



## Intramolecular Excited-State Proton Transfer in Biologically Active Flavones: A Combined Experimental and Theoretical Study

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The absorption and fluorescence characteristics of two biologically active flavone derivatives 6-hydroxy-7,3',4',5'-tetramethoxyflavone (6HTMF) and 7-hydroxy-6,3',4',5'-tetramethoxyflavone (7HTMF) were studied as a function of acidity (pH/H<sub>0</sub>) of the solution. Dissociation constants were determined for ground and first excited singlet states and the results were compared with those obtained from Forster-Weller calculations. The acidity constants for these two compounds (6HTMF and 7HTMF) obtained from fluorimetric titrations are in poor agreement with those obtained from Forster-Weller data but are in good agreement with ground state pK<sub>a</sub> values indicated the deactivation of excited state (S<sub>1</sub>) before the prototropic equilibrium is complete. The broad band emission of both the flavones in acidic aqueous solution suggests their use as tuneable dye lasers. 6-Hydroxy-7,3',4',5'-tetramethoxyflavone undergoes adiabatic photo-dissociation in the singlet excited state indicating the formation of an exciplex or a photo-tautomer. The stability of photo-tautomer was confirmed by calculated value of enthalpy of formation using AM1 method.

**Keywords:** Flavones, Dissociation constants, Prototropic equilibrium, AM1 method, Phototautomer.

### INTRODUCTION

Flavones are well-known for their efficient biological use in medicinal industry as highly potential anticancer, anti-inflammatory and anti-allergic agents [1-5]. These properties are attributed to the structure of flavones which is based on their chemical behaviour. On going from ground state to excited state, there is a drastic change in these chemical properties. One such characteristic change on electronic excitation is in fluorescence intensity at different pH values. Solvent and pH dependent fluorescence spectroscopy has proved to be a valuable tool for studying primary photochemical processes, in particular, photo-dissociations and photo-protonations. The fluorescence based methods are also useful in understanding highly selective protein interactions and properties [6-8]. Since many transitions are accompanied by extensive reorganization of the molecular electron density, the acidity or basicity of such molecules may vary significantly in different electronic states. Therefore, the response of an otherwise neutral molecule to an incoming proton may strongly depend on the nature of the particular electronic state the acceptor molecule is in. Just as the absorbance

of the acidic and basic forms of an acid-base system provides information concerning the extent of dissociation of the unexcited molecules, so does the fluorescence of the acidic and basic forms of excited molecules. Fluorescence, in comparison to absorbance, is thus a measure by which the change in acidity upon excitation can be detected. After Weber's study on fluorescence intensity variation with pH [9] and Forster's method to calculate pK<sub>a</sub> in excited state [10], Weller used the method of fluorimetric titrations [11]. Acidity constant values can, therefore, be calculated using Forster-Weller method by taking fluorescence intensity as a function of pH or Hammett's acidity scale (H<sub>0</sub>). An interesting phenomenon is phototautomerism. Following charge transfer excitation, a molecule is protonated at one site and deprotonated at another site to form a tautomer. This often results in unusually large Stokes shift in the fluorescence band.

In continuation of our earlier work on the photophysical properties of certain novel flavone compounds [12-15], we have investigated the effect of pH on absorption and fluorescence spectra of the compounds 6-hydroxy-7,3',4',5'-tetramethoxyflavone (6HTMF) and 7-hydroxy-6,3',4',5'-tetrame-

