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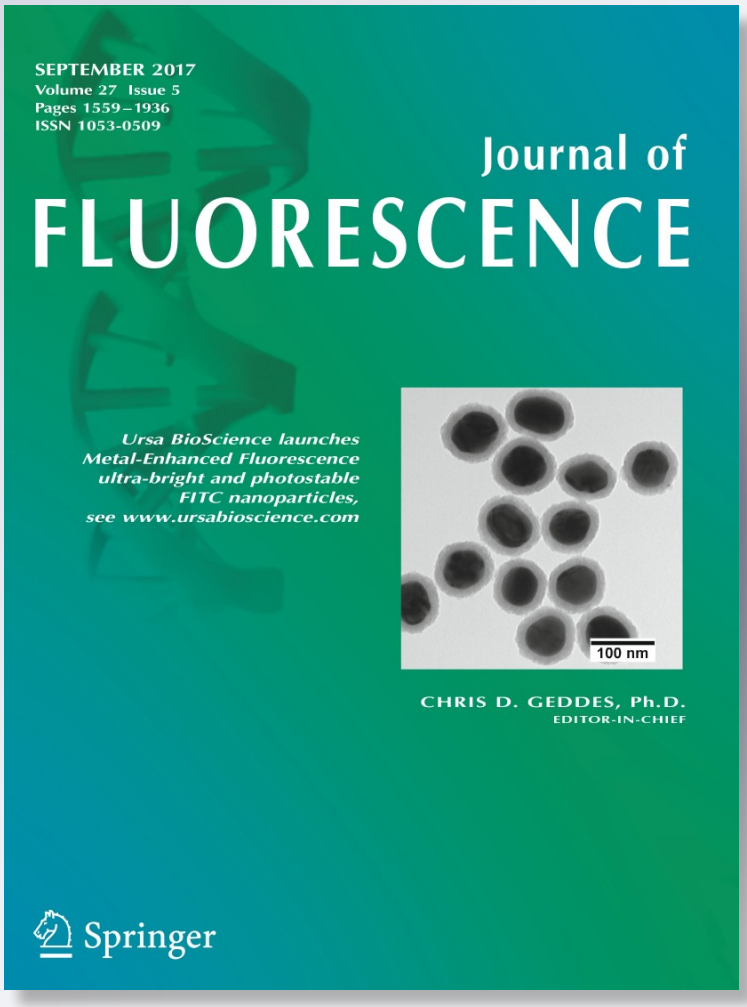

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ORIGINAL ARTICLE

Analysis of Fluorescence Quenching for Newly Synthesized Biologically Active 3(*2H*)-pyridazinone Derivative by Aniline

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Abstract Herein, we have studied the analysis of fluorescence quenching for newly synthesized biologically active 3(*2H*)-pyridazinone derivative 5-(5-bromo-2-hydroxy-phenyl)-2-phenyl-*2H*-pyridazin-3-one [BHP] by various concentrations of aniline using UV-Visible spectroscopy, fluorescence spectroscopy and time-correlated single photon counting technique in five different solvents namely, methanol, ethanol, propan-2-ol, dimethylsulfoxide and ethyl acetate at room temperature. The fluorescence intensity of BHP molecule decrease with increasing in the aniline concentration and it is studied using the Stern-Volmer relation. The obtained Stern-Volmer plots were found to be linear in all the five solvents. The various parameters responsible for the fluorescence quenching such as quenching rate parameters (k_q), diffusion rate parameter (k_d) and the probability of quenching per encounter (p) were experimentally calculated in all five solvents. An activation energy of quenching (E_a) was calculated using the values of activation energy of diffusion (E_d) and p. It was found that the values of E_a are greater than E_d in all five solvents studied. Further, it is inferred that the fluorescence quenching reactions in BHP molecule are more significantly affected by activation energy processes.

