Analysis of Fluorescence Quenching of BPBD by Aniline in Toluene

J Thipperudrappa[*] and S M Hanagodimath[†]

Abstract

Fluorescence quenching of 2-(4'-t-Butylphenyl)-5-(4"-biphenylyl)-1,3,4-oxadiazole (BPBD) by aniline in toluene has been carried out at room temperature by steady state and time resolved fluorescence spectroscopy. The Stern-Volmer plot by steady state method has been found to be non-linear showing a positive deviation, whereas by time-resolved method it is linear. In order to interpret these results we have used the ground state complex and sphere of action static quenching models. Using these models various rate parameters have been determined. Based on these models, with finite sink approximation model, we conclude that positive deviation Stern-Volmer plot is due to the simultaneous presence of dynamic and static quenching processes.

Keywords: Fluorescence quenching, stern–volmer plot, BPBD, static quenching, dynamic quenching, finite sink approximation model.

1. Introduction

Fluorescence quenching of organic fluorophores in solution by various chemical species like aniline, bromobenzene, carbon tetra chloride, eththyltrithiocarbonate, halide ions, metal ions etc. has

[*] Department of Physics, BNM Institute of Technology, Bangalore-560 070, India; jtrphy2007@gmail.com.

[†] Department of Physics, Gulbarga University, Gulbarga-585 106, India; smhmath@rediffmail.com.

been studied by several investigators [1-11]. The accessibility of fluorophores to quenchers can be used to determine the location of probes on macromolecules. This leads to the importance of quenching mechanism not only in physical science but also in chemical, biological and medical sciences. The study has been mainly to understand the nature of bimolecular reactions taking place both under steady state and transient conditions. The fluorescence in bimolecular liquid systems is quenched due to several mechanisms such as static and dynamic quenching, excimer and exciplex formation, charge transfer processes etc.

2-(4'-t-Butylphenyl)-5-(4"-biphenylyl)-1, 3, 4 -oxadiazole (BPBD) finds various applications as a laser dye and as electron transport layer in Polymer Light Emitting Devices (PLEDs) [12]. It also has potential applications in Quantum dots-based light emitting diodes (QLEDs), solar cells and Organic light emitting diode (OLEDs) as commonly used organic [13], wavelength shifter in the form of liquid and solid solutions in Cherenkov counters [14] and as a scintillation fluid for scintillation counting of polyacrylamide gel slices after electrophoresis [15]. All these applications of BPBD are based on its high quantum yield/fluorescence. The quenching of its fluorescence by a quencher leads to the reduction in efficiency of devices which make use BPBD as their counterpart. Hence, fluorescence quenching of BPBD by a chemical species in general, by aniline in particular has practical importance.

In the present study, we have used steady state and time-resolved experimental methods to investigate the quenching of BPBD by aniline in toluene with a view to understand the nature of quenching mechanism.

2. Materials and methods

The solute BPBD was obtained from Sigma –Aldrich, USA and is of scintillation grade. The molecular structure of BPBD is shown in Fig. 1. The quencher aniline was analytical grade and used after double distillation. The toluene was of spectroscopic grade and is used without any further purification. The solutions were prepared at very low fixed concentration of BPBD (1×10^{-5} M) to avoid self absorption effects and varying the quencher concentration (0.000M

to 0.010M). Fluorescence spectrophotometer F-2000 was used for fluorescence intensity measurements, with perpendicular geometry. BPBD has maximum absorption of 305nm in toluene. Therefore, it was excited at 305 nm and fluorescence spectrum was recorded in the absence and presence of quencher. The experimental values are reproducible within 5% of the experimental error. The Measurement of fluorescence lifetime of BPBD with and without quencher was made by using single photon9 counting time-domain spectrofluorimeter (Edinburgh Instrument, UK, Model EI-199). Hydrogen was used to fill the lamp of the single photon counting (SPC) system at 0.6 atm pressure, keeping the electrode separation 0.7 mm, applying the discharge voltage of about 7 kV and pirating the lamp at a frequency 30 kHz. With this setting, lamp pulses having pulse width of about 1ns at FWHM were obtained.
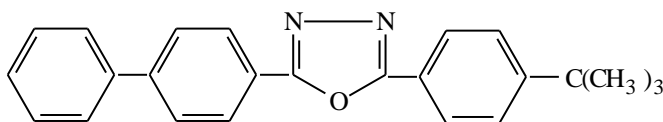