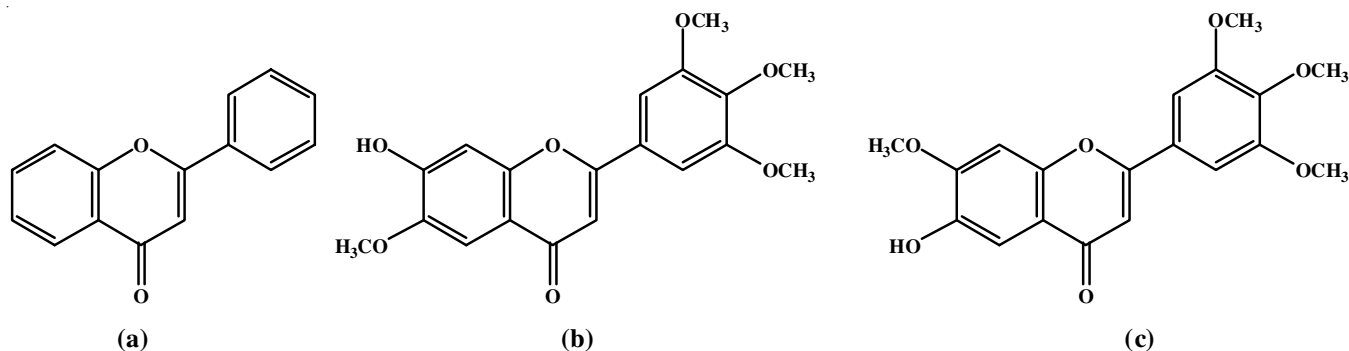


Fig. 1. Molecular structures of (a) flavone, (b) 6HTMF and (c) 7HTMF respectively

thoxyflavone (7HTMF) experimentally, as well as theoretically.

## EXPERIMENTAL

The flavones (6HTMF and 7HTMF) were synthesized according to the standard reported methods [16-19] (Fig 1). Their structures and purity were confirmed on the basis of their known melting points and their  $^1\text{H}$  NMR spectra. The absorption and fluorescence spectra were measured on a Kontron UV-930 spectrophotometer and Jasco FP-770 spectrofluorimeter respectively. All the solvents used, such as, benzene, ethyl acetate, acetonitrile, ethanol, methanol and triple distilled water were of spectroscopic grade. Analytical grade  $\text{H}_2\text{SO}_4$  and NaOH were used to prepare acidic and basic solutions. A modified Hammett acidity scale [20] for solutions below pH 1.5 (using  $\text{H}_2\text{SO}_4\text{-H}_2\text{O}$  mixture) was employed. Hammett's acidity function ( $H_0$ ) serves to define as a measure of the tendency for the solution in question to transfer a proton to an uncharged or charged base molecule; increasingly negative values corresponding to higher acidity. Stock solutions were prepared in methanol (10 %) and diluted with different pH/ $H_0$  solutions. Fluorimetric titrations were performed to calculate  $\text{pK}_a$  values using the wavelengths of isobestic points for excitation. These  $\text{pK}_a$  values were used in calculation of  $\text{pK}_a^*$  values by Forster cycle method. Austin Model 1 (AM1) semi-empirical molecular orbital calculations were carried out to estimate the stability of the anionic, cationic and neutral species of both the systems and phototautomer of 6HTMF compound. These theoretical calculations have been carried out by using Hyperchem 7.52 version program [21].

## RESULTS AND DISCUSSION

Absorption and fluorescence spectra of the flavones (6HTMF and 7HTMF) have been studied at different pH ranging from 12.25 to  $H_0$ -7.80 (Figs. 2-9, Tables 1-3). From the absorption spectra of 6HTMF and the absorption and fluorescence spectra of 7HTMF, three species are evident, namely the anion (A), the neutral molecule (N) and the cation (C) (Figs. 8 & 9 and Scheme-I). In contrast, four distinct fluorescence bands are observed in fluorescence spectra of 6HTMF on increasing the acidity of the solution from pH 12.25 to  $H_0$ -6.50. These bands have maxima at 594, 572, 532 and 562 nm and are assigned as anion (A), neutral molecule (N), cation (C) and a phototautomer (PT). The band at 562 nm is observed at pH 0.18 in addition to a peak at 536 nm and has been ascribed to an

