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FLUORESCENCE QUENCHING OF 1,4-BIS [2-(2-METHYLPHENYL) ETHENYL]-BENZENE BY ANILINE IN BENZENE-ACETONITRILE MIXTURES

J. THIPPERUDRAPPA[a*] AND S.M. HANAGODIMATH[b]

[a] Department of Physics, BNM Institute of Technology, Bangalore-560070, India.
[b] Department of Physics, Gulbarga University, Gulbarga-585 106, India.

ABSTRACT

Fluorescence quenching of 1,4-bis [2-(2-methylphenyl) ethenyl]-benzene (Bis-MSB) has been studied in solvent mixtures of benzene (BN) and acetonitrile (ACN) at room temperature with a view to understand the role of dielectric constant on fluorescence quenching. The quenching is found to appreciable and a positive deviation from linearity was observed in Stern-Volmer plot in all the solvent mixtures. The positive deviation is maximum in pure benzene and it decreases with increase in percentage of acetonitrile in benzene. Various rate parameters for the quenching process have been determined by sphere of action static quenching model and finite sink approximation model. The magnitudes of these rate parameters indicate that positive deviation in the Stern-Volmer plot is due to both static and dynamic quenching processes. The Stern-Volmer quenching constant decreases with increase in dielectric constant of solvent mixture. This dependence of Stern-Volmer quenching constant with dielectric constant of solvent mixture is explained based on the possibility of formation of dispersion of Bis-MSB microaggregates which is non-fluorescent in high dielectric constant solvent mixtures.

Keywords: Fluorescence quenching; Stern–Volmer plot; Bis-MSB; Static quenching; Dynamic quenching; finite sink approximation model.

1. INTRODUCTION

Fluorescence quenching of fluorophores in solution by various quenchers like aniline, bromobenzene, carbon tetra chloride, eththyltrithiocarbonate, halide ions, metal ions etc. has been studied by several investigators by steady state (Roy R and Mukherjee S, 1986; Hanagodimath S M et al. 1990; Behera P K et al. 1993; Hariharan C and Mishra A K, 2000; Thipperudrappa et al. 2006 ; Thipperudrappa et al. 2007) and transient methods (Suresh kumar H M et al. 2002; Shailaja M K et al. 2000). The accessibility of fluorophores to such quenchers can be used to determine the location of probes on macromolecules to quenchers. Such studies aim at

understanding the nature of bimolecular reactions taking place both under steady state and transient conditions. This leads to the importance of quenching mechanism not only in physical science but also in chemical, biological, and medical sciences. In almost all the cases the experimental results follow the linear Stern-Volmer (S-V) relation. But in some cases, it has been observed that the experimental results show positive deviation from a linear S-V relation. This positive deviation is attributed to various processes like intersystem crossing, formation of charge transfer complexes both at ground and excited states, static and dynamic quenching etc. Apart from this, the dielectric constant, viscosity etc. of solvent medium and the range of quencher concentration are

expected to play a role in this mechanism. In the present study we have used steady state experimental setup to investigate the quenching of 1,4-bis [2-(2-methylphenyl) ethenyl]-benzene (Bis-MSB) by aniline in different solvent mixtures of BN and ACN with a view to understand the nature of quenching mechanism involved in this system. By choosing two aprotic solvents BN and ACN with widely different dielectric constants ε, a good range of solvent polarity variation (2.28 – 37.12) was obtained. We have used the literature values of dielectric constants for all the solvent mixtures

2.1. Materials

The solute 1,4-bis [2-(2-methylphenyl) ethenyl]-benzene (Bis-MSB) was obtained from Sigma – Aldrich, USA and is of scintillation grade. The quencher aniline was used after double distillation. The solvents benzene and acetonitrile were of spectroscopic grade and were used without any further purification. The solutions were prepared at very low fixed concentration of Bis-MSB ($5 \times 10^{-6}M$/L) to avoid self absorption effects and varying the quencher concentration ($0.008M$ to $0.040M$) in all the solvent mixtures.

