

Effect of Solvent Polarity on the Fluorescence Quenching of Biologically Active 5BAMC by Aniline in Binary Solvent Mixtures

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Abstract The fluorescence quenching of 5, 6-benzo-4-azidomethyl coumarin (5BAMC) by aniline have been carried in different solvent mixtures of benzene (BN) and acetonitrile (AN) at room temperature by steady state fluorescence measurements. The quenching is found to be appreciable and a positive deviation from linearity was observed in the Stern-Volmer plot for the fluorophore in all the solvent mixtures. Various parameters for the quenching process have been determined using the extended S-V equation and have been found to be dependent on the solvent polarity. Further, with the use of finite sink approximation model, it is concluded that the bimolecular reactions quenching reactions are diffusion limited, and the distance parameter R' and mutual diffusion coefficient D are estimated independently.

Keywords 5, 6 benzo-4-azidomethyl coumarin · Fluorescence quenching · Diffusion limited · Finite sink approximation model · Static and dynamic quenching

Introduction

The fluorescence quenching of organic molecules by different quencher like aniline and carbon tetrachloride etc has been a subject of continued investigation for the past couple of decades to understand the nature of bimolecular quenching reactions taking place both steady state and transient conditions. There are wide varieties of quenching process that includes excited-state reactions, molecular rearrangements, ground-state complex formation and energy transfer, intersystem crossing, singlet-to-triplet excitation and excited-states. Apart from this, the polarity of the solvent medium and the range of quencher concentration also expected to play an important role. The quenching technique can be used to determine the accessibility of the quencher to fluorophore, monitor conformational changes, monitor association reactions of fluorescence of one of the reactants that changes upon binding. There are two basic types of quenching: static and dynamic. Both types require an interaction between the fluorophore and quencher. The fluorescence quenching has been widely studied both as a fundamental phenomenon and also for the application of fluorescence to biochemical problems. Fluorescence quenching process depends upon the nature of fluorophore and quencher molecule [1–34, 37, 38].

The fluorescence quenching of organic molecules in solution by various quenchers like aniline, bromobenzene, carbon tetrachloride, ethyltrithiocarbonate, halide ions, metal ions, etc. has been studied by several investigators by steady state and transient methods [1–34]. In almost all the cases the experimental results follow the linear Stern-Volmer (S-V) relation and is given by

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \quad (1)$$

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Where I_0 and I are fluorescence intensities in the absence and presence of quencher respectively. K_{SV} is the Stern-Volmer constant and $[Q]$ is the quencher concentration. But in some cases, it has been observed that the experimental results show positive deviation from a linear S-V relation [8–20]. 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 polarity of the solvent mixtures and the range of quencher concentration are expected to play a role in these mechanisms.

Families of coumarin dyes are applicable in different fields of science and technology [29–34]. They exhibit strong fluorescence in the UV and VIS region that makes them suitable to use as a colorants, dye laser media and as nonlinear optical chromophores. In medicine, coumarin derivatives are used as anticoagulants, as a fluorescent indicators for the physiological pH region and as fluorescent probes to determine the rigidity and fluidity of living cells and its surrounding medium. Coumarins and its derivatives has been a subject of considerable interest in numerous fields [8, 9, 11]. Due to these interesting properties associated with coumarins, we undertook in studying the fluorescence quenching of 5BAMC by aniline in different solvent mixtures of benzene (BN)—acetonitrile (AN) with a view to understand the nature of quenching mechanisms. In the present study we have used steady state technique by choosing two aprotic solvents BN–AN 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 [8, 16] of dielectric constants for all the solvent mixtures. The various rate parameters responsible for fluorescence quenching have been determined by sphere of action static quenching model and finite sink approximation model.