Fig.1. Molecular structure of BPBD

3. Results and discussion

The steady state fluorescence intensities I_0 and I were measured in the absence and presence of quencher respectively in toluene. The fluorescence spectrum of BPBD in the presence of different concentrations of quencher aniline is shown in Fig. 2. From Fig. 2, it is clear that fluorescence of BPBD is efficiently quenched by the addition of aniline. Further, fluorescence lifetime of BPBD was measured in toluene in the absence and presence of quencher. The fluorescence decay curves of BPBD with and without quencher are double exponential according to

$$I(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) \tag{1}$$

where the subscripts 1 and 2 refer to the shorter and longer pre-exponential components respectively of the decay and, a_1 and a_2 are the corresponding relative amplitudes. The lifetime values were obtained by fitting experimental decay profiles to double exponential function (using reconvolution procedure) to get $\chi 2$ values very close to unity. The average lifetime <τ> in each case is calculated according to equation <τ> = a_1τ1 + a_2τ2. Where τ1 and τ2 are the fluorescence lifetimes corresponding to amplitudes a_1 and a_2 respectively. The corresponding data is given in Table 1. From Table 1, it can be observed that lifetime of BPBD decreases with increase in quencher concentration.
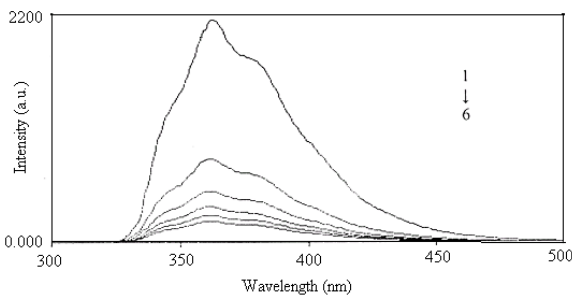

Fig. 2. Fluorescence emission spectra of BPBD in the presence of aniline in toluene at Concentrations of aniline (in M/L) (1) 0.000 (2) 0.002 (3) 0.004 (4) 0.006 (5) 0.008 (6) 0.010.

Quencher concentration (Q) M/L	τ_1 (ns)	a_1	τ_2 (ns)	a_2	Average Lifetime τ (ns)
0.000	4.315	0.004	0.908	0.996	0.920
0.002	1.978	0.017	0.705	0.983	0.726
0.004	1.688	0.056	0.605	0.944	0.666
0.006	1.344	0.045	0.606	0.955	0.639
0.008	1.413	0.024	0.596	0.976	0.615
0.010	0.880	0.122	0.503	0.878	0.549

Table 1: Fluorescence Lifetime Data of BPBD as a Function of Quencher Concentration in Toluene at Room Temperature.

The Stern-Volmer (S-V) plots I_0/I (or τ_0/τ) versus [Q] were plotted according to the equation [16],

$$\frac{I_0}{I}\left(or\ \frac{\tau_0}{\tau}\right) = 1 + K_{SV}[Q] \tag{2}$$

where K_{SV} is the S-V quenching constant and [Q] is the quencher concentration. Eqn. (1) is applicable as long as the experimental results show linear variation. The S-V plot by steady state method in our case is found to be nonlinear showing positive deviation (Fig. 3). Whereas the S-V plot from time resolved method (τ_0/τ versus [Q]) is found to be linear (Fig. 4). It is observed that the phenomenon of quenching by time resolved method follows the S–V relation. This suggests the presence of dynamic quenching. However, the positive deviation in the S-V plot from steady state measurements implies that the quenching is not purely dynamic and may be due to the presence of other parallel quenching mechanisms such as formation of either ground state complex, transient quenching process, static quenching process etc. [11].
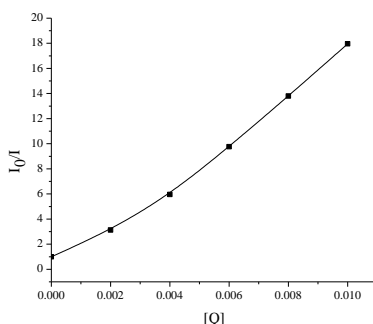