Keywords 3(*2H*)-pyridazinone · Activation energy · Fluorescence quenching · Material diffusion · Stern-Volmer relation

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Introduction

The 3(*2H*)-pyridazinones are the pyridazine derivatives which contain two adjacent nitrogen atoms at the one and two positions in a six-membered ring and a carbonyl group at the three position and they have different functionalities in their structure [1, 2]. A substantial number of pyridazinones derivatives are reported to possess antimicrobial, antitubercular, analgesic, anti-inflammatory, cyclooxygenase inhibitor, antidiabetic, antihypertensive, antiplatelet, anticancer, antifungal, antidepressant–anxiolytic, anticonvulsant, bronchodilatory (asthma), anti-allergic, antifeedant, inhibition of linolenic acid, activity for neurological disorders and many other properties [3]. Some of the major pyridazinone derivatives which have appeared in the market are indolidan, bemoradan, pimobendan, levosimendan as antihypertensive, minaprine as an antidepressant, emorfazone as anti-inflammatory and azanrinone as a cardiotonic [3].

Recently, spectroscopic and quantum chemical investigations on 3(*2H*)-pyridazinone derivatives such as levosimendan (IUPAC name: 2-[[4-[(4*R*)-4-methyl-6-oxo-4,5-dihydro-1*H*-pyridazin-3-yl]phenyl]hydrazinylidene]propanedinitrile) and bromopyrazone (IUPAC name: 1-phenyl-4-amino-5-bromopyridazon-(6)) compounds have been reported in the literature [4, 5]. In addition, the detailed structural, conformational, spectroscopic, electronic and nonlinear optical properties of the 3(*2H*)-pyridazinone derivatives namely flufenpyr (IUPAC name: {2-chloro- 4-fluoro-5-[5-methyl-6-oxo-4-(trifluoromethyl)-1(6H)-pyridazin-1-yl] phenoxy}acetic acid)) used in agriculture as a herbicide and amipizone (IUPAC name: 6-(p-(2-chloropropionylamino)phenyl)-5-methyl-4,5-dihydropyridazin-3- one)) designed to be antithrombotics and an inhibitor for platelet aggregations and the cardiovascular system) compounds estimated at the B3LYP (Becke's three-parameter

exact exchange functional (B3) combined with the gradient-corrected correlational functional of Lee, Yang and Parr (LYP)), B3PW91(Becke's three parameters incorporating Perdew and Wang's 1991 gradient-corrected exchange and correlation functions and includes 20% Hartree − Fock exchange) and HSEH1PBE (Heyd − Scuseria − Ernzerhof hybrid combined with Perdew, Burke and Ernzerhof's exchange and correlation functions) levels of theory with the basis set 6-311G (d, p) [6]. Soliman et al. reported the molecular structure, spectroscopic properties, NLO (nonlinear optical), HOMO (highest occupied molecular orbital) – LUMO (lowest unoccupied molecular orbital) and NBO (natural bond orbital) analysis of 6-hydroxy-3(2H)-pyridazinone [7]. Recently, we have reported the photophysical properties particularly fluorescence quantum yield and ground and excited state dipole moments of 3(*2H*)-pyridazinone derivatives [8, 9]. However, there are no reports on the fluorescence quenching of these derivatives. So, we are the first to report the fluorescence quenching of said molecule.

The phenomenon of fluorescence quenching competes with the spontaneous emission and causes the reduction in the fluorescence intensity and lifetime of the fluorescence molecules [10]. Generally, it is a process in which the electronic excitation energy of an excited molecule is transferred to a quencher molecule via several mechanisms such as diffusion, charge transfer and energy transfer etc., it leads to the non-fluorescent emission of quencher molecule. Under steady state illumination, the rate of formation of an excited molecule M^* is equal to its rate of deactivation and the concentration of excited molecule $[M^*]$ remains constant.

$$d[M^*]/dt = 0 \tag{1}$$

In the absence of any bimolecular step, the concentration of M^* is given below

$$[M^*] = \frac{R_a}{k_f + k_{IC} + k_{ISC}} = \frac{1}{k_f + \sum k_i} \tag{2}$$

where

Ra Rate of formation of the activated molecule.
k_f Rate constant for fluorescence.
$\sum k_i$ Sum of the rate constants for all the unimolecular deactivation steps such as internal conversion (k_{IC}) and intersystem crossing (k_{ISC}) which originate from this state.