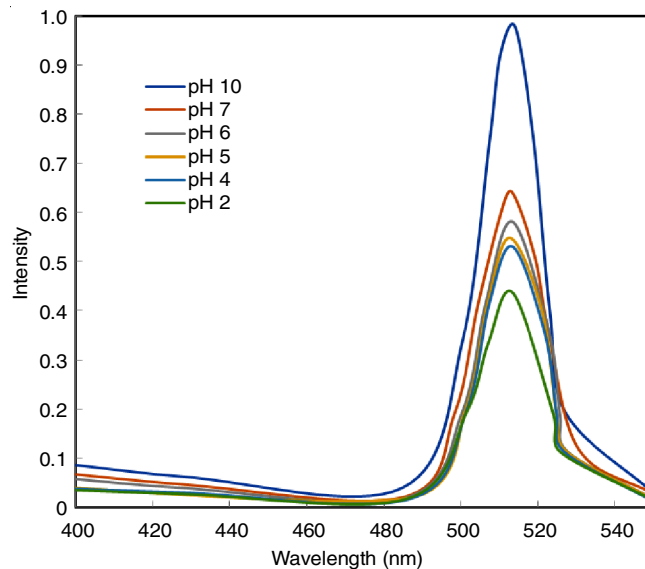


Fig. 2. Fluorescence spectra of 7HTMF at different pH values. Excited at isobestic wavelength 302 nm

TABLE-1  
SOLVENT AND ACIDITY DEPENDENT ABSORPTION, EXCITATION AND EMISSION MAXIMA ( $\lambda_{\text{max}}$ /nm) OF FLAVONE

Solvent pH/ $H_0$	$\lambda_{\text{max}}^{\text{abs}}$	Assigned species	$\lambda_{\text{max}}^{\text{exc}}$	$\lambda_{\text{max}}^{\text{flu}}$	Assigned species
7.00	304	N	—	—	—
0.00	306	C	330	405	C
$H_0$ -0.31	310	C	330	405	C
$H_0$ -1.01	324	C	328	405	C
$H_0$ -2.41	345	C	330	405	C
$H_0$ -4.46	348	C	350	405	C
$H_0$ -7.05	350	C	355	405	C
$H_0$ -9.85	355	C	355	405	C

TABLE-2  
SOLVENT AND ACIDITY DEPENDENT ABSORPTION, EXCITATION AND EMISSION MAXIMA ( $\lambda_{\text{max}}$ /nm) OF 7-HYDROXY-7,3',4',5'-TETRAMETHOXYFLAVONE

Solvent pH/ $H_0$	$\lambda_{\text{max}}^{\text{abs}}$	Assigned species	$\lambda_{\text{max}}^{\text{exc}}$	$\lambda_{\text{max}}^{\text{flu}}$	Assigned species
12.25	375	A	283	572	A
10.14	376	A	283	572	A
7.06	328	N	277	560	N
5.00	328	N	265	536	C
0.18	329	N	265	536	C
$H_0$ -0.31	393	C	260	536	C
$H_0$ -1.72	393	C	260	535	C
$H_0$ -4.46	393	C	260	535	C