2.2. Experimental

(Bilal A et al. 2001). The various rate parameters responsible for fluorescence quenching have been determined by sphere of action static quenching model and finite sink approximation model. In light of these rate parameters and dependence of quenching rate parameter on the dielectric constant of the solvent mixture the possible quenching mechanisms are discussed.

2. MATERIALS AND METHODS

Fluorescence spectrophotometer F-2000 was used for fluorescence intensity measurements, with perpendicular geometry. Fluorescence measurements were made by taking fresh solution each time in a rectangular quartz cell having an airtight stopper. The solute has maximum absorption around 350 nm in all solvent mixtures. The solute was excited at 350 nm and the fluorescence spectrum was recorded. First the fluorescence intensity I_0 was measured without the quencher and then the fluorescence intensity I was measured at different quencher concentrations and at a fixed solute concentration. The experimental values are reproducible within 5% of the experimental error.

2.3. Theoretical Background

The dynamic process in which quenching mechanism is mainly due to collision is governed by the linear S-V equation

$$\frac{I_0}{I} = 1 + K_{sv}[Q] \tag{1}$$

where I_0 is the fluorescence intensity of the solute in the absence of quencher, I is the fluorescence intensity in the presence of quencher, K_{sv} (= $k_q \tau_0$, k_q is quenching rate parameter) is the S-V constant and τ_0 is the lifetime of the solute molecule in the absence of quencher. Equation (1) is applicable as long as the experimental results show linear variation. The departure from linearity in S-V plot, suggests that quenching mechanism is not purely collisional and this may be attributed either to the ground state complex formation or to the sphere of action static quenching model (Behera P K et al. 1993). In order to see whether the ground state complex formation is partly playing a role, one can use extended S-V equation (Zeng H and Durocher G, 1995) given by

$$[(I_o / I) - 1]/[Q] = (K_{sv} + k_g) + (K_{sv}k_g)[Q] \tag{2}$$

where K_{sv} and k_g are S-V and ground state association constants respectively. From equation (2), the values of K_{sv} and k_g can easily be determined

by least square fit method. The ground state complex formation takes place if quenching constant k_q (= K_{sv}/τ) obtained from equation (2) agrees well with

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that obtained from the lower portions of the plot according to equation (1) i.e. in the low concentration region in the plot of I_0/I against [Q], which is linear. In that case static quenching is very low. Apart from this the ground state complex formation may be noticed if there is a change in the absorption and fluorescence spectra even at higher concentrations of quencher. In order to see whether static quenching process is playing a role, one can use "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 (Frank J M and Wawilow S J, 1931; Moon A Y et al. 1965) to describe this static quenching process, all leading to the following modified form of the S-V equation.

$$(I_o / I) = \frac{1 + K_{sv} [Q]}{W} \tag{3}$$

where K_{sv} has its usual meaning as explained earlier and [Q] is the quencher concentration. The Smoluchowski's diffusion controlled equation containing transient term is given by

$$K_d = 4\pi N' R D + 4R^2 N' (\pi D)^{1/2} t^{-1/2} \tag{4}$$

where N' is the Avogadro's number per millimole, R is the encounter distance i.e. the sum of the radii of the solute and quencher molecules and t is the time. The retention of the later term of Eq. (4) leads to an additional factor, W in Eq. (3). This additional factor W is given by

$$W = e^{-V[Q]} \tag{5}$$
$$\ln(1/W) = V[Q]$$

where V is the static quenching constant and it represents an active volume element surrounding the excited solute molecule. Instantaneous (static) quenching occurs in a randomly distributed system when a quencher happens to reside within a sphere of action with a volume 'V/N'' and radius 'r' i.e

$$V / N' = (4\pi r^3) / 3 \tag{6}$$

surrounding a solute molecule at the time of excitation.

