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Binding of ruthenium(II)-polypyridyl complexes with gallic acid and quercetin in Triton X-100

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ABSTRACT

The binding of $[Ru(bpy)_3]^{2+}$ (bpy = 2,2'-bipyridine), $[Ru(dmbpy)_3]^{2+}$ (dmbpy = 4,4'-dimethyl-2,2'-bipyridine) and $[Ru(dtbpy)_3]^{2+}$ (dtbpy = 4,4'-di-ter-butyl-2,2'-bipyridine) complexes with polyphenols (gallic acid and quercetin) have been studied in Triton X-100 at pH 11 by means of absorption spectral techniques. The absorption and emission maximum of these complexes are in the range of 449-457 nm and 612-639 nm respectively. The excited state lifetime of $[Ru(bpy)_3]^{2+}$, $[Ru(dmbpy)_3]^{2+}$ and $[Ru(dtbpy)_3]^{2+}$ complexes are 600, 350 and 654 ns. The binding constant (K_b) of these complexeswith gallic acid and quercetin are determined from the Benesi-Hildebrand equation using absorption intensity data. The observed binding constant values are sensitive to the nature of the ligand, medium and the structure ofgallic acid and quercetin. Quercetin binds strongly with Ru(II) complexes than that of gallic acid owing to the presence of more phenolic–OH groups. The ground-state interactions between the polyphenols and the bipyridyl rings of Ru(II) complexes are hydrophobic in nature. Structural effect, hydrophobic effect and the medium seem to play a vital role on the binding of the gallic acid and quercetinwith Ru(II) complexes.

Keywords: Ruthenium(II)-polypyridyl complexes; Polyphenols; Benesi-Hildebrand equation; Binding constant; Structural effect

1. Introduction

Polyphenols have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favourable biological effects including antioxidant properties. Phenolic acids and flavonoids constitute one of the most common and widespread groups of substances in flowering plants, occurring in all vegetative organs and fruits. The antioxidant property of polyphenols is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, singlet oxygen quenchers and metal chelators [1]. The antioxidant activity of polyphenols depends on the number of hydroxyl groups that are strengthened by steric hindrance [2]. In addition to antioxidant and free-radical scavenger properties, polyphenols have numerous other biological activities, such as antihistamine activity, as well as anti-inflammatory, protecting against cardiovascular diseases and anticancer activity [3].

Gallic acid (3,4,5-trihydroxybenzoic acid) is a basic structural unit of hydrolysable tannins widely distributed in the plant kingdom especially in tanniferous plants. Gallic acid is a strong chelating agent and forms stable complexes with iron [4]. The degree of chelation increases as the pH increases. The pK_a values of gallic acid are 4 (carboxylic acid), 8.7, 11.4 and > 13 (phenolic - OH groups) [5]. Quercetin (3,3',4',5,7-pentahydroxy flavone) is particularly interesting because it is one of the most biologically active and common dietary flavonols. Quercetin has two different pharmacophores, the catechol group (ring B) and the benzo-γ-pyrone derivative (ring A and C), of which the catechol moiety is the most reactive one where deprotonation occurs easily [6]. The pK_{a1} and pK_{a2} values of quercetin are 5.87 and 8.48 [7].

The development of transition metal complexes that target and interact non-covalently with proteins and antioxidants is an emerging field that links inorganic chemistry with chemical and synthetic biology [8]. Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry. The metal complexes as therapeutic agents for treatment of different diseases have been extensively studied [9]. As they generally have different mechanism of activity from the organic compounds, the development of metal complexes provides an alternative route of novel drug [10]. Among the transition metal complexes ruthenium(II)-polypyridyl complexes ($[Ru(NN)_3]^{2+}$) have particularly drawn significant interest for developing new diagnostic and therapeutic agents that can recognize and cleave DNA. Ru(II)-ploypyridyl complexes undergo binding with DNA, RNA and proteins and act as therapeutic agents [11]. Polyphenols with gallol or catechol groups are generally the most potent antioxidants, primarily because of the large iron-binding stability constants for these groups. Polyphenols containing catechol (quercetin) and gallol (gallic acid) groups have very different activities, depending on the metal ion [12].

