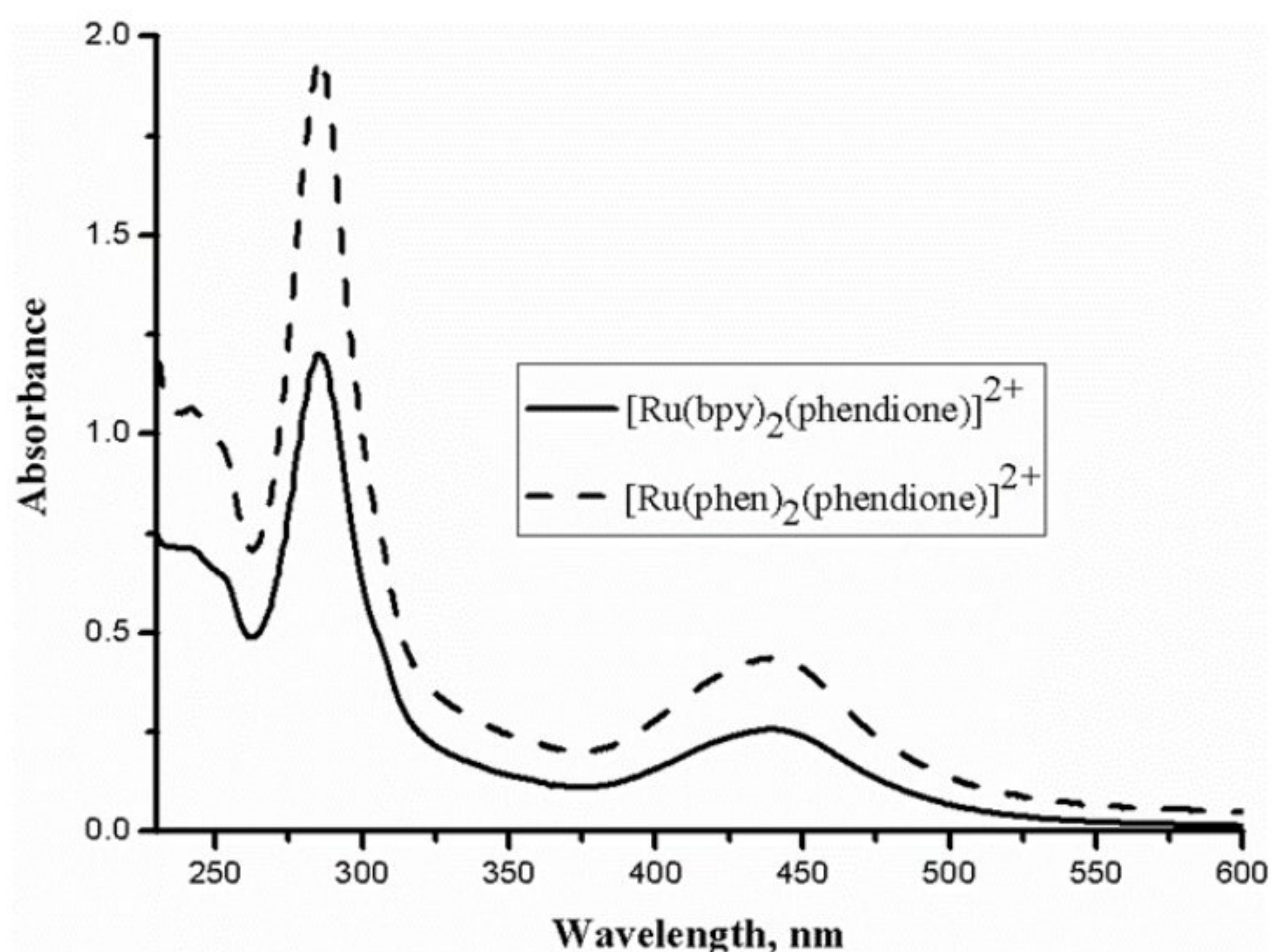


Fig.2 Absorption spectra of Ru(II) complexes in aqueous medium

The absorption spectral studies of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes in aqueous medium with the incremental addition of histidine and tyrosine shows a slight increase in the MLCT absorption maxima, indicates the formation of ground state complexes (**Fig. 3**). 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.4**). 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 $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes are hydrophobic or π -stacking in nature [11]. As the extent of π - π stacking interactions between the ligands of Ru(II)-complexes and the amino acids increase the binding becomes stronger.

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

Amino acids	Binding Constant (K_b) M^{-1}	
	$[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$	$[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$
Tyrosine	3.365×10^3	5.660×10^4
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 $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with histidine are 1.477×10^4 and $1.926 \times 10^6 \text{ M}^{-1}$. The K_b values of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with tyrosine are 3.365×10^3 and $5.660 \times 10^4 \text{ M}^{-1}$. The results reveal that histidine binds strongly with $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes.