As W depends on the quencher concentration [Q] the S-V plots for a quencher with a high quenching ability generally deviate from linearity. Thus it is worth rewriting Eq. (3) as

$$[1 - (I/I_o)]/Q = K_{sv}(I/I_o) + (1-W)/[Q] \tag{7}$$

From equation (7) one can easily calculate K_{sv} and the values of W by least square fit method. The static quenching constant V and the values of 'r' of sphere of action can also be calculated using equations (5), (6) and (7). If the distance between the quencher

molecule and the excited molecule lies between the encounter distance and the kinetic distance, the static effect takes place especially in the case of steady state experiments irrespective of ground state complex formation provided reactions are limited by

diffusion (Andre et al. 1978; Zeng H and Durocher G, 1995). In order to find whether the reactions are diffusion limited one can invoke the Finite Sink Approximation Model. Keizer (Keizer J. 1982; Keizer J. 1983; Keizer J. 1987) has proposed a nonequilibrium statistical modification of the Smoluchowski-Collins-Kimball (SCK) expression to fit the I_0/I ratio in fluorescence quenching. In the case of SCK model time dependent rate coefficient k(t) for diffusion-limited reactions is given by (Joshi G C et al. 1990)

$$k(t) = a + b \exp(c^2 t) erfc(ct^{1/2}) \tag{8}$$

where

$$a = k_a [1 + \frac{k_a}{4\pi N' RD}]^{-1} \tag{9}$$

$$b = k_a [1 + \frac{4\pi N' RD}{k_a}]^{-1} \tag{10}$$

$$c = [1 + \frac{k_a}{4\pi N' RD}] \frac{D^{1/2}}{R} \tag{11}$$

Integration of Eq. (8) between the limits [Q] (∞) at r → ∞ and [Q] [R] at r = R provides the well known expression

$$1/k_d = (1/k_q) + (1/k_a) \tag{12}$$

where $k_d = 4\pi N' DR$ and k_a is the activation energy controlled rate constant describing the reaction of encountered pairs at a reactive distance R and D is the sum of the diffusion coefficients of the solute and quencher molecules. Following this expression, k_q is independent of [Q]. But for efficient quenching process in liquids, k_q is often observed to increase with [Q]. This might be attributed as discussed above to static quenching of solute molecule, in the vicinity of [Q], transient effects arising from an initial time dependence of the concentration gradient or combination of them. But if one assumes that only the first encounter is of interest in the case of efficient fluorescence quenching an initial average separation distance r_0 can be defined (sink radius) so that the diffusive region of interest for a first encounter is in the range   R≤ r ≤ r_0 such that all subsequent encounters are eliminated. Integration of the flux equation between the limits [Q](r_0) at r_0 and [Q] (R) at R leads to modification of expression (12) as

$$\frac{1}{k_q} = \frac{1 - (R/r_o)}{k_d} + \frac{1}{k_a} \tag{13}$$

This equation reduces to the reaction limited form ($k_q = k_a$) both for inefficient quenching ($k_a \ll k_d$) and for quenching in pure quenching solvents where R = r_o. In the diffusion controlled limit ($k_a \gg k_d$) Eq. (13) reduces to

$$k_q = \frac{k_d}{1 - R/r_o} \tag{14}$$

and k_q depends on the quenching concentration through r_o. Since the sink radius (r_o) is identified to the most probable nearest neighbour initial separation, the appropriate distribution requires that [16] $r_o = (2\pi N' [Q])^{-1/3}$.

Replacing r_o in Eq. (13) and k_d by its value (4πN'DR) and dividing everywhere by the fluorescence lifetime of solute in the absence of quencher (τ) one obtains the modified S-V relationship as (Frank J M and Wawilow S J, 1931).

$$K_{sv}^{-1} = (K_{sv}^{o})^{-1} - \frac{(2\pi N')^{1/3}}{4\pi N' D \tau}[Q]^{1/3} \qquad (15)$$

$$\text{Where} \quad K_{sv}^{o} = \frac{4\pi N' DR \quad \tau k_a}{4\pi N' DR \quad + \quad k_a}$$