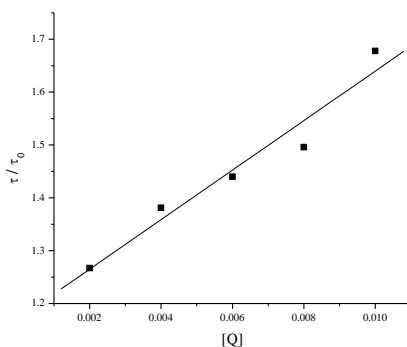**Fig. 3.** S-V plot from steady state measurements

Fig. 4. S-V plot from transient measurements

3.1. Ground state complex formation model

The possibility of ground-state complex formation has been analyzed by using the extended S-V equation given by the equation [3]

$$[(I_o / I) - 1]/[Q] = (K_{sv} + k_g) + (K_{sv}k_g)[Q] \qquad (3)$$

where K_{SV} and k_g are S-V and ground-state association constants respectively. According equation (3) the plot of $[(I_0/ I)- 1]/[Q]$ versus $[Q]$ was found to be linear and is shown in Fig. 5(a). The correlation is good with correlation coefficient of 0.91. From this plot, the values of K_{sv} and k_g were determined. These values are tabulated in Table 2. Also, the value of K_{sv} was determined from the lower quencher concentration region of the plot I_0/I versus $[Q]$ (Fig. 3). Corresponding values of k_q were determined according to equation $k_q = K_{sv} / \tau_0$. The value of τ_0 was taken as 0.92ns for BPBD in the absence of quencher. The value of k_q determined from ground state complex formation model is represented as k_q^a and one determined from lower concentration region of the plot I_0/I versus $[Q]$ is represented as k_q^b. From Table 2, it is observed that the values of k_q's determined from two methods are not same. Therefore, fluorescence quenching due to ground state complex formation is ruled out in the present case. Also, we have observed that, the absorption spectrum of BPBD in toluene in the absence and presence of quencher do not show any observable difference in the shape or the peak position. This clearly indicates that the fluorescence quenching of BPBD has occurred without involving ground-state complex formation [11]. Alternatively, there may be transient component present in dynamic quenching. Transient fluorescence quenching occurs in those cases where quencher resides within a sphere of action with a volume surrounding the fluorophore at the time of relaxation. However, the presence of transient quenching is ruled out in the present case as we have observed a perfect linear plot for τ_0/τ against $[Q]$ (Fig. 4). Hence, the positive deviation could be due to the presence of static quenching. The possibility of static quenching process was analysed using sphere of action static quenching model [1, 2, 17].
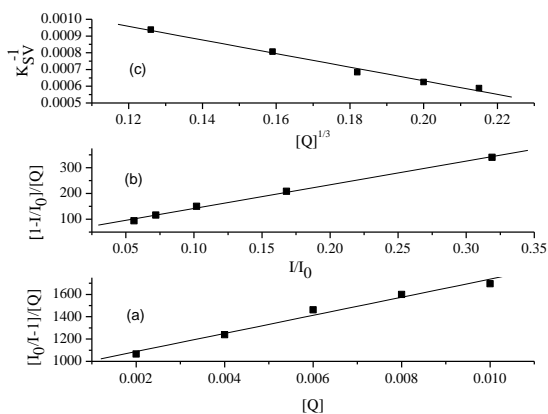
Fig. 5. Plots of (a) [(I0/I)-1]/[Q] against [Q], (b) [1-(I/I0)]/[Q] against I/I0 and (c) Ksv-1 versus [Q]1/3

k_g (M^{-1})	k_q^a x 10^{-10} ($M^{-1}s^{-1}$)	k_q^b x 10^{-10} ($M^{-1}s^{-1}$)	K_{sv}^c (M^{-1})	k_q^c x10^{10} ($M^{-1}s^{-1}$)	Range of W	V (M^{-1})	r (Å)
98.12	89.95			99.98	0.50-0.90	68.72	30.09
		153.58	919.88				

Table 2: The values Ksv, kg, kq, Range of W, V and R.