The $[Ru(NN)_3]^{2+}$complexes have been extensively used as probes in micellar media and the photophysical properties vary enormously with the nature of the surfactant and concentration [13]. Many researchers [14,15] have attempted a systematic investigation of binding, partitioning and photosensitization of these $[Ru(NN)_3]^{2+}$ complexes in homogeneous and micro-heterogeneous media. The electron transfer reactions in micellarmedium can be either enhanced or slowed down compared to the reactions in aqueous solutions. The presence of hydrophobic groups like alkyl and aryl in the ligands of Ru(II) lead to strong binding of $[Ru(NN)_3]^{2+}$ with micelles through hydrophobic interaction. The strength of binding depends on the combination of electrostatic attractions or repulsions and hydrophobic effects. Bowers et al. [16] studied the surface and aggregation behaviour of aqueous solutions containing Ru(II) metallosurfactants. Based on the literature survey, the present study concentrates on the binding studies of gallic acid and quercetin on $[Ru(NN)_3]^{2+}$complexes in Triton X-100 at pH 11. In order to know the role of Triton X-100 in this binding reaction, the observed results are compared with the results obtained from aqueous medium at pH 11.

2. Experimental Section

$RuCl_3.3H_2O$, ligands (2,2′-bipyridine (bpy), 4,4′-dimethyl-2,2′-bipyridine (dmbpy), 4,4′-di-tert-butyl-2,2′-bipyridine (dtbpy)) and the polyphenols (gallic acid and quercetin) were procured from Sigma–Aldrich. HPLC grade solvents were used throughout the study for the synthesis of complexes as well as for binding studies. The double-distilled deionized water was used for the binding studies. The three $[Ru(NN)_3]^{2+}$ complexes {where NN = 2,2′-bipyridine (bpy), 4,4′-dimethyl-2,2′-bipyridine (dmbpy), 4,4′-di-t-butyl-2,2′-bipyridine (dtbpy)} were synthesized by reacting $RuCl_3.3H_2O$ with the corresponding ligands according to the procedure previously described [17].

The absorption spectral measurements were carried out using SYSTRONICS 2203 double beam spectrophotometer. Emission intensity measurements were carried out and the emission spectra were recorded using ELICO SL 174 spectrofluorometer. Excited state lifetime of the complex was made with laser flash photolysis technique using an Applied Photophysics SP-Quanta Ray GCR-2(10) Nd:YAG laser as the excitation source. The binding

of $[Ru(NN)_3]^{2+}$ complexes with various concentrations (2×10^{-5} - 1.4×10^{-4} M) of gallic acid and quercetin in Triton X-100 at pH 11 has been studied by absorption spectral technique.Phenolate ions of the gallic acid and quercetin for the binding studies were prepared by mixing the corresponding polyphenols with NaOH and the pH of the solution was maintained at 11 to confirm that the quencher was present as phenolate ions. The binding constant (K_b) of the $[Ru(NN)_3]^{2+}$ complex with gallic acid and quercetin was determined from the Benesi-Hildebrand equation using absorption intensity data .

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

where ΔA is the change in absorption of the complex with different concentrations ([G]) of polyphenols. The plots of $1/\Delta A$ 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 structure of the ligands and the polyphenols used in the present study are shown in **Fig. 1**. The photophysical properties like absorption and emission spectral data and the excited state lifetime of $[Ru(NN)_3]^{2+}$ complexes in aqueous and Triton X-100 at pH 11 are given in **Table 1**. The photophysical properties of the $[Ru(NN)_3]^{2+}$ complexes change from homogeneous to microheterogeneous medium.

Table 1 Absorption, emission spectral data and excited state lifetime of $[Ru(NN)_3]^{2+}$ complexes in aqueous and Triton X-100, at pH 11.

