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EFFECT OF PH ON THE BINDING OF TRIS(2,2'-BIPYRIDYL)RUTHENIUM(II) COMPLEX WITH POLYPHENOLSIN AQUEOUS MEDIUM

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Abstract-The binding of$[Ru(bpy)_3]^{2+}$ (bpy = 2,2'- bipyridine) complex with polyphenols (gallic acid and quercetin) in aqueous medium at different pH (7, 11 and 12.5) has been studied by absorption spectral techniques. The complex shows absorption and emission maximum at 451 and 612 nm and it shows a lifetime of614 ns in aqueous medium. The binding constant (K_b) for these reactions in aqueous medium is determined from the Benesi-Hildebrand plot using absorption intensity data. The observed binding constant values of gallic acid and quercetin with $[Ru(bpy)_3]^{2+}$ complex is highly sensitive to the pH of the medium and the substituents present in the polyphenol.Quercetin binds strongly with this complex than gallic acid owing to the presence of more phenolic –OH groups. The binding constant values for gallic acid and quercetin with $[Ru(bpy)_3]^{2+}$ complex at pH 12.5 is higher than that of pH 11 and pH 7. The binding constant increases with increase in the pH of the medium. Structural effects and the pH of the medium seem to play a vital role on the binding of the gallic acid and quercetin with this complex.
Keywords: Polyphenols; Benesi-Hildebrand equation; Binding constant; Structural effect; Solvent effect

Introduction

Polyphenols have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favorable biological effects including antioxidant properties.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]. In addition to antioxidant andfree-radical scavenger properties, polyphenolshavenumerous other biological activities, such as antihistamineactivity, as well as anti-inflammatory, vasodilatory, and protecting against cardiovascular diseases [2]. These biological properties are attributed mainlyto their powerful antioxidant and antiradical activity.The antioxidant activity of polyphenols depends on the number of hydroxyl groups that are strengthened by steric hindrance [3].

Polyphenols are classified into two groups termed flavonoids and non-flavonoids. Flavonoids are some of the most common phenolics, widely distributed in plant tissues and are derived from the aromatic amino acids, phenyalanine and tyrosine, and have three-ringed structures [4]. Phenolic acids are one of the other main phenolic classes within the plant Kingdom and occur in the form of esters, glycosides or amides, but rarely in free form.Phenolic acids have a carboxyl group attached or linked to benzene ring.Polyphenols with gallol group (gallic acid) or catechol group (quercetin) are generally the most potent antioxidants, primarily because of their large iron-binding stability constantand have different activities, depending on the metal ion [5].

Gallic acid is found in almost all plants, plants known for their high gallic acid content include gallnuts, grapes, tea, hops and oak bark. Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect the cells against oxidative damage and it shows cytotoxicity against cancer cells, without harming healthy cells. Gallic acid molecule is essentially planar and has two intramolecular hydrogen bonds between hydroxyl groups, the hydrogen atoms of the three hydroxyl groups are oriented in the same direction around the ring and form intra-and intermolecular hydrogen bonds. The crystal structure is stabilized by all available intermolecular hydrogen bonds.Gallic acid is a strong chelating agent and forms complexes of high stability with iron [6]. 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). Thus, under the two pH conditions (9 and 11), the carboxylic acid aswell as a phenolic–OH of the semiquinone will be ionized [7]

Quercetin is a plant-derived flavonoid, specifically a flavonol, used as a nutritional supplement. Quercetin consists of 3 rings and 5 hydroxyl groups and it may have anti-inflammatory and antioxidant properties. It has being investigated for a wide range of potential health benefits and it reduces the risk of certain cancers.The oxidation potential of a polyphenol provides an estimate of the energy required to donate an electron; the lower

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the oxidation potential, lesser the energy required to donate an electron, hence it undergoes oxidation easily. The mechanism of oxidation of polyphenols and their stability in solution depend on pH [8]. The pK_{a1} and pK_{a2} values of quercetin are 5.87 and 8.48 [9].