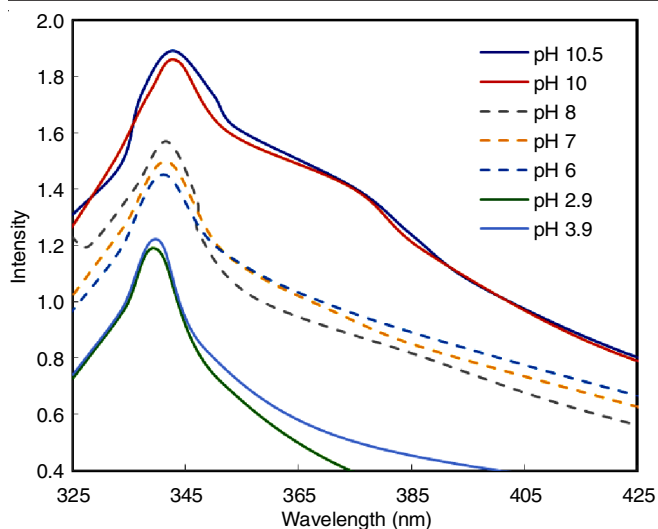
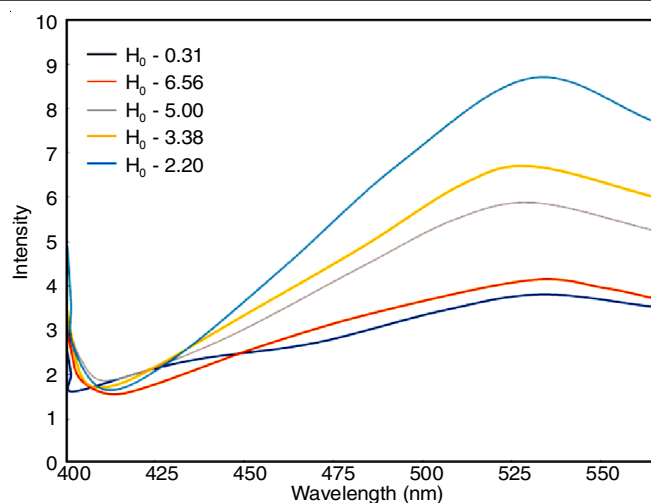
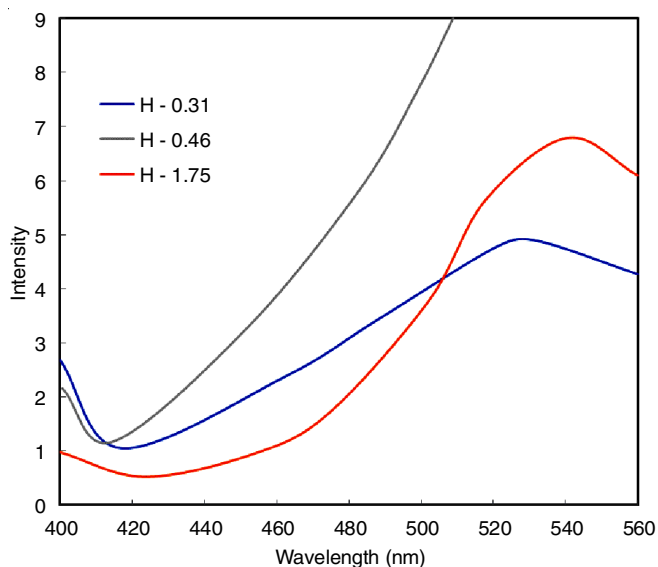
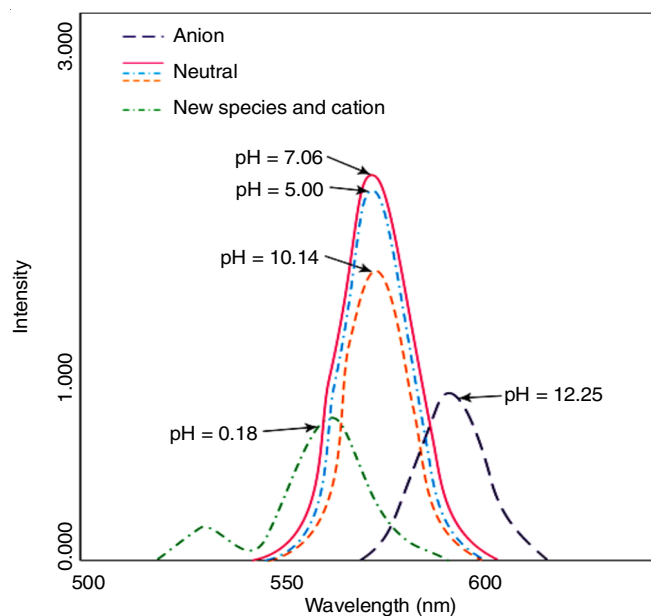
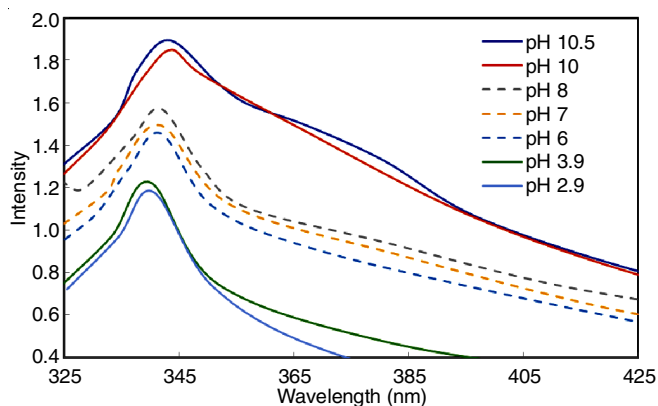
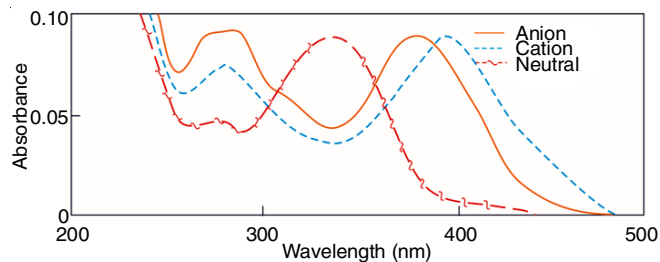
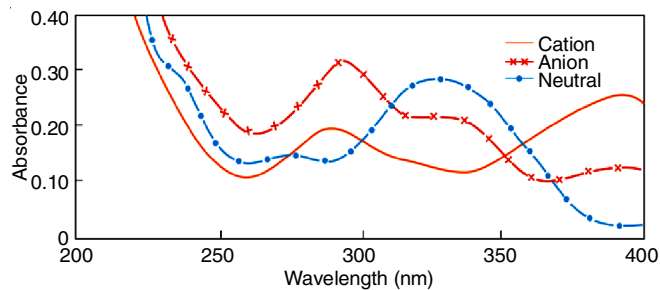