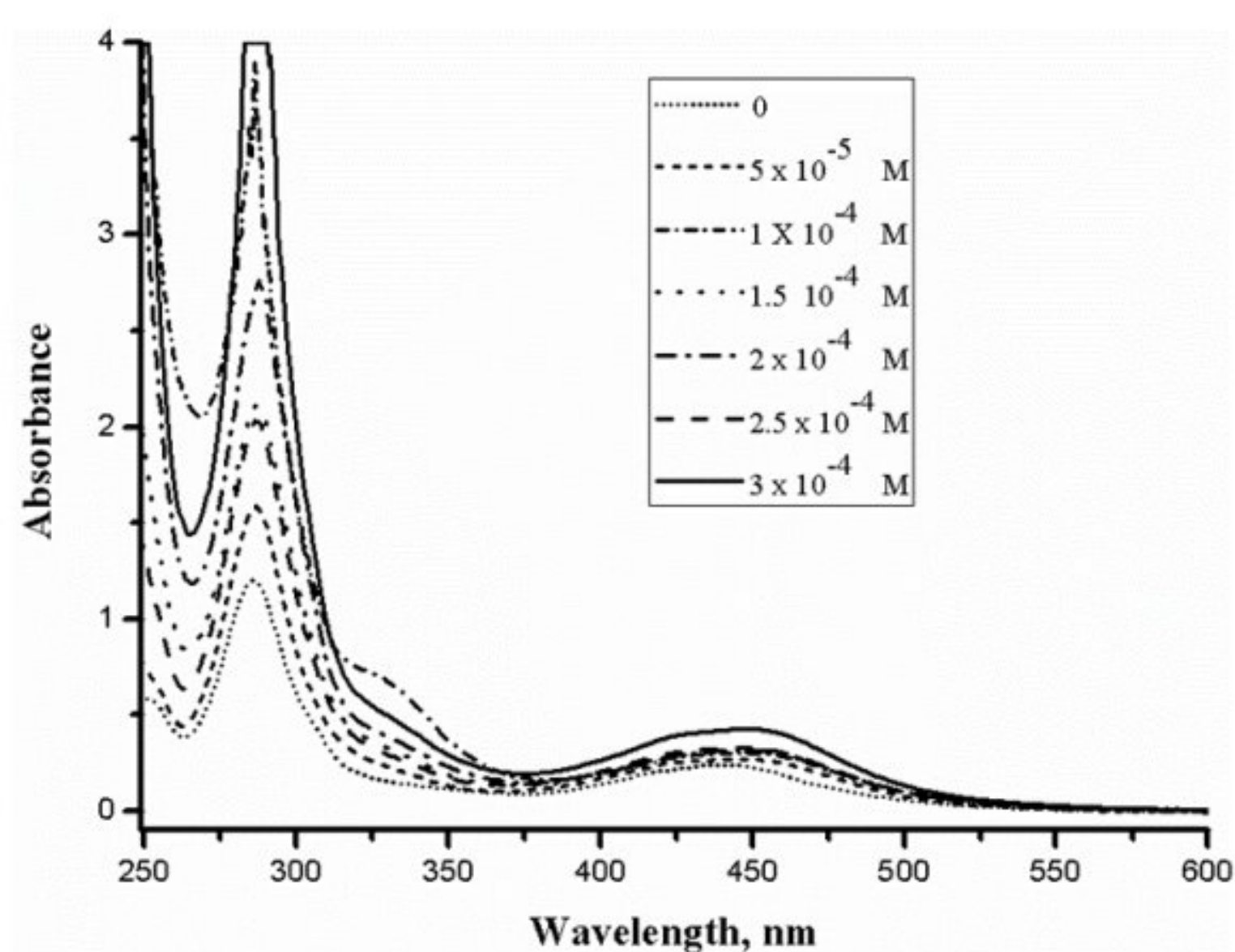


Fig. 3 Absorption spectra of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ complex with incremental addition of histidine