If another molecule Q is added to the solution which quenches the fluorescence intensity of molecule M by bimolecular quenching step, then:

$$M^* + Q \rightarrow M + Q$$
$$\text{Reaction rate} = k[M^*][Q] \tag{3}$$

Then the concentration of fluorophore (excited molecule) $[M^*]$ in the presence of the quencher is given below

$$[M^*] = \frac{R_a}{k_f + \sum k_i + k[Q]} \tag{4}$$

If $[M^*]^0$ and $[M^*]$ are the fluorophore concentrations in the absence and presence of quencher, the respective quantum yields F_0 and F are given below

$$F_0 = \frac{k_f[M^*]^0}{R_a} = \frac{k_f}{k_f + \sum k_i} \tag{5}$$

$$F = \frac{k_f[M^*]}{R_a} = \frac{k_f}{k_f + \sum k_i + k[Q]} \tag{6}$$

then the ratio of two quantum yields gives:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] \tag{7}$$

Equation (7) is known as the Stern- Volmer [S-V] equation. where

$$\tau_0 = \frac{1}{(k_f + \sum k_i)}$$
$$K_{sv} = k_q \tau_0$$

F_0 and F are the fluorescence intensities without and with quencher respectively.

K_{SV} is the S-V constant and $[Q]$ is the quencher concentration. The S-V constant K_{SV} can be estimated from the slope of the linear plot of F_0/F versus $[Q]$. k_q is the S-V quenching rate parameter and τ_0 is the fluorescence lifetime of the solute in the absence of quencher.

The fluorescence quenching of organic molecules in solvents by various quenchers like carbon tetrachloride [11, 12],

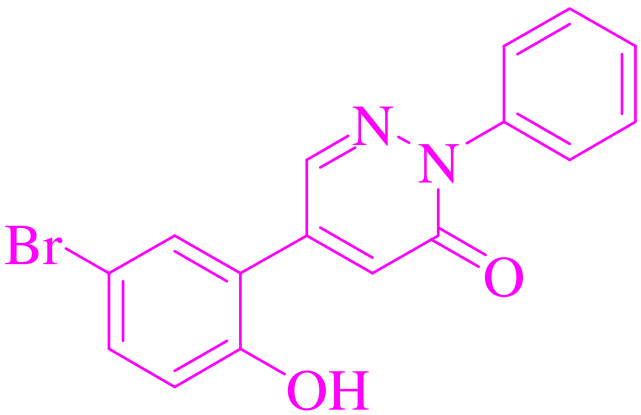

Fig. 1 Molecular structure of 5-(5-bromo-2-hydroxy-phenyl)-2-phenyl-*2H*-pyridazin-3-one [BHP]

aniline [13–15], oxygen [16], TiO_2 nanoparticles [17, 18] and silver nanoparticles [19] have been a subject of continued investigators for last two decades. The aim of above-said investigations is to know the whether the fluorescence quenching is dynamic or static in nature and these results of interactions between biologically active molecules with biosystems and nanoparticles may shine in the area of biological applications. In general, the fluorescence quenching phenomenon is very useful in the field of physical science, chemical science and medical science [20, 21].

In view of the biological importance of 3(*2H*)-pyridazinone derivatives and fluorescence quenching process, we have studied the fluorescence quenching of newly synthesized