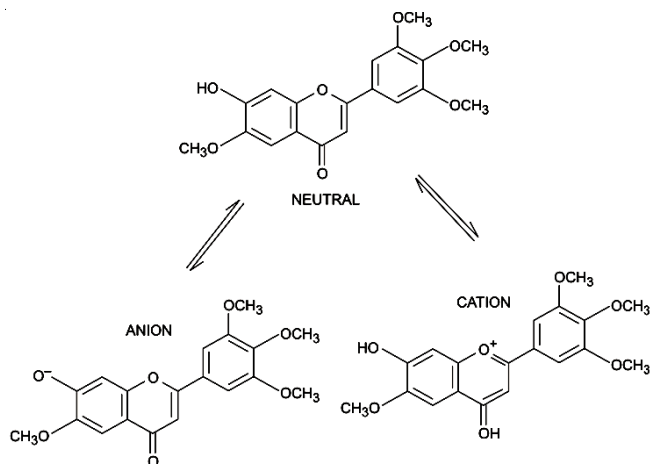
Fig. 3. Fluorescence spectra of 7HTMF at different pH/ $H_0$ Fig. 6. Fluorescence spectra of 6HTMF at different pH/ $H_0$ Fig. 4. Fluorescence spectra of 7HTMF at different pH/ $H_0$ Fig. 7. Fluorescence spectra of 6HTMF at different pH/ $H_0$ 