Complex	Absorption maximum (nm)		Emission maximum (nm)		Excited state lifetime (ns)	
	Aqueous	Triton X-100	Aqueous	Triton X-100	Aqueous	Triton X-100
$[Ru(bpy)_3]^{2+}$	451	449	612	613	614	600
$[Ru(dmbpy)_3]^{2+}$	457	456	625	625	380	360
$[Ru(dtbpy)_3]^{2+}$	457	456	626	639	510	654

The absorption spectral studies of $[Ru(NN)_3]^{2+}$ complexes with the incremental addition of gallic acid and quercetin showing a slight increase in the MLCT absorption maximum, indicates the formation of ground state complex (**Fig. 2**). Gallic acid and quercetin have weak absorption at 454 and 426 nm [18]. Gallic acid and quercetin bind with the $[Ru(NN)_3]^{2+}$ complexes in aqueous and Triton X-100 medium, since gallic acid and quercetin have weak absorption close to the region where Ru(II) complex have strong MLCT absorption. The absorption spectra of $[Ru(NN)_3]^{2+}$ complexes with gallic acid and quercetin shows a hypsochromic shift of 2 to 3 nm, due to the formation of ground state complex. The association of gallic acid and quercetin with $[Ru(NN)_3]^{2+}$ complexes in the ground state may be due to the static nature of quenching. The association constant (K_a) of $[Ru(NN)_3]^{2+}$ complexes with gallic acid and quercetin calculated from the Benesi–Hildebrand plot (**Fig.3**) using the Benesi–Hildebrand equation for the absorption spectral data in aqueous and Triton X-100 medium at pH 11 is given in **Table 2**. The K_a obtained for gallic acid and quercetin with $[Ru(NN)_3]^{2+}$ complexes is in the order of 10^2–10^4 M^{-1}.

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Table 2 Association constant, $K_a(M^{-1})$ calculated from absorption spectral data for gallic acid and quercetin with $Ru(NN)_3]^{2+}$ complexes in aqueous and Triton X-100, at pH 11

Complex	Aqueous		Triton X 100	
	Gallic acid	Quercetin	Gallic acid	Quercetin
$[Ru(bpy)_3]^{2+}$	2.9×10^3	4.0×10^4	2.5×10^2	2.4×10^3
$[Ru(dmbpy)_3]^{2+}$	8.2×10^3	5.7×10^4	4.7×10^2	5.2×10^3
$[Ru(dtbpy)_3]^{2+}$	8.9×10^3	9.0×10^4	6.4×10^2	6.8×10^3