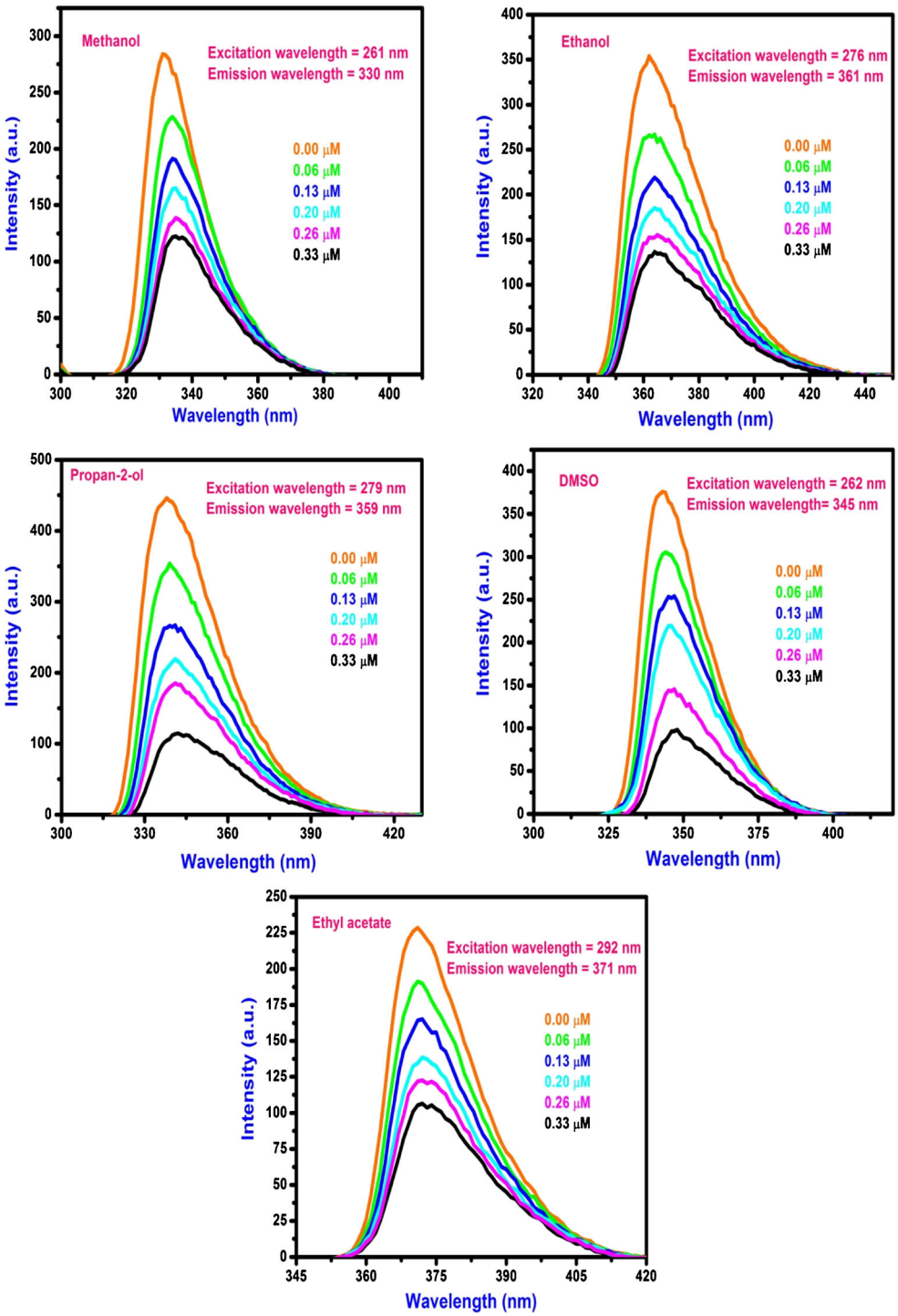

Fig. 2 Fluorescence spectra of BHP molecule along with their corresponding excitation and emission wavelengths in five solvents for different concentrations of aniline

biologically active 3(*2H*)-pyridazinone derivative 5-(5-bromo-2-hydroxy-phenyl)-2-phenyl-*2H*-pyridazin-3-one [BHP] by the various concentration of aniline in five different solvents. Further, we have estimated various fluorescence quenching rate parameters responsible for fluorescence quenching using the Stern-Volmer relation.