Fig. 5. Fluorescence spectra of 6HTMF at different pH values. Excited at isobestic wavelength 320 nm

Fig. 8. Absorption spectra of 7HTMF at different pH/ $H_0$ Fig. 9. Absorption spectra of 6HTMF at different pH/ $H_0$ 

intramolecular phototautomer whose resonance structures are shown in the **Scheme-II**. This new species (phototautomer) should be formed in an adiabatic reaction during the life time of excited states.



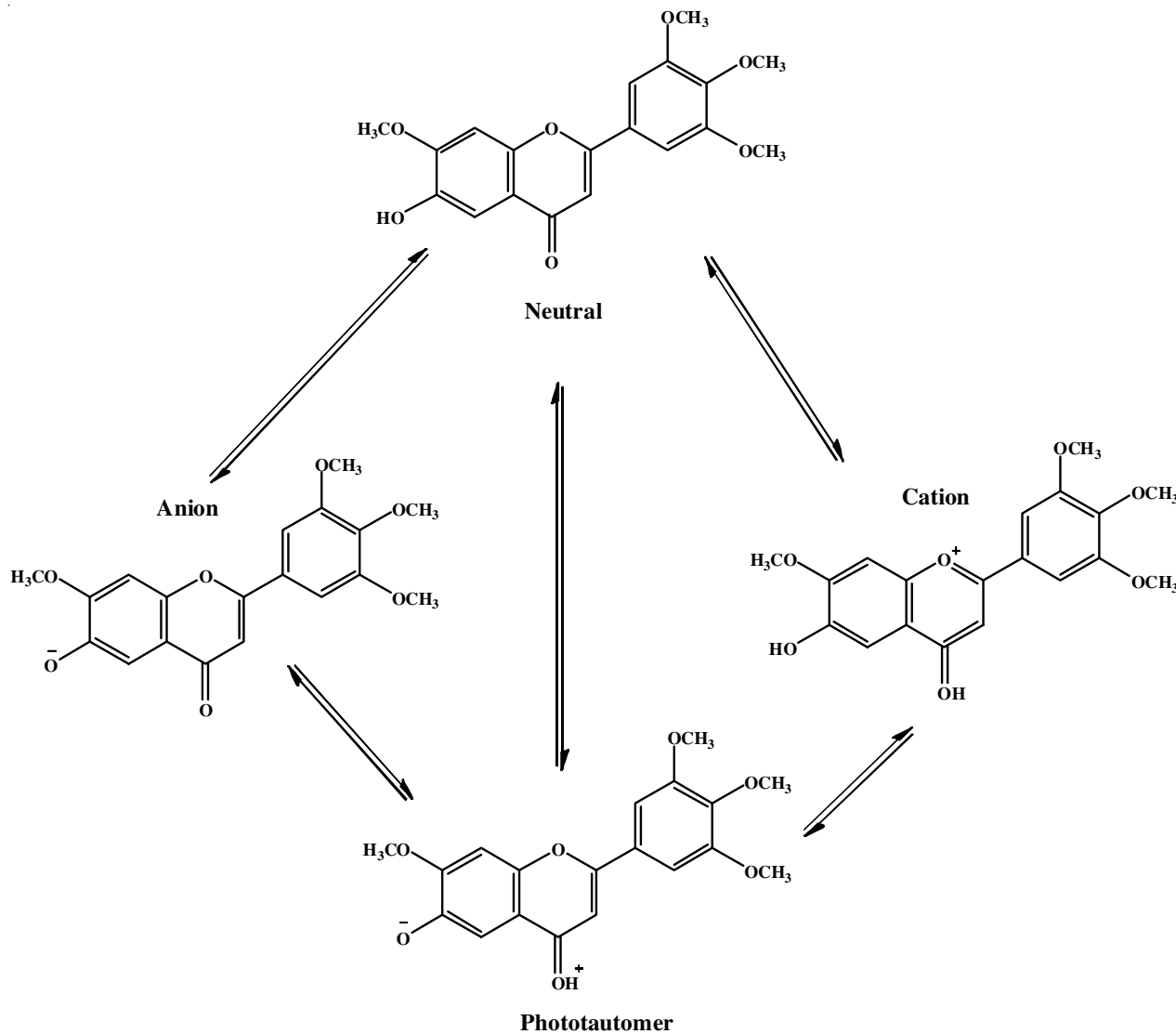
Scheme-I: Resonating structures of 7HTMF

The existence of new species (phototautomer) in case of 6HTMF has been further confirmed by AM1 calculated values of enthalpy of formation and HOMO-LUMO energy gap (Tables 5 and 6) based on Frontier orbital theory [22]. The calculated values support the phototautomer to be more stable than cationic

Solvent pH/ $H_0$	$\lambda_{\text{max}}^{\text{abs}}$	Assigned species	$\lambda_{\text{max}}^{\text{exc}}$	$\lambda_{\text{max}}^{\text{flu}}$	Assigned species
12.25	294	A	294	594	A
10.14	294	A	284	574	N
7.06	325	N	284	574	N
5.00	326	N	284	574	N
0.18	326	N	278	562,532	PT, C
$H_0$ -0.31	393	C	260	532	C
$H_0$ -2.20	393	C	260	532	C
$H_0$ -3.38	393	C	260	531	C
$H_0$ -5.00	393	C	260	531	C
$H_0$ -6.56	393	C	260	532	C

or anionic species. However, for 7HTMF, cation is found to be most stable due to lowest value of energy gap and highly negative enthalpy of formation values (Fig. 10).