Metal complexes play an essential role in agriculture, pharmaceutical and industrial chemistry. 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]. Many researchers have proved that binding of a drug to metalloelement enhances its activity and in many cases the complex possesses even more significant activity than the parent compound [11]. Among the transition metal complexes ruthenium(II)-polypyridyl complexes have particularly drawn significant interest for developing new diagnostic and therapeutic agents that can recognize and cleave DNA. These complexes undergo binding with DNA, RNA and proteins and act as therapeutic agents [12].

Ruthenium polypyridyl complexes are the most investigated one in the fields of solar energy conversion [13], artificial photosynthesis [14], optical sensing [15], and luminescent probes for characterizing microheterogeneous environments, owing to their favorable photophysical properties, excited state reactivity, and chemical stability [16].Based on the literature survey, the present study concentrates on the binding of $[Ru(bpy)_3]^{2+}$ (bpy = 2,2'- bipyridine) complex with polyphenols (gallic acid and quercetin) in aqueous medium at different pH (7, 11 and 12.5). The binding constant (K_b) for this reaction is determined from the Benesi-Hildebrand plot using absorption intensity data.

Experimental Section

$RuCl_3.3H_2O$, ligand (2,2'- bipyridine) and the polyphenols (gallic acid and quercetin) were purchased from Sigma- Aldrich. HPLC grade solvents were used throughout the study for the synthesis of complex as well as for quenching studies. The complex, $[Ru(bpy)_3]Cl_2$ was synthesized according to the procedure previously described [17].

Samples of the complex, $[Ru(bpy)_3]^{2+}$ as well as the polyphenols in aqueous medium were freshly prepared for each measurement. Absorption spectra were measured using SYSTRONICS 2203 double beam spectrophotometer. Emission spectrumwas recorded using JASCO FP-6300 spectrofluorometer. All the spectral measurements were carried out at 298 K. Excited state lifetime was made with laser flash photolysis technique using an Applied Photophysics SP-Quanta Ray GCR-2(10) Nd:YAG laser as the excitation source.Transient spectra were obtained by a point-to-point technique, monitoring the absorbance changes (A) after the flash at intervals of 10 nm over the spectral range 300-700 nm, averaging at least 30 decays at each wavelength.

The binding of $[Ru(bpy)_3]^{2+}$ complex with various concentrations(1×10^{-5} - 6×10^{-5} M) of polyphenols, gallic acid and quercetin in aqueous medium at various pH has been studied by absorption spectral technique. Phenolate ions for the binding studies were prepared by mixing the corresponding polyphenol with NaOH at various pH (7, 11 and 12.5). The binding constant (K_b) of the $[Ru(bpy)_3]^{2+}$ complex with gallic acid and quercetin were determined from the Benesi-Hildebrand equation using absorption intensity data.

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 complex and the polyphenols(gallic acid and quercetin) used in the present study are shown in **Fig.1**. The absorption spectrum of $[Ru(bpy)_3]^{2+}$ complex in aqueous medium shows a high energy absorption at 286 nm corresponding to the ligand centered π - π^* transition and the low energy absorption at 451 nm assigned to the $d\pi$ - π^* metal to ligand charge transfer (MLCT) transition (**Fig.2**). The MLCT transition involves electronic excitation from the metal orbital [$d\pi$ (Ru)] to the ligand centered acceptor π^* orbitals (ligand). The emission maximum of Ru(II) complex originates from the $d\pi$ - π^* ^{3}MLCT transition. The $[Ru(bpy)_3]^{2+}$ complex shows an emission maximum and excited state lifetime at 612 nmand 614 ns respectively.