Experimental

Materials

The biologically active 3(*2H*)-pyridazinone derivative 5-(5-bromo-2-hydroxy-phenyl)-2-phenyl-*2H*-pyridazin-3-one [BHP] molecule is synthesized according to reference [22], the completion of the reaction, purity of the molecule was checked by TLC (Thin-layer chromatography). Further, purity as 100% was confirmed by recording the spectrum using Agilent-single Quartz LC-MS (Liquid chromatography–mass spectrometry). The elemental analysis was carried out using Heraus CHN rapid analyzer. The melting point was determined by using a Shital melting-point apparatus. Functional groups were analyzed with Nicolet-5700 Fourier transform infrared (FTIR) spectrophotometer. Structural confirmation of molecule was analyzed with ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400-MHz spectrometer using $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard and were discussed in reference [22]. The molecular structure of BHP is shown in Fig. 1. Solvents methanol, ethanol, propan-2-ol, dimethylsulfoxide (DMSO) and ethyl acetate (Solvents are chosen based on the high solubility of BHP molecule) were of spectroscopic grade and they were obtained from S.D. Fine Chemicals Ltd., India. The quencher aniline of ACS grade (>99.5% assay) was double distilled and tested for its purity before use. Solutions were prepared to keep the concentration of solute BHP fixed 1×10^{-5} M and varying quencher concentrations (0.00 μM, 0.06 μM, 0.13 μM, 0.20 μM, 0.26 μM and 0.33 μM) in all five different solvents.

Spectroscopic Measurements

In all the spectroscopic measurements quartz cuvettes were used. For the absorption spectra we have used two sides transparent, then for fluorescence spectra and fluorescence lifetimes, we have used four sides transparent cuvette. These cuvettes are having 1 cm width and 5 cm height.

- **Absorption spectra**: Absorption spectra were recorded using UV-visible spectrophotometer [Model: Hitachi U-3310 at USIC, K U Dharwad, India] in the range of 200 nm – 800 nm with bandwidth 1.0 nm and deuterium lamp used as a light source.
- **Fluorescence spectra:** Fluorescence spectra were recorded in absence and presence of the quencher using fluorescence spectrophotometer [Model: Hitachi F-7000 at USIC, K U Dharwad, India] in the range of 300 nm – 600 nm with bandwidth 1 nm, voltage 400 V and Xenon lamp used as a light source.
- **Fluorescence lifetimes:** Fluorescence lifetimes were recorded in the absence of the quencher using time-correlated single photon counting technique (TCSPC) [Model: ISS 90021 at USIC, K U Dharwad, India] in the bi-exponential fitting having chi-square value nearly unity using laser diodes as a source.

All these spectroscopic measurements were carried out at room temperature [300 K]. The experimental values are reproducible within 5% of the experimental error.

Table 1 Fluorescence intensities of BHP molecule for different concentrations of aniline in five different solvents

Solvent	Quencher concentration (μM)	Fluorescence intensity (a.u.)
Methanol	0.00	281.888
	0.06	226.897
	0.13	187.165
	0.20	160.335
	0.26	135.963
	0.33	123.163
Ethanol	0.00	352.935
	0.06	260.772
	0.13	214.691
	0.20	185.445
	0.26	153.249
	0.33	133.219
Propan-2-ol	0.00	444.279
	0.06	348.607
	0.13	266.002
	0.20	215.237
	0.26	179.642
	0.33	113.858
DMSO	0.00	375.321
	0.06	303.982
	0.13	252.095
	0.20	216.425
	0.26	141.841
	0.33	097.999
Ethyl acetate	0.00	227.367
	0.06	189.137
	0.13	162.375
	0.20	137.525
	0.26	121.316
	0.33	105.030