Two excited state acidity constant ( $pK_a^*$ ) values governing the A-N-C equilibria were estimated to be 3.4 and 3.37 for 7HTMF and 3.4 and 1.7 for 6HTMF respectively, using the



Scheme-II: Resonating structures of 6HTMF

TABLE-4  
GROUND AND EXCITED STATE ACIDITY CONSTANTS ( $pK_a$ ) OF FLAVONES AND THEIR CONJUGATE ACIDS AT 298 K

System	Species	$pK_a$	Foster-Weller calculations			$\Delta pK_a$	$pK_a^*/pK_a(FT)$
			$V_{abs}$	$V_{fl}$	$V_{av}$		
Flavone	N	–	32895	–	–	–	–
	C	-1.33	28169	–	–	9.92	8.59/-1.10
6HTMF	A	–	25641	16835	21238	–	–
	N	7.70	30769	17422	24096	6.00	1.70/7.50
	C	-0.85	25445	18793	22119	4.25	3.40/f
7HTMF	A	–	26667	17482	22075	–	–
	N	7.80	30488	17857	24173	4.40	3.40/7.80
	C	-1.05	25445	18692	22069	4.42	3.37/f

A = Anion; N = Neutral; C = Cation; f = Flat curve;  $pK_a(FT)$  = By fluorimetric titration

TABLE-5  
THEORETICAL PROPERTIES OF 7-HYDROXY-7,3',4',5'-TETRAMETHOXYFLAVONE AND ITS CONJUGATE ACIDS IN GASEOUS PHASE

Species	$\lambda_{max}$ (nm)	HOMO (eV)	LUMO (eV)	$\Delta E$ (eV)	Enthalpy of formation ( $H_f$ ) (kcal/mol)
Anion	284.4	-9.237	-1.035	8.202	-156.1
Neutral	283	-9.066	-0.836	8.131	-184.2
Cation	339.8	-7.750	-0.141	7.609	-194.5

TABLE-6  
THEORETICAL PROPERTIES OF 6-HYDROXY-7,3',4',5'-TETRAMETHOXYFLAVONE AND ITS CONJUGATE ACIDS IN GASEOUS PHASE

Species	$\lambda_{max}$ (nm)	HOMO (eV)	LUMO (eV)	$\Delta E$ (eV)	Enthalpy of formation ( $H_f$ ) (kcal/mol)
Anion	243	-9.074	-0.928	8.146	-156.8
Neutral	285	-8.861	-0.958	7.902	-180.6
Cation	298	-8.737	-0.940	7.797	-114.6
Phototautomer	396	-8.094	-1.261	6.833	-164.3