R_S = 4.31 Å　　　　R_Q = 2.84 Å　　　R (= R_S+R_Q) = 7.15 Å

[a] Bimolecular quenching rate parameter determined from ground state complex formation model.

[b] Bimolecular quenching rate parameter determined from linear portion (low concentration) of the plots I_0/I versus [Q].

[c] Dynamic quenching constant and Bimolecular quenching rate parameters determined from sphere of action static quenching model.

3.2. Sphere of action static quenching model

According to this model, the instantaneous or static quenching occurs if the quencher molecule is very near to, or in contact with the fluorescent molecule at the exact moment it happens to be excited. This was explained by the fact that only a certain fraction 'W' of the excited state is actually quenched by the collisional mechanism. Some molecules in the excited state, the fraction of

which is (1-W) are de-activated almost instantaneously after being formed, because a quencher molecule happens to be randomly positioned in the proximity at the time the molecules are excited and interacts very strongly with them. Several models were employed to describe this static quenching process, all leading to the following modified form of the S-V equation [16].

$$[1-(I/I_o)]/Q = K_{sv}(I/I_o)+(1-W)/[Q] \qquad (4)$$

According to equation (3), the plot of $[(1-I/I_0)]/[Q]$ against I/I_0 is found to be linear (Fig. 5(b)). The value of K_{SV} is determined by least square fit method and hence k_q is determined using the relation $k_q = K_{SV}/\tau_0$. These values are given in Table 2. The intercept of least square fit line of Fig. 5(b) is equal to $(1-W)/[Q]$. From this intercept, the static quenching constant 'V' and radii 'r' of sphere of action (or kinetic distance) is determined according to equations $\ln(1/W) = V[Q]$ and $V/N' = (4/3)\pi r^3$, where N' is Avogadro's number per millimole. The respective data is collected in Table 2. It is observed that the value of K_{SV} is rather larger relative to the value of V, which explains the lack of the changes in absorption spectrum in the presence of quencher. In order to compare the radius 'r' of sphere of action with encounter distance or reactive distance R, i.e., the sum of the radii of the BPBD (R_S) and the quencher molecule (R_Q), the radii of BPBD and aniline were determined by the method suggested by Edward [18] and are given at the bottom of Table 2. It is known that the static effect takes place in case of steady state experiments irrespective of ground state complex formation, when the distance between the quencher molecule and the excited molecule lies between the reactive distance R and kinetic distance r, provided the reactions are diffusion limited [19, 20]. From Table 2 it is evident that, the value of kinetic distance 'r' is greater than the reactive distance R, indicating the role of static quenching process in the present case. Further, to find out whether the reaction is diffusion limited, we have used the finite sink approximation model.

3.3. Finite sink approximation model

According to this model, the modified S-V equation is [21]

$$K_{sv}^{-1} = (K_{sv}^{o})^{-1} - \frac{(2\pi N')^{1/3}}{4\pi N' D \tau}[Q]^{1/3} \tag{5}$$

where K_{sv}^{o} is the S-V constant at [Q] = 0 and is given by $K_{sv}^{o} = \dfrac{4\pi N' DR\tau k_a}{4\pi N' DR + k_a}$, D is mutual diffusion coefficient, k_a is activation energy controlled rate constant and other terms have their usual meanings. According to equation (5), the plot of K_{sv}^{-1} against $[Q]^{1/3}$ which is shown in Fig. 5(c) is found to be linear with negative slope. The value of D is calculated from the slope and the distance parameter R' is calculated from the intercept according to equation $K_{sv}^{0} = 4\pi N' DR'\tau$. These values are given in Table 3. For $R' > R$, the bimolecular reactions of fluorescence quenching are said to be diffusion limited, if the value of k_q (k_q^c) determined from Eqn. (3) is greater than $4\pi N'R'D$ [20, 22]. From the Tables 2 & 3, it is observed that $k_q^c > 4\pi N'R'D$. Therefore, the reaction is diffusion limited.