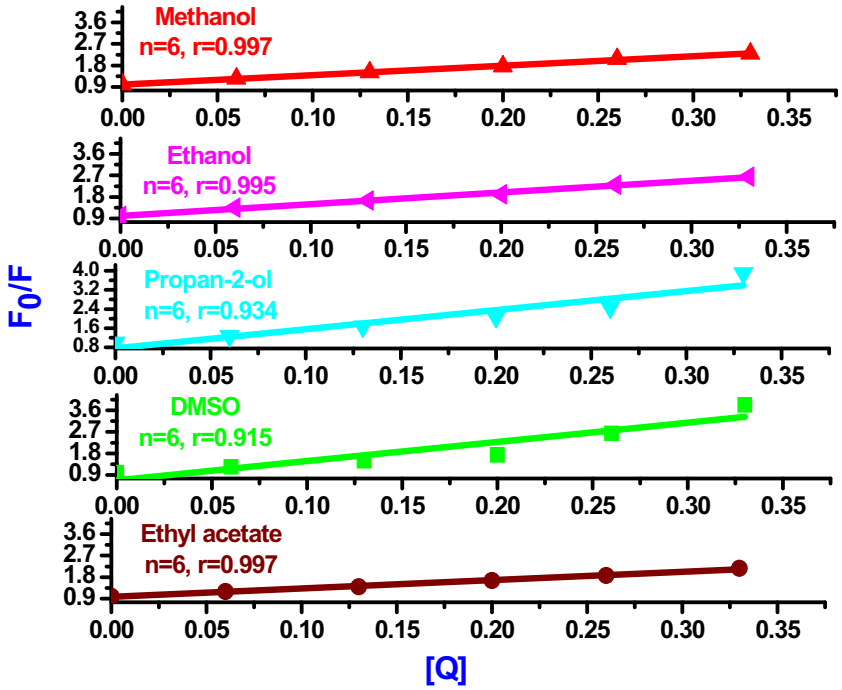

Fig. 3 Stern-Volume plots for BHP molecule in five different solvents

Results and Discussion

Fluorescence spectra of BHP molecule along with their corresponding excitation and emission wavelengths in methanol, ethanol, propan-2-ol, DMSO and ethyl acetate solvents for various quencher concentrations (aniline) were recorded and are shown in Fig. 2. Initially, the fluorescence intensity F_0 was measured without the quencher and then the fluorescence intensity F was measured for various concentrations of aniline. The experimentally measured values of fluorescence intensity for BHP molecule in five different solvents for various concentrations of aniline and are shown in Table 1. Using the experimentally measured values of F and F_0, S–V plots were plotted and are shown in Fig. 3. From Fig. 3 it is found that S-V plots are linear with intercept nearly unity. An observed linear S-V plot indicates that the fluorescence quenching in said molecule is purely dynamic in nature. Using the least square fit method the obtained values of slope, intercept and correlation coefficient of S-V plots were shown in Table 2. The slope value of S-V plot gives the S-V constant (K_{SV}). Further, the fluorescence lifetime decay curve without quencher (τ_0) for BHP molecule in methanol, ethanol, propan-2-ol, DMSO and ethyl acetate solvents were recorded and shown in Fig. 4. The corresponding values of fluorescence lifetime are shown in Table 3. The quenching rate parameter k_q is measured using the experimentally measured values of K_{SV} and τ_0 and is given by an Eq. (8) and these measured values are shown in Table 3.

$$k_q = \frac{K_{SV}}{\tau_0} \tag{8}$$

In order to study the diffusion process of fluorescence quenching mechanism in BHP molecule, we have estimated the diffusion coefficient of solute D_S and the quencher D_Q can be estimated using the Stoke's Einstein Eq. (9) [23].

$$D = \frac{KT}{a\pi\eta R} \tag{9}$$

Table 2 Values of slope (K_{SV}), intercept and correlation coefficient (r) of S-V plots for BHP molecule in five different solvents

Molecule	Solvent	(K_{SV}) (M^{-1})	Intercept	Correlation coefficient
BHP	Methanol	3,956,970	0.998	0.997
	Ethanol	4,886,160	1.011	0.995
	Propan-2-ol	7,986,090	0.900	0.934
	DMSO	8,021,710	0.903	0.915
	Ethyl acetate	3,488,190	0.979	0.997