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

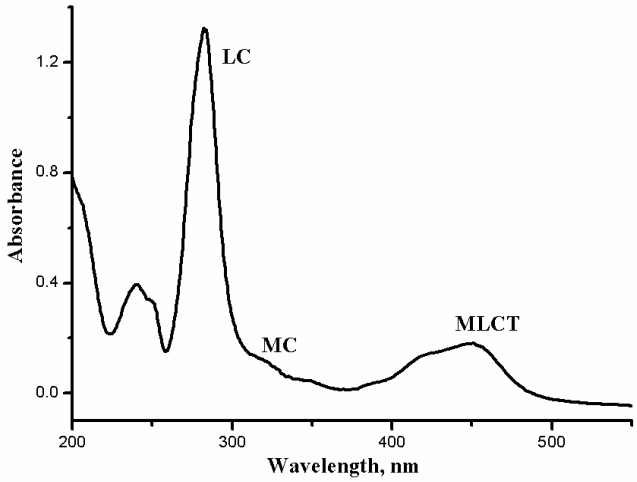

Fig.2 Absorption spectrum of $[Ru(bpy)_3]^{2+}$ complex in aqueous medium

The absorption spectral studies of $[Ru(bpy)_3]^{2+}$ complex with the incremental addition of gallic acid and quercetin shows a slight increase in the MLCT absorption maximum, indicates the formation of ground state complex (**Fig.3**). Gallic acid and quercetin have weak absorption at 454 and 426 nm [18]. Gallic acid and quercetin bind with the $[Ru(bpy)_3]^{2+}$ complex in aqueous medium at various pH since, gallic acid and quercetin have weak absorption close to the region where Ru(II) complexes have strong MLCT absorption. The binding constant (K_b) of this complex with gallic acid and quercetin are determined from the Benesi-Hildebrand plot (**Fig.4**). The K_b calculated from the Benesi-Hildebrand plotat various pH are represented in **Table 1**.The ground-state interactions between polyphenols and the bipyridyl rings of $[Ru(bpy)_3]^{2+}$ complex 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 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.The $K_b$calculated for $[Ru(bpy)_3]^{2+}$ complexin gallic acid and quercetin depends on the pH of the medium. The $K_b$of $Ru(bpy)_3]^{2+}$ complex with gallic acid at pH 7, 11 and 12.5 are 1.1 x 10^2, 2.9 x 10^3 and 2.4 x 10^4 respectively. The $K_b$of $Ru(bpy)_3]^{2+}$ complex with quercetinat pH 7, 11 and 12.5 are 1.7 x 10^3, 4.0 x 10^4 and 6.2 x 10^4respectively. The binding process is highly sensitive to the pH of the medium. As far as phenols are concerned they are in the undissociated form at low pH, i.e., pH < pK_a, but they are in the form of phenolate ions at high pH, i.e., pH > pK_a. Further in more acidic condition, the reducing capacity of the phenols may be suppressed due to protonation, whereas in more basic condition, the reducing capacity of the phenols enhances due to the formation of phenolate ions [8,18]. Therefore, in the present study the $K_b$of the complex with polyphenols at pH 7 and 11 is less than that of at pH 12.5. As the pH of the medium increases more and more phenolae ions are formed and this phenolate ion binds with the complex in the ground state. Hence at pH 12.5, all the phenolic–

OH groups in the polyphenols are exist as phenolate ions, this phenolate ions bind with the complex and thus increase the binding constant.

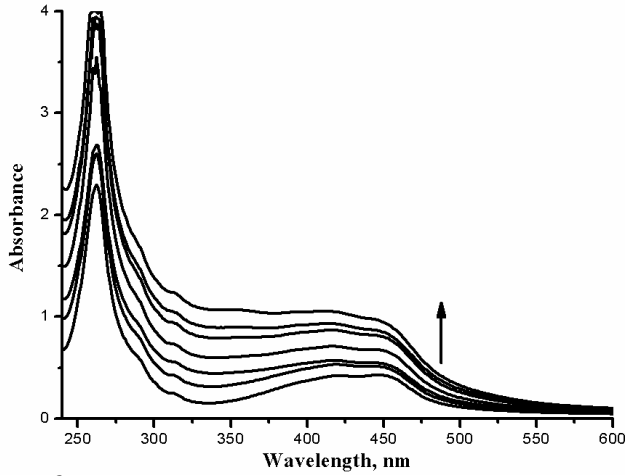

Fig.3 Absorption spectrum of $[Ru(bpy)_3]^{2+}$complex with incremental addition of gallic acid in aqueous mediam at pH 12.5