A plot of K_{sv}^{-1} against $[Q]^{1/3}$ becomes linear with negative slope. Mutual diffusion coefficient D becomes directly accessible from the slope of the graph exemplified in Eq. (15) and K_{sv} is obtained at [Q] = 0 regardless of the relative magnitudes of k_a and k_d (=4πN'DR), irrespective of quenching is diffusion limited or not. From K_{sv} we only have access to R' which is a composite of R and k_a through Eq. (16).

$$K_{sv}^{0} = 4\pi N' DR' \tau \qquad (16)$$

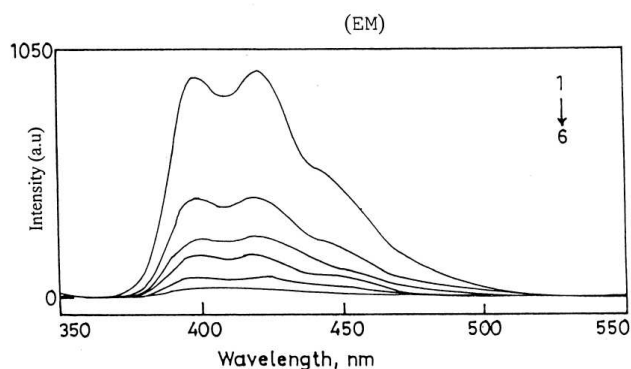where R' is the distance parameter and has the same meaning as in the long-time SCK model and is given as

$$R' = R[1 + 4\pi RDN'/k_a]^{-1} \qquad (17)$$

Then according to the theory discussed above if ka is greater than kd (i.e Eq.12) then the reactions are said to be diffusion limited (i.e. for R'<R). But for R'>R, the bimolecular reactions of fluorescence quenching are said to be diffusion limited if the values of k_q determined from Eq.(7) are greater than 4π N'R'D.
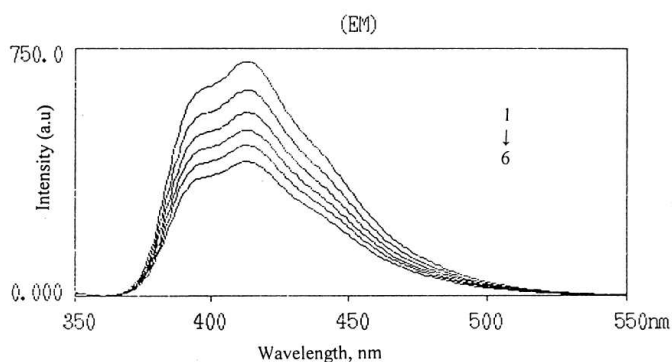
3. RESULTS AND DISCUSSIONS

The fluorescence intensities I and I_0 were measured with and without quencher (aniline) respectively at a fixed Bis-MSB concentration in solvent mixtures of BN and ACN. The quencher concentration was varied from 0.000-0.040 M/L in all the solvent mixtures. The typical emission spectrum of Bis-MSB in pure BN and ACN at different quencher concentrations are shown in figures 1 and 2 respectively. From figures 1 and 2 it can be seen that the nature of emission spectrum in benzene and acetonitrile is not same. In case of pure benzene emission spectrum is dual in nature with two emission peaks around 400nm and 420nm. But in case of pure acetonitrile, emission spectrum is almost broad with emission peak only at 420nm. This type of difference in the emission spectrum in non polar solvent n-heptane and polar solvent acetonitrile was observed by others (Maiti M et al. 1999) for 4-methoxy-benzo[b] thiophene (4MBT). But it can be seen that with the addition of quencher there is no noticeable spectral shift in the emission spectra at 420 nm.