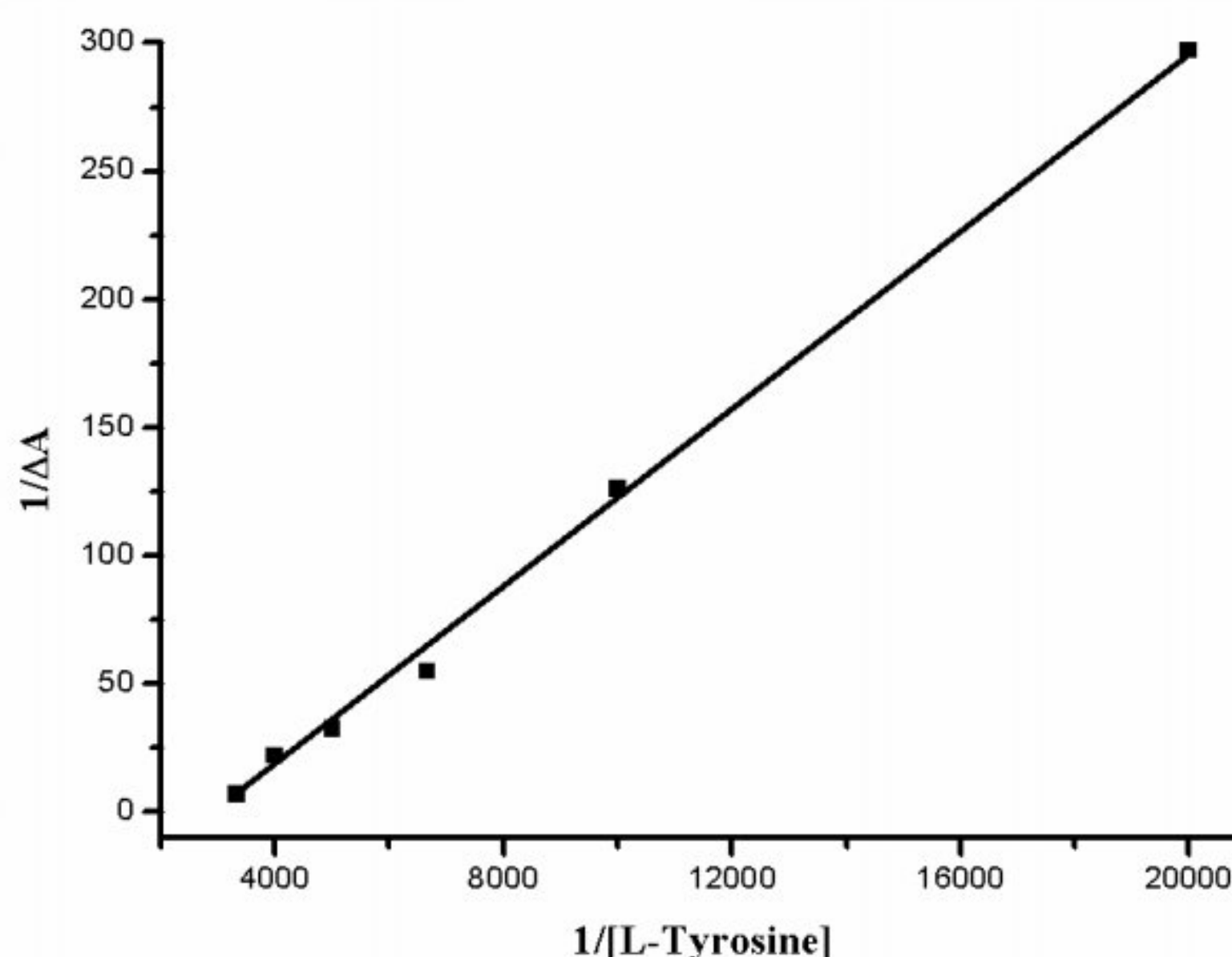


Fig. 4 Benesi-Hildebrand plot of $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complex with incremental addition of tyrosine.

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 and are hydrophilic in nature [12]. The protonated form shows hydrophilic nature whereas the non-protonated form shows both aromatic and hydrophobic nature. As the pH increase the imidazolite ion formation also increases and at pH greater than 7, the non-protonated form dominates and prefers hydrophobic environment. Histidine has three binding sites they are carboxylate oxygen, imidazole nitrogen and amino nitrogen [13]. The imidazole ring of histidine is aromatic in all pH, the increase in binding nature of amino acids on Ru(II) complexes is based on the factors of aromatic planarity and hydrophobicity [14]. 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 characters depending on the environment [15]. The ring is aromatic and hydrophobic, but the hydroxyl substituent is hydrophilic. Tyrosine -OH group exist as phenoxide ion at pH 12.5. 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. 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.

The K_b values of $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complex with the histidine and tyrosine is higher than that of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ complex. The ground-state interactions between the $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with the amino acids are π - π stacking in nature. The π - π interactions of $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complex is more than of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ complex, therefore, it binds strongly with the amino acids. Hence, the K_b values of $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complex is higher when compared to that of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ complex. This can be explained from the K_b values, the K_b of $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complex with histidine is $1.926 \times 10^6 \text{ M}^{-1}$ whereas for $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ complex is $1.477 \times 10^4 \text{ M}^{-1}$. Thus, the K_b of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ 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 $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with histidine and tyrosine in aqueous medium at pH 12.5 has been investigated by UV-Visible absorption spectral techniques. The K_b values of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complexes with histidine are 1.477×10^4 and $1.926 \times 10^6 \text{ M}^{-1}$. 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 $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ complex with the amino acids is higher than that of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ complex because of more π - π interactions. The K_b of $[\text{Ru}(\text{bpy})_2(\text{phendione})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{phendione})]^{2+}$ 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 biologically important amino acids with the Ru(II) complexes.

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