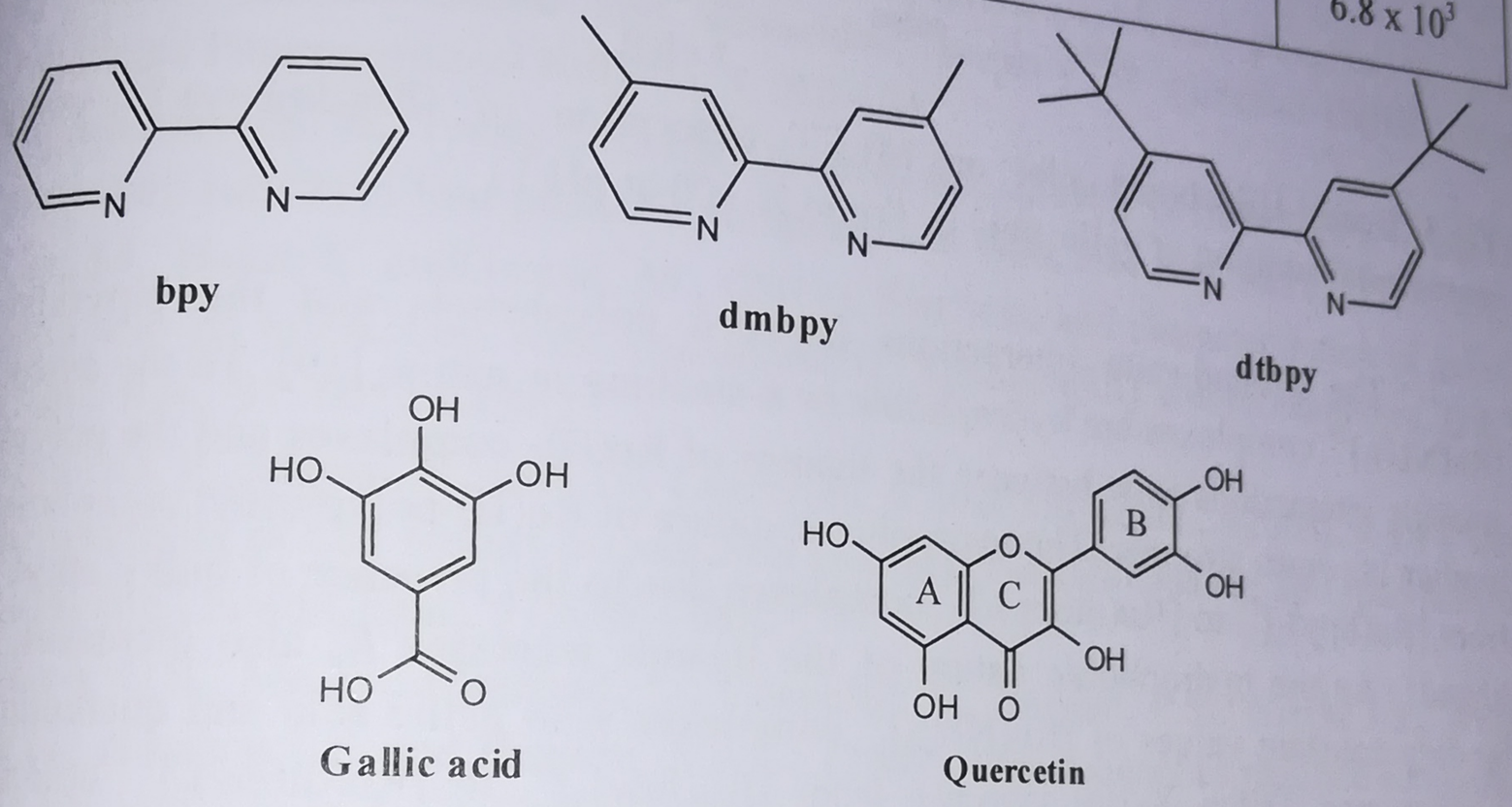

Fig.1 Structure of the ligands and the polyphenols

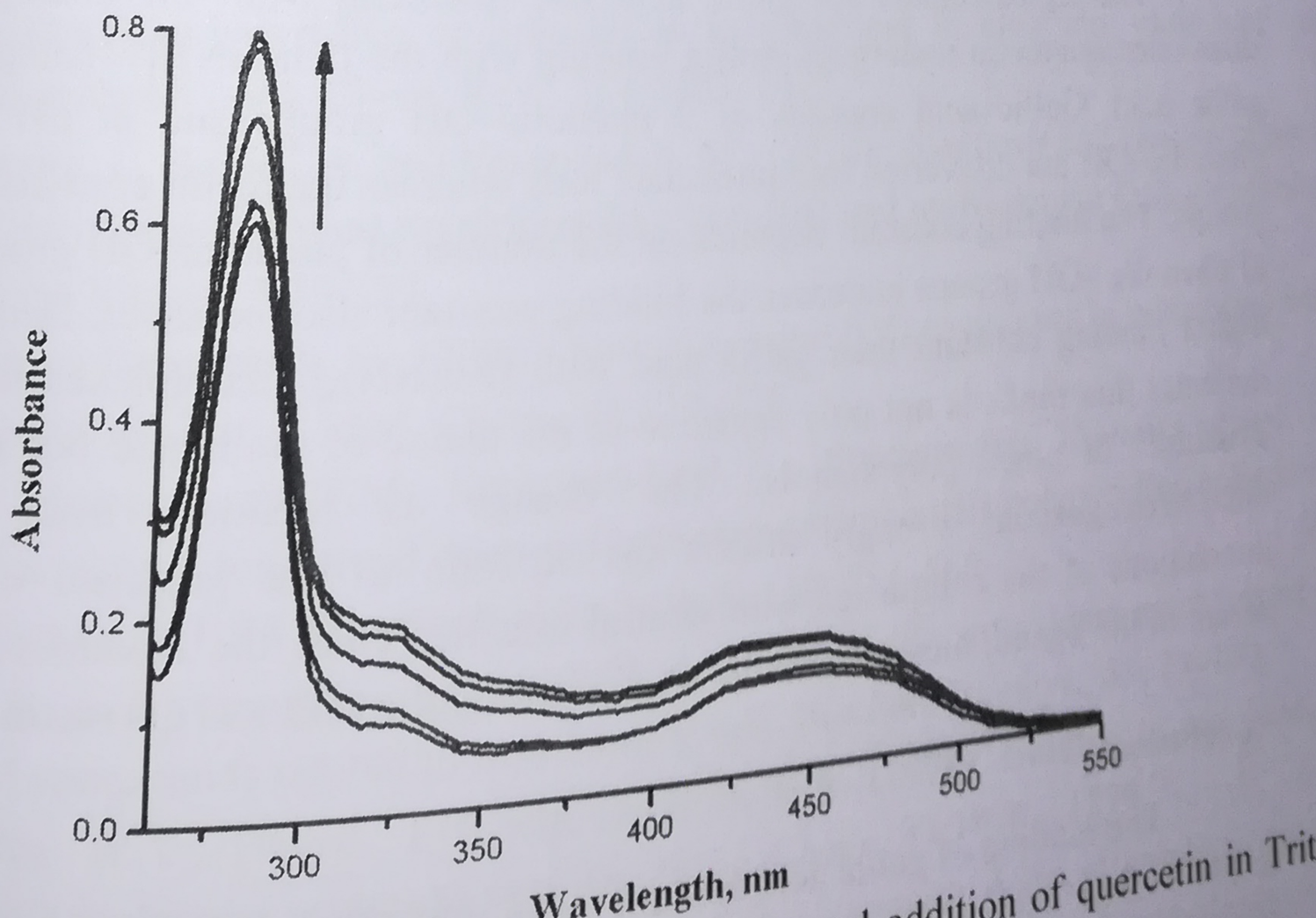

Fig.2 Absorption spectrum of $[Ru(bpy)_3]^{2+}$ with incremental addition of quercetin in Triton X-100 at pH 11