Figure 1
*Emission spectra of Bis-MSB in the presence of aniline in 100%
BN at 27°C. Conc. of aniline (in M/L) (0.000(1) to 0.040(6)).*

Figure 2
*Emission spectra of Bis-MSB in the presence of aniline in 100%
ACN at 27°C. Conc. of aniline (in M/L) (0.000 (1) to 0.040 (6)).*

The S-V plots obtained by using the experimentally determined values of I_0 and I are found to be nonlinear in all the solvent mixtures showing positive deviation and are shown in fig. 3. Similar experimental results were also observed by others (Hariharan C and Mishra A K, 2000; Thipperudrappa et al. 2006 ; Thipperudrappa et al. 2007). The positive deviation is maximum in pure benzene and it decreases with increase in percentage of acetonitrile in benzene. Thus, the positive deviation from linearity suggests that quenching is not purely collisional and reveals the role of the static quenching process. This was explained by sphere of action static quenching model.
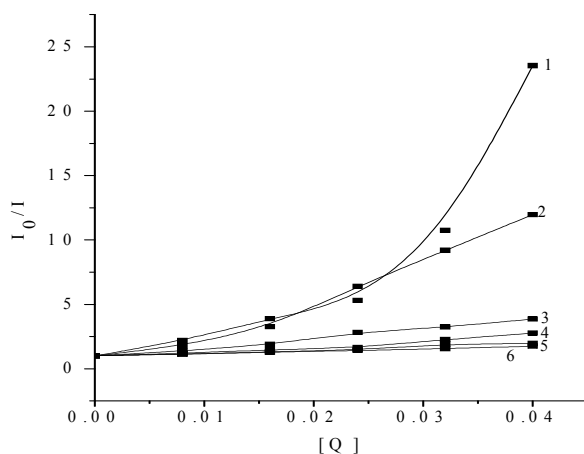
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Figure 3.
Stern-Volmer (S-V) plots for Bis-MSB+ aniline system in different mixtures of BN and ACN solvents; 1) 100%BN   2) 80%BN   3) 60% BN   4) 40% BN   5) 20% BN   6) 100% ACN.

Figure 4
shows the plots of [1-(I/I$_0$)]/[Q] against I/I$_0$ for Bis-MSB for different solvent mixtures of BN and ACN which are linear according to equation (7).
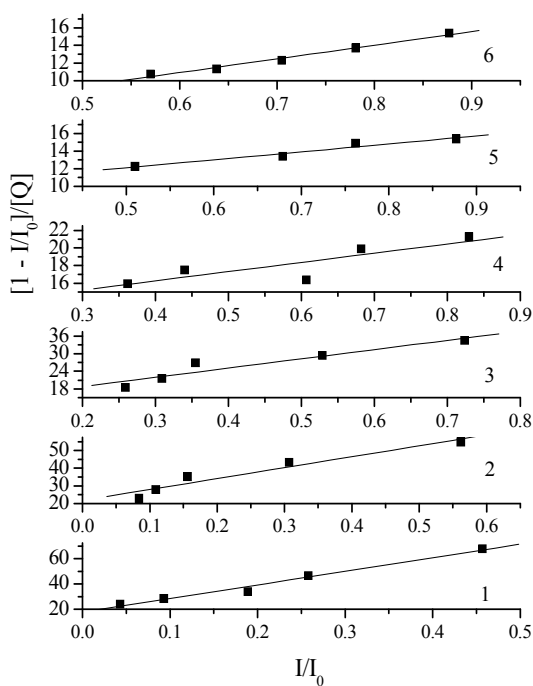

Figure 4
Plots of [1-(I/I$_0$)]/[Q] against I/I$_0$ for Bis-MSB + aniline system in different mixtures of BN and ACN solvents; 1) 100%BN   2) 80%BN   3) 60% BN   4) 40% BN   5) 20% BN   6) 100% ACN.

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The dynamic quenching constant K_{sv} is determined in all the cases by least square fit method using Eq. 7 and the values are given in the Table 1. Fluorescence lifetime τ_0 of the solute studied is obtained from the catalogue of the Koch-Light laboratories limited, England and is given at the bottom of the Table 1. Bimolecular quenching rate parameter k_q was determined from the relation $k_q = K_{sv}/\tau_0$ (Table 1). Here high values of k_q indicate efficient quenching of fluorescence. In order to support static and dynamic effects, we have determined the magnitudes of static quenching constant V and radii r of sphere of action (or kinetic distance) using the sphere of action model. With the use of Eq. (5) and (6) the values of V and r are determined by least square fit method in all the solvent mixtures and are given in Table 1.