Table 1Binding constant, K_b (M^{-1}) for gallic acid and quercetin with $Ru(bpy)_3]^{2+}$ complex in aqueous medium at different pH

pH	Binding constant, K_b (M^{-1})	
	Gallic acid	**Quercetin**
7	1.1×10^2	1.7×10^3
11	2.9×10^3	4.0×10^4
12.5	2.4×10^4	6.2×10^4

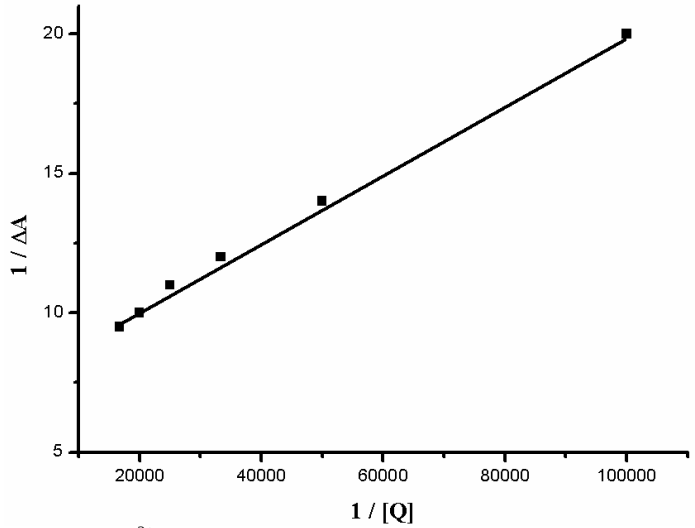

Fig.4 Benesi-Hildebrand plot for $[Ru(bpy)_3]^{2+}$ complex with incremental addition of quercetin in aqueous medium at pH 11

Gallic acid consist of three phenolic–OH groups, in alkaline medium especially above pH 11 the three phenolic–OH groups get ionized. Quercetin has two different pharmacophores, the catechol group in ring B and the three hydroxyl groups in rings A and C, the pK_a values of quercetin are 5.87 and 8.48. 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 $[Ru(bpy)_3]^{2+}$ complexat various pH. Thus the binding constant not only depends on the pH of the medium but also depends on the substituent present in the polyphenols.

Conclusion

The present investigation deals with the binding of polyphenols with $[Ru(bpy)_3]^{2+}$ complex in aqueous medium at different pH. The binding constant of the $[Ru(bpy)_3]^{2+}$ complex with gallic acid and quercetin are determined from the Benesi-Hildebrand plot. The binding constant depends on the pH of the medium and the nature of

substituents present in the polyphenols.The binding constant depends on the number of phenolic–OH groups. As the number of phenolic–OH groups increases the binding constant also increases. Quercetin shows higher binding constant than gallic acid due to the presence of more number of phenolic–OH groups. Moreover the K_b depends on the pH of the medium, as the pH of the medium increases binding constant also increases. This study confirms the structural effect and the effect of pH on the binding of biologically important phenolate ions with $[Ru(bpy)_3]^{2+}$ complex.

References

[1] Kaviarasan, S., Naik, G.H., Gangabhagirathi, R.., Anuradha, C.V. and Priyadarsini, K.I., In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. Food Chem., 2007, 103, 31 – 37.

[2] Souza, L.P., Calegari, F., Zarbin, A.J.G., Júnior, L.H.M. and Bergamini, M.F., Voltammetric determination of the antioxidant capacity in wine samples using a carbon nanotube modified electrode.J. Agric. Food Chem., 2011, 59, 7620 –7625.

[3] Janeiro, P., Novak, I., Seruga, M. and Brett, A.M.O., Electroanalytical oxidation of *p*-coumaric acid. Anal. Lett., 2007, 40, 3309–3321.

[4] Routray, W. and Orsat, V., Review: Microwave-assisted extraction of flavonoids. Food Bioprocess Technol., 2012, 5, 409 – 424.