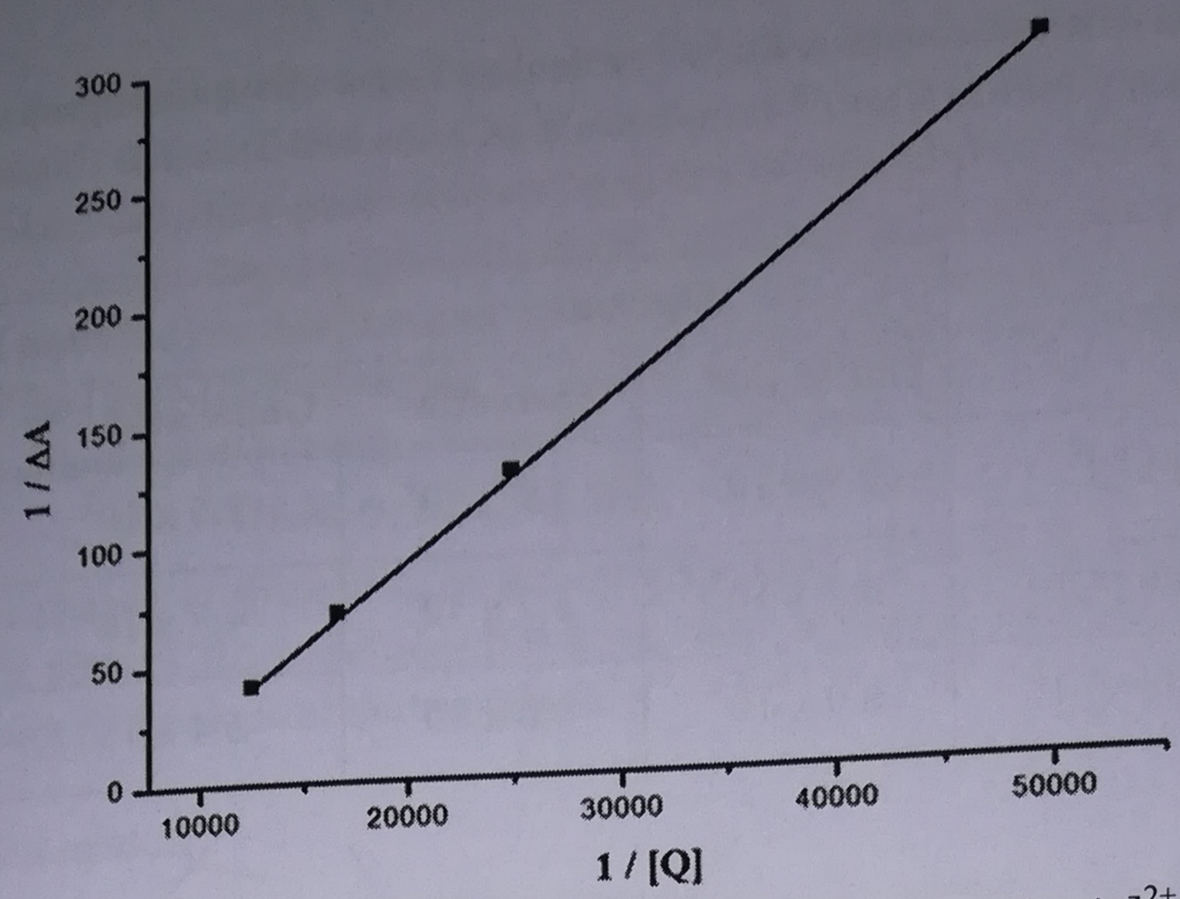

Fig.3 Benesi-Hildebrand plot on MLCT absorption of $[Ru(dmbpy)_3]^{2+}$ complex with incremental addition of gallic acid in Triton X-100 at pH 11

The ground-state interactions between polyphenols and the bipyridyl rings of $[Ru(NN)_3]^{2+}$ complexes are hydrophobic or π-stacking in nature [19]. To the extent that π - π stacking interactions exist between the ligands of Ru(II)- complexes and the polyphenols, the binding becomes stronger. The hydrophobic nature of Ru(II)-polypyridyl complexes increases from $[Ru(bpy)_3]^{2+}$ to $[Ru(dtbpy)_3]^{2+}$ complexes due to the presence of bulky alkyl substituted ligands. As the hydrophobic nature of the ligands increases K_b also increases. Hence the binding constant values of $[Ru(NN)_3]^{2+}$ complexes with gallic acid and quercetinin aqueous medium and Triton X-100 increase from $[Ru(bpy)_3]^{2+}$ to $[Ru(dtbpy)_3]^{2+}$ complexes. This results show that K_b is sensitive to the hydrophobic nature of the ligands.

The K_b calculated for gallic acid and quercetin from the absorption spectral data shows that quercetin undergoes strong binding with the $[Ru(NN)_3]^{2+}$ complexes than that of gallic acid. Gallic acid consists of 3 phenolic–OH groups and at pH 11 almost all the phenolic–OH are converted into phenolate ions whereas quercetin consists of 4 phenolic–OH groups. The binding constant depends on the number of phenolic–OH groups. As the number of phenolic –OH groups increases the binding constant also increases. Hence quercetin shows higher binding constant than gallic acid with $[Ru(NN)_3]^{2+}$ complexes in both media. This indicates that theK_b is not only sensitive to the nature of the ligand but also depends on the structure of the polyphenols. The change in medium from homogeneous to microheterogeneous strongly affects the K_b due to the presence of the hydrophobic interactions of the complexes with neutral micelle. Thus, the $K_b$values are sensitive to the nature of the ligand, medium and the structure of gallic acid and quercetin.

Conclusion

The binding of gallic acid and quercetin with $[Ru(NN)_3]^{2+}$ complexes in Triton X-100 at pH 11 has been studied by absorption spectral techniques. The K_b of the $[Ru(NN)_3]^{2+}$ complexes with gallic acid and quercetin is determined from the Benesi-Hildebrand plot. The K_b depends on the medium, hydrophobic nature of the ligands and the number of phenolic–OH groups of the polyphenols. As the number of phenolic–OH groups increases the binding

constant also increases. Quercetin shows higher binding constant than that of gallic acid due to the presence of more number of phenolic–OH groups. This study confirms the structural and hydrophobic effects and the effect of medium on the binding of biologically important phenolate ions with $[Ru(NN)_3]^{2+}$ complexes.

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