K_{sv}^{o} (M⁻¹)	D x10⁵ (cm²s⁻¹)	R' (Å)	4π N'R'D x10⁻¹⁰ (M⁻¹s⁻¹)
689.65	55.05	17.98	74.93

Table 3: The values of K_{sv}^{o} , Mutual Diffusion Coefficient D, Distance Parameter R' and 4πN'DR'.

4. Conclusion

From the present investigation we conclude that: (1) the S-V plot shows positive deviation leading to high value of kq, indicating efficient fluorescence quenching. (2) Ground state complex formation is not playing a role in quenching mechanism. (3) The linear S-V plot from time resolved method indicates the absence of transient fluorescence quenching. (4) The value of kinetic distance 'r' is greater than the encounter distance R, suggesting the role of static quenching (5) The value of k_q is greater than 4πN'R'D,

indicating the biomolecular reaction is diffusion limited. In view of the above facts we conclude that fluorescence quenching of BPBD by aniline in toluene is due to simultaneous presence of dynamic and static quenching processes.

5. Acknowledgement

References

[1] H. R. Deepa, J. Thipperudrappa and H. M. Suresh Kumar, "Fluorescence quenching studies of 6,7,8,9-tetrahydro-6,8,9-trimethyl-4-(trifluoromethyl)-2H-pyrano[2,3-b][1,8]naphthyridin-2-one by aromatic amines in alcohols," *Int. J. Phy. & Appl.*, vol. 4(2), p. 157, 2012. ISSN 0974-3103.

[2] H. R. Deepa, J. Thipperudrappa, and H. M. Suresh Kumar, " A study on fluorescence quenching of LD-425 by aromatic amines in 1,4-dioxane-acetonitrile mixtures," *J. Lumin.*, vol. 132, p. 1382, 2012. http://dx.doi.org/10.1016/j.jlumin.2012.01.008.

[3] B. G. Evale, and S. M. Hanagodimath, "Static and dynamic quenching of biologically active coumarin derivative by aniline in benzene–acetonitrile mixtures," *J. Lumin.*, vol. 130, p. 1330, 2010. http://dx.doi.org/10.1016/j.jlumin.2010.03.011.

[4] Y. Ni, S. Su, and S. Kokot, "Spectrometric studies on the interaction of fluoroquinolones and bovine serum albumin," *Spectrochim. Acta Part A*, vol. 75, p. 547, 2010. http://dx.doi.org/10.1016/j.saa.2009.11.014.

[5] B. G. Evale, and S. M. Hanagodimath, "Effect of temperature and quencher on the fluorescence of 4-(5-methyl-3-furan-2-yl-benzofuran-2-yl)-7-methyl-chromen-2-one in different solvents" *Spectrochim. Acta Part A*, vol. 75, p. 1592, 2010. http://dx.doi.org/10.1016/j.saa.2010.02.024.

[6] S. M. Hanagodimath, B. Siddlingeshwar, J. Thipperudrappa, S. K. B. Hadimani, "Fluorescence-quenching studies and temperature dependence of fluorescence quantum yield, decay time and intersystem crossing activation energy of TPB" *J. Lumin.*, vol. 129, p.335, 2009. http://dx.doi.org/10.1016/j.jlumin.2008.10.016.

[7] R. M. Melavanki, R. A. Kusanur, J. S. Kadadevaramath, and M. V. Kulakarni, "Quenching mechanisms of 5BAMC by aniline in different solvents using Stern–Volmer plots", *J. Lumin.*, vol. 129, p.1298, 2009. http://dx.doi.org/10.1016/j.jlumin.2009.06.011.