Table 1

The values of Dielectric constant ε, Stern–Volmer constant K_{sv}, bimolecular quenching rate parameter k_q, static quenching constant V and kinetic distance r for different solvent mixtures.

Solvent mixture	ε	K_{sv} (M^{-1})	k_qx10^{-10} (M^{-1}s^{-1})	V (mol^{-1}dm^3)	r (Å)
100%BN	2.28	107.96	10.15	30.09	22.85
80%BN	10.67	61.86	5.82	51.00	27.24
60%BN	15.18	31.45	2.95	17.32	19.01
40%BN	24.30	15.47	1.45	16.34	18.64
20%BN	30.46	10.47	0.98	8.91	15.23
100%ACN	37.12	9.00	0.84	1.81	8.958

R_Y (Bis-MSB) = 4.24 Å; R_Q (aniline) = 2.84 Å; τ_0 = 1.063 ns, R (= R_Y + R_Q) = 7.08 Å

The radii of the solute (R_Y) and the quencher (R_Q) molecules were determined by adding the atomic volumes of all the atoms constituting the molecule as suggested by Edward (Edward J T. 1970) and are given at the bottom of Table 1. From these values of R_Y and R_Q, the sum of the molecular radii R of Bis-MSB and aniline is determined. This sum of the molecular radii is referred to as encounter distance. This value is then compared with the values of 'r' to verify whether the reaction is due to sphere of action model. From Table 1, it can be observed that the values of kinetic distance 'r' are greater than the encounter distance R. Therefore, the static effect takes place irrespective of ground state complex formation provided reactions are limited by diffusion indicating the sphere of action model holds well for the present system. Further it may also be noted that a positive deviation in S-V plot is expected when both static and dynamic quenching occurs simultaneously (Behera P K et al. 1993).In order to find whether the reactions are diffusion limited, we have invoked the Finite Sink Approximation Model. Figure 5 shows the plots of K_{sv}^{-1} against $[Q]^{1/3}$ in solvent mixtures according to eqn.15. From fig.5 we can see that all the plots in solvent mixtures are almost linear and small deviation may be due to experimental uncertainties. Hence, the linear dependence of K_{sv}^{-1} on $[Q]^{1/3}$ within the error limits is confirmed (Frank J M and Wawilow S J, 1931). Then, from the least square fit, values of K_{sv}^o (Stern-Volmer constant at $[Q] = 0$) and mutual diffusion coefficients D were determined. Using these values of K_{sv}^o and D, the distance parameter R' was determined according to equation (16) and the values are given in the Table 2.
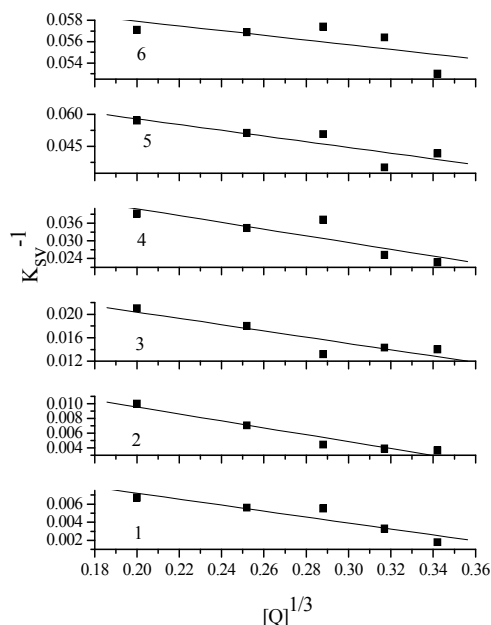
Figure 5.
Plots of K_{sv}^{-1} versus $[Q]^{1/3}$ for Bis-MSB + aniline system in different mixtures of BN and ACN solvents; 1) 100%BN AN 2) 80%BN 3) 60% BN 4) 40% BN 5) 20% BN 6) 100% ACN