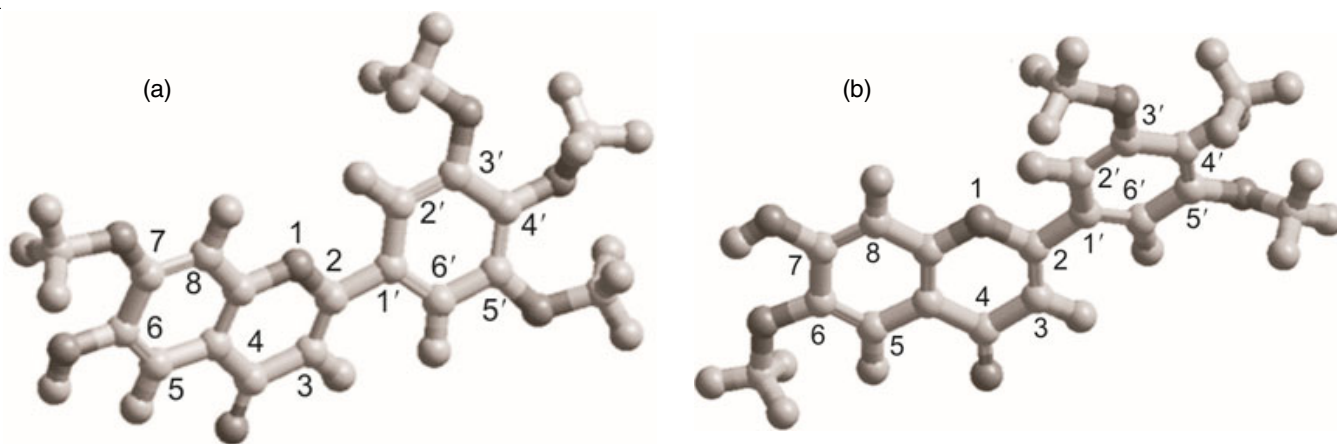


Fig. 10. Optimized geometry of neutral species of (a) 6HTMF and (b) 7HTMF

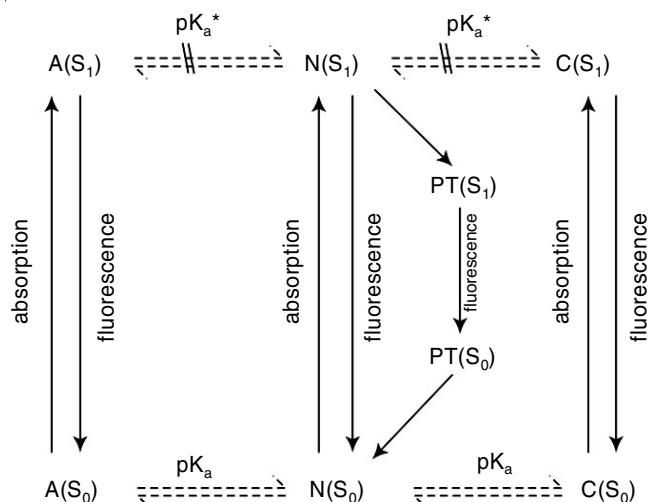
Forster cycle method. The  $pK_a$  values obtained for both the systems by fluorescence titration (FT) method (7.7 and 7.5, Table-4) are in poor agreement with those obtained from Forster cycle method (3.4 and 3.4) but are in good agreement with ground state  $pK_a$  values (7.8 and 7.7). This indicates deactivation of the excited state ( $S_1$ ) before the prototropic equilibrium is complete. The fluorimetric titration method is found to be less accurate because it is applicable only when there are two species in equilibrium. But in the flavones studied here, more than two species are in equilibrium with each other.

A broad band emission of these compounds in acidic aqueous solution suggests that they can be used as tunable dye lasers

although the low values of fluorescence quantum yields of flavones [13] have adverse effect on their laser dye properties. Intensity of fluorescence maxima for both the flavones first increases on changing pH from 1 to 10 and decreases sharply from pH 10 to 12.25 (Figs. 2, 5 and 7). This behaviour indicates small energy difference between the singlet and triplet excited states giving rise to more efficient intersystem crossing (Scheme-III).

### Conclusion

The fluorimetric titration (FT) method for calculating  $pK_a^*$  values is found to be less accurate because it is applicable only



**Scheme-III:** Despite the predicted high basicity of N(S<sub>1</sub>), the excited state process N(S<sub>0</sub>)→N(S<sub>1</sub>)→C(S<sub>1</sub>)→fluorescence plays no significant role in moderately acidic solutions. The path N(S<sub>0</sub>)→N(S<sub>1</sub>)→PT(S<sub>1</sub>)→fluorescence is more favourable. The photodissociation of N(S<sub>1</sub>) to form fluorescent A(S<sub>1</sub>) predicted by Forster-Weller estimations occurs only to a very small extent

in cases of equilibrium containing two species. Analysis of absorption spectra for different pH indicates the existence of three species (*i.e.* anion, neutral and protonated form) of both the compounds whereas, fluorescence spectra analysis and enthalpy of formation values obtained from theoretical calculations confirm the existence of a new emitting species (photo-tautomer) for the 6HTMF molecule. This broadening of fluorescence spectra in both the compounds and formation of photo-tautomer on excitation (in case of 6HTMF) suggests their use as tuneable dye lasers. The decrease in intensity of fluorescence spectra of both the compounds in higher alkaline medium is probably due to more efficient intersystem crossing.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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