[5] Farhan, S.A. Study on the interaction of copper (II) complex of morin and its antimicrobial effect. Int. J. Chem. Sci., 2013, 11, 1247 – 1255.

[6] Li, A.S., Bandy, B., Tsang S.S. and Davison, A.J., DNA-breaking versus DNA-protecting activityof four phenolic compounds in vitro. Free Rad. Res., 2000, 33, 551 – 566.

[7] Can, M., Bulut, E. and Ozacar, M. Synthesis and characterization of gallic acid resin and its interaction with Palladium(II), Rhodium(III) chlorocomplexes. Ind. Eng. Chem. Res., 2012, 51, 6052 – 6063.

[8] Sokolová, R., Ramešová, S., Degano, I., Hromadova, M., Gal, M. and Zabka, J., The oxidation of natural flavanoid quercetin. Chem. Commun., 2012, 48, 3433 – 3435.

[9] Harris, C.S., Mo, F., Migahed, L., Chepelev, L., Haddad, P.S., Wright J.S., Willmore, W,G., Arnason J.T. and Bennett, S.A.L. Plant phenolicsregulate neoplastic cell growth and survival: a quantitative structure-activity and biochemical analysis, Can. J. Physiol. Pharmacol., 2007, 85, 1124 – 1138.

[10] Kostova, I. and Momekov, G. New zirconium (IV) complexes of coumarins with cytotoxic activity. Eur. J. Med. Chem., 2006. 41, 717 – 726.

[11] Rajeshirke, M., Shah, R., Yadav, P. and Purohit, N.V. Synthesis and antioxidant activity of metal (II) complexes of isocoumarin derivatives. Der Pharmacia Sinica., 2012, 3, 239 – 248.

[12] Maksimoska, J., Feng, L., Harms, K., Yi, C., Kissil, J., Marmorstein, R. and Meggers, E. Targeting large kinase active site with rigid, bulky octahedral ruthenium complexes. J Am Chem Soc., 2008, 130, 15764 – 15765.

[13] Chitra, S., Somasundaram, N. and Easwaramoorthy, D., Ruthenium dyes with donor and acceptor moieties for TiO_2 solar cells. Arch. Appl. Sci. Res., 2013, 5, 112–119.

[14] Kalyanasundaram, K. and M. Graetzel, Artificial photosynthesis: biomimeticapproaches to solar energy conversion and storage. Curr.Opin. Biotechnol., 2010, 21, 298–310.

[15] Lainé, P.P., Campagna, S. and Loiseau, F., Conformationally gatedphotoinduced processes within photosensitizer–acceptor dyadsbased on ruthenium(II) and osmium(II) polypyridyl complexes with an appended pyridinium group. Coord. Chem. Rev., 2008, 252,2552–2571.

[16] Sun, Y., Collins, S.N., Joyce, L.E. and Turro, C., Unusual photophysicalproperties of a ruthenium(II) complex related to$[Ru(bpy)_2(dppz)]^{2+}$. Inorg. Chem., 2010, 49, 4257–4262.

[17] Swarnalatha, K., Rajkumar, E., Rajagopal, S., Ramaraj, R., Lu, Y.L., Lu, K.L and Ramamurthy, P., Photoinduced electron transfer reactions of ruthenium(II) complexes containing 2,2′-bipyridine-4,4′-dicarboxylic acid with phenols Steric and charge effects. J. Photochem. Photobiol. A Chem.,2005, 171, 83 – 90.

[18] Ramešová, S., Sokolová, R., Degano, I., Bulíčková, J., Žabka, J. and Gál, M., On the stability of the bioactive flavonoids quercetin and luteolin under oxygen-free conditions. Anal. Bioanal. Chem., 2012, 402, 975 – 982.

[19] Li, C. and Hoffman, M.Z., Oxidation of phenol by singlet oxygen photosensitized by the tris(2,2′-bipyridine)ruthenium(II) ion. J. Phys. Chem. A, 2000, 104, 5998 – 6002.