According to Joshi et al. (Joshi G C et al. 1990) the bimolecular reactions are said to be diffusion limited, if the values of k_q (determined from equation (7)) are greater than $4\pi N'R'D$. Hence the values of $4\pi N'R'D$ are calculated using the experimentally determined values of R' and D and are given in Table 2. We see from Table 2, that the values of k_q are greater than $4\pi N'R'D$ in 100% BN, 80% BN and in other solvent mixtures k_q is approximately equal. However, in case of 100%ACT experimentally determined value of distance parameter R' (= 2.256 Å) is less than the encounter distance R (= 7.08 Å), it is possible to calculate the activation energy controlled rate constant k_a according to equation (17) and is found to be 2.216 x 10^{10} M^{-1}s^{-1}. Then, if k_a is greater than $4\pi N'R'D$ then the reactions are said to be diffusion limited (Zeng H and Durocher G, 1995), which is true for 100%ACN.

Table 2

The values of K_{sv}^{o} (steady state quenching constant at [Q] = 0), mutual diffusion co-efficient D, distance parameter R', $4\pi N'R'D$ and activation energy controlled rate constant k_a for different solvent mixtures.

Solvent mixture	K_{sv}^{o} (dm³mol⁻¹)	Dx10⁵ (cm²s⁻¹)	R' (Å)	$4\pi N'R'D$x10⁻¹⁰ (M⁻¹s⁻¹)	k_ax10⁻¹⁰ (M⁻¹s⁻¹)
100%BN	72.46	5.88	15.31	6.82	-
80%BN	52.63	4.12	15.87	4.95	-
60%BN	32.25	3.62	11.05	3.03	-
40%BN	15.67	1.68	11.56	1.47	-
20%BN	11.82	1.44	10.16	1.11	-
100%ACN	16.05	8.84	2.25	1.51	2.216

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Variation of K_{SV} with dielectric constant of solvent mixture

From the previous discussion, we observed that fluorescence quenching of Bis-MSB by aniline is due to the simultaneous presence of both dynamic and static quenching components. From Table 1 it can be seen that the values of dynamic quenching constant K_{SV} and static quenching constant V decreases with increase in dielectric constant. This dependence of quenching constants with dielectric constant of solvent mixture may be attributed to the formation of non-fluorescent dispersion of Bis-MSB micro aggregates in high dielectric constant medium similar to dispersion of DPH micro aggregates in water (high dielectric constant) (Sujatha J and Mishra A K, 1996). Since Bis -MSB possesses, a very rigid polyene structure with a rod-like shape of length 12 Å which is comparable to that of DPH which is of length 11 Å, we expect the formation of dispersion of Bis-MSB micro aggregates in high dielectric constant solvent acetonitrile. This is also evident from figure (3) that fluorescence emission is maximum in low dielectric constant solvent benzene and minimum in high dielectric constant solvent acetonitrile. As the non-fluorescent dispersion of Bis-MSB increases, the possibility of dynamic quenching and static quenching processes with quencher decreases with fluorescent molecules. This leads to the less quenching efficiency in high dielectric constant solvent acetonitrile compared to low dielectric constant solvent benzene.

4. CONCLUSIONS

From the above discussion we observe that: (1) the S-V plots show positive deviation leading to high values of k_q, indicating efficient fluorescence quenching. (2) The dependence of Stern-Volmer constant on dielectric constant of the solvent mixture indicates the possibility of formation of non-fluorescent dispersion of Bis-MSB microaggregates in high dielectric constant medium. In view of the above facts we conclude that quenching reaction is diffusion limited and both static and dynamic quenching processes are partly playing a role in this system.

5. ACKNOWLEDGEMENTS

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