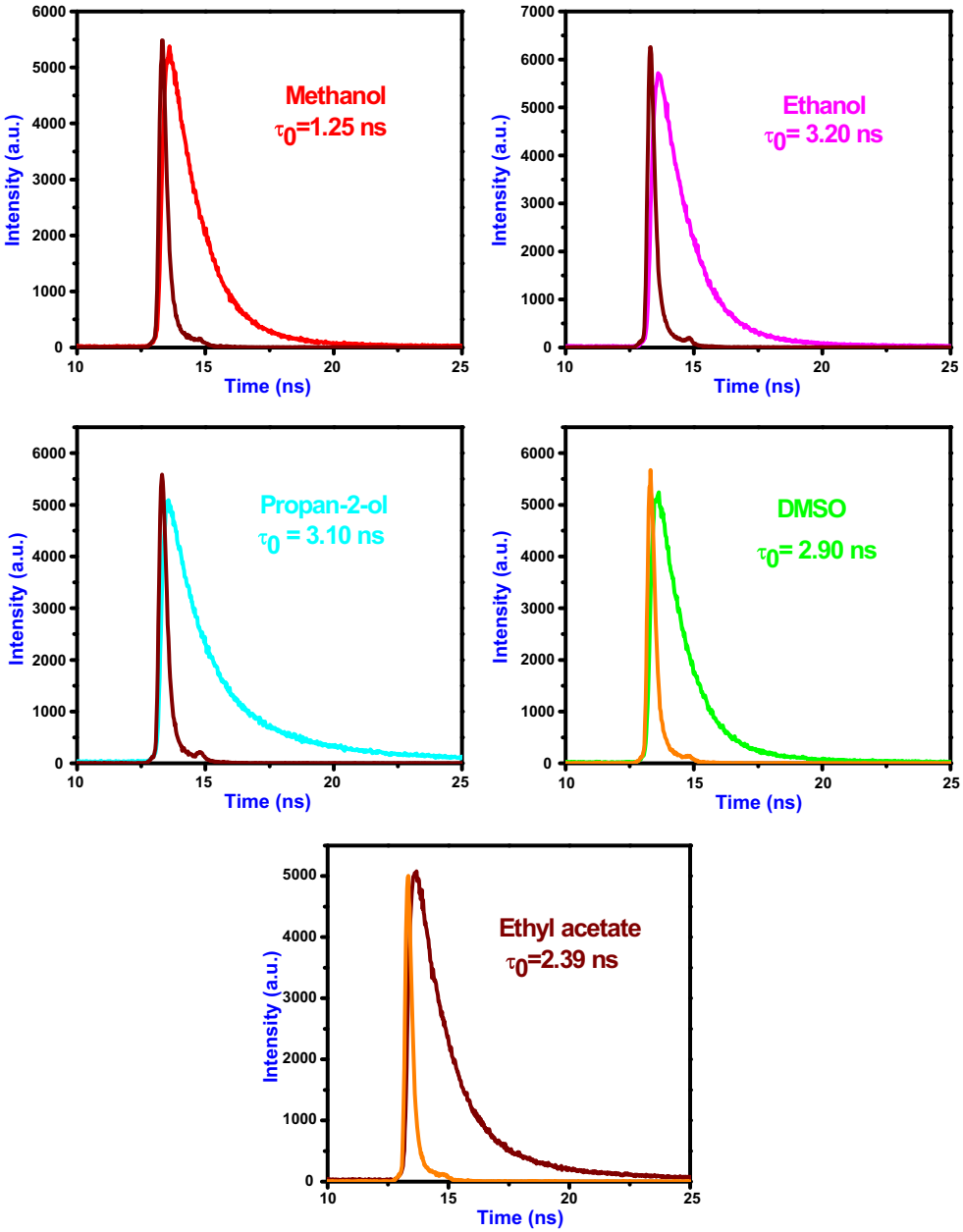

Fig. 4 The fluorescence lifetime decay curves for BHP molecule without quencher in five different solvents

Table 3 Values of slope (K_{SV}), lifetime of solute in absence of quencher and quenching rate parameter (k_q) for BHP molecule in five different solvents

Molecule	Solvent	K_{SV} (M^{-1})	τ_0 (ns)	$k_q \times 10^{15}$ (M^{-1} s^{-1})
BHP	Methanol	3,956,970	1.25	3.165
	Ethanol	4,886,160	3.20	1.526
	Propan-2-ol	7,986,090	3.10	2.576
	DMSO	8,021,710	2.90	2.736
	Ethyl acetate	3,488,190	2.39	1.459

where

K Boltzmann constant,
T Temperature
η Viscosity of the solvent
a The Stoke's Einstein number $a = 6$ for solute and $a = 3$ for quencher [24].