[8] C. Tablet and M. Hillerbrand, "Quenching of fluorescence of 3-carboxy-5,6-benzocoumarin by aromatic amines", *J. Photochem. Photobiol. A Chem.*, vol. 189, p. 73, 2007. http://dx.doi.org/10.1016/j.jphotochem.2007.01.009.

[9] D. S. Biradar, J. Thipperudrappa and S.M. Hanagodimath, "Fluorescence quenching studies of 1, 3 – diphenyl benzene,", *Spectrosc. Lett.*, vol. 40, p. 559, 2007. http://dx.doi.org/10.1080/00387010701301147.

[10] C. Hariharan, A. K. Mishra, "Quenching of liquid scintillators by chloroalkanes and chloroalkenes", *Radiat. Measure.* vol. 32, p. 113, 2000. http://dx.doi.org/10.1016/S1350-4487 (99)00246-2.

[11] P. K. Behera and A. K. Mishra, "Static and dynamic model for 1-napthol fluorescence quenching by CCl_4 in dioxane-acetonitrile mixtures", *J. Photochem. Photobiol. Chem. Part A* **,** vol. 71, p. 115, 1993. http://dx.doi.org/10.1016/1010-6030(93)85061-C.

[12] G. Santos, F. J. Fonseca, A. M. Andrade, I. Grova, L. Akcelrud, L. Pereira, "Polymer light emitting diode using PFT--poly(9,9'-n-dihexil-2,7-fluorenodiilvinylene-alt-2,5thiophene, *J.Nanosci. Nanotechnol.*, vol. 10, p. 2776, 2010. http://dx.doi.org /10.1166/ jnn.2010.1410.

[13] B. Debasis, Q. Lei, T. Teng-Kuan,H. H. Paul, "Quantum Dots and Their Multimodal Applications: A Review", *Material.*, vol. 3, p. 2260, 2010. http://dx. doi.org/10.3390/ma3042260.

[14] M. Takiue, and H. Ishikawa, "Improvement of Cherenkov counting method (author's transl)", *Radioisotopes.*, vol. 27, p. 123-127, 1978. ISSN:0033-8303.

[15] G. Gezelius, "A convenient, efficient and inexpensive method for scintillation counting of polyacrylamide gel slices after electrophoresis", *Analy. Biochem*, vol. 80, p. 627, 1977. http://dx.doi.org/10.1016/0003-2697(77)90688-1.

[16] J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Ed., Springer, New York, 2006.

[17] M. N. Swaminathan, and N. Radha," Static and dynamic quenching model for 4-amino diphenyl fluorescence quenching by CCl4 in hexane", *Spectrochim. Acta Part A*, vol. 60, p.1839, 2004. http://dx.doi.org/10.1016/j.saa.2003.09.023.

[18] J. T. Edward, "Molecular volumes and the Stokes–Einstein equation", *J. Chem. Edu.*, vol. 47, p. 261, 1970. http://dx.doi.org /10.1021/ed047p261.

[19] J. C. Andre, M. Niclause, and W.R. Ware, "Kinetics of partly diffusion controlled reactions I. Transient and apparent transient effect in fluorescence quenching", *Chem. Phys.*, vol. 28, p. 371, 1978. http://dx.doi.org/10.1016/0301-0104 (78)80014-7.

[20] H. Zeng and G. Durocher, "Analysis of fluorescence quenching in some antioxidants from nonlinear Stern–Volmer Plots", *J. Lumin.*, vol. 63, pp. 74-84, 1995. http://dx.doi.org/10.1016/0022-2313(94)00045-E.

[21] J. Keizer, "Diffusion effects on rapid bimolecular reactions", *Chem. Rev.*, vol. 87, p. 167, 1987. http://dx.doi.org /10.1021/ cr00077a009.

[22] G. C. Joshi, R. Bhatnagar, S. Doraiswamy, and N. Periaswamy, "Diffusion controlled reactions: Transient effects in the fluorescence quenching of indole and N-acetyltryptophanamide in water", *J. Phys. Chem.*, vol. 94, p. 2908, 1990. http://dx.doi.org /10.1021/j100370a033.