Since the radii of the solute molecule have a larger size than the solvent molecule. $R = R_S + R_Q$ is the sum of the radii of solute and quencher are estimated using the method as

Table 4 Values of viscosity of solvent (η), diffusion coefficient of solute (D_S), diffusion coefficient of quencher (D_Q) and sum of the diffusion coefficients ($D = D_S + D_Q$) for BHP molecule in five different solvents

Molecule	Solvent	$\eta \times 10^{-3}$ (pascal)	$D_S \times 10^{-5}$ (cm^2 s^{-1})	$D_Q \times 10^{-5}$ (cm^2 s^{-1})	$D \times 10^{-5}$ (cm^2 s^{-1})
BHP	Methanol	0.54	1.03	2.86	3.89
	Ethanol	0.98	0.57	1.58	2.72
	Propan-2-ol	2.04	0.27	0.76	1.03
	DMSO	1.99	0.28	0.78	1.06
	Ethyl acetate	0.42	1.32	3.68	5.00

For BHP molecule: R_S = 3.92 A° and for aniline R_Q = 2.82 A°

suggested by Edward [25] and these values are shown at the bottom of Table 4. The diffusion coefficients D_S for solute and D_Q for quencher were calculated using Eq. (9) with help of literature values of viscosity [13] of the respective solvents and the estimated values of radii and these values are shown in Table 4. In this calculation, the viscosity of the solute and the quencher are taken as equal to the viscosity of the medium, because their concentrations are very small. Thus, using values of D and R the values of diffusion rate parameter k_d was estimated using an Eq. (10) and these values are shown in Table 5.

$$k_d = 4\pi N' DR\left[1 + \frac{R}{(2D\tau_0)^{1/2}}\right] \tag{10}$$

where N' is the Avogadro's number per millimole.

Further, using the values of k_q and k_d the probability of quenching per encounter p was estimated using an Eq. (11)

$$p = \frac{k_q}{k_d} \tag{11}$$

The calculated values of p in all five solvents are shown in Table 5. From Table 5, it is observed that the probability of quenching per encounter is less than unity and this fact may infer that the fluorescence quenching process in BHP molecule is governed by activation energy other than the material diffusion. In solution, the value of activation energy for diffusion E_d and activation energy for quencher E_a are needed in order to study the role of an activation process. Using the values gas constant R and calculated values of p and E_d, E_a is determined according to Eq. (12).

$$E_a = E_d + RT\ln\left(\frac{1}{p} - 1\right) \tag{12}$$

According to Eq. (12), the probability of quenching per encounter p is related to the activation energy. The calculated values of E_a are shown in Table 5. From Table 5, it is observed that values of E_a are greater than the E_d in all five solvents studied. It indicates that in bimolecular fluorescence quenching reaction, the influences of activation energy for quencher process is more than the activation energy for diffusion.

Conclusion

From the present discussions of fluorescence quenching of BHP molecule by various concentrations of aniline in five different solvents namely methanol, ethanol, propan-2-ol, DMSO and ethyl acetate. Herein, we noted that;

- The said molecule undergoes fluorescence quenching by aniline in all five different solvents at room temperature.
- S-V plot is linear in all five different solvents.

Table 5 Values for the diffusion rate parameter (k_d), the probability of quenching per encounter (p), the activation energy for diffusion (E_d) and activation energy for quenching (E_a) for BHP molecule in five different solvents

Molecule	Solvent	$k_d \times 10^{15}$ (M^{-1} s^{-1})	p	E_d (k calM^{-1})	E_a (k calM^{-1})
BHP	Methanol	19.75	0.1602	3.049	7.090
	Ethanol	13.81	0.1105	3.784	8.873
	Propan-2-ol	5.23	0.4924	3.072	3.145
	DMSO	5.38	0.5085	4.214	4.330
	Ethyl acetate	25.39	0.0575	2.544	9.370

- The values of quenching rate parameter (k_q) are greater than diffusion rate parameter (k_d) in all five different solvents.
- The value of probability of quenching per encounter is less than unity in all five different solvents.
- The value of activation energy for quenching process is larger than the activation energy for diffusion in all five different solvents.

In view of these facts, it may be inferred that the fluorescence quenching of BHP molecule by the various concentrations of aniline in five different solvents is not solely due to diffusion but there is also a contribution of activation energy.

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