Materials and methods

The solute 5BAMC was synthesized using standard methods [29, 35, 36]. Molecular structure of the solute is shown in Fig. 1. The solvents benzene and acetonitrile were of spectroscopic grade and were used without any further purification. The quencher aniline was double distilled and tested for its purity before use. The solutions were prepared

Fig. 1 Molecular structure of 5BAMC

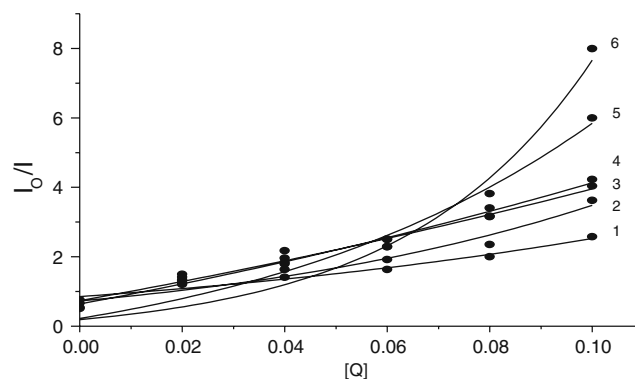
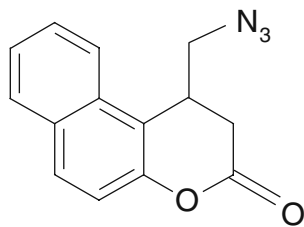


Fig. 2 Stern-Volmer (S-V) plots from steady state fluorescence emission intensity measurements for 5,6, benzo-4-azidomethyl coumarin + aniline system in different mixtures of benzene (BN) and acetonitrile (AN) solvents; 1) 100%BN + 0%AN 2) 80%BN + 20% AN 3) 60% BN + 40% AN 4) 40% BN + 60% AN 5) 20% BN + 80% AN and 6) 0% BN + 100% AN

keeping the concentration of 5BAMC fixed (1×10^{-4} M/L) and varying the quencher concentration (0.00–0.10M) in all the BN-AN solvent mixtures. Hitachi 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. Solute has a maximum fluorescence corresponds to 350 nm. 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.

Results and discussion

The S-V plots obtained by using the experimentally determined values of I_0 and I are found to be nonlinear in all the solvents showing positive deviation and are shown in Fig. 2. Similar experimental results were also observed by others [8–20]. Thus, 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. According to this model 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 deactivated 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

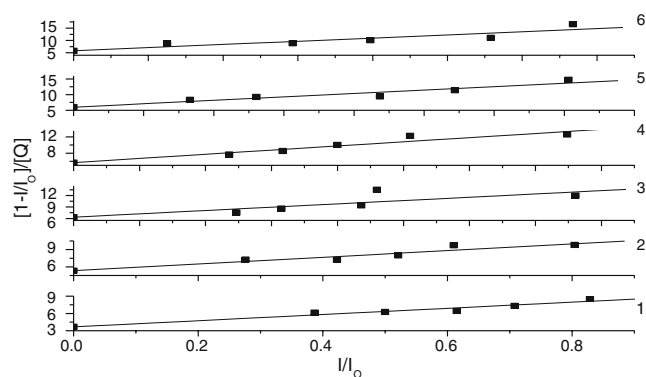


Fig. 3 Plots of $[1 - I/I_0]/[Q]$ against I/I_0 for 5,6, benzo-4-azidomethyl coumarin + aniline system in different mixtures of benzene (BN) and acetonitrile (AN) solvents; 1) 100%BN + 0%AN 2) 80%BN + 20% AN 3) 60% BN + 40% AN 4) 40% BN + 60% AN 5) 20% BN + 80% AN 6) 0% BN + 100% AN

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 [8, 16, 17]

$$\frac{I_0}{I} = \frac{1 + K_{SV}[Q]}{W} \quad (2)$$

Where W is given by

$$W = \exp(-V[Q]) \quad (3)$$

Where V is the static quenching constant that 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 ’ [$V/N' = 4\pi r^3/3$] 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 Eq. 2 is written as

$$[1 - (I/I_0)]/[Q] = K_{SV}(I/I_0) + (1 - W)/[Q] \quad (4)$$

Figure 3 shows the plots of $[1 - I/I_0]/[Q]$ against I/I_0 for 5BAMC with aniline as quencher for different solvent mixtures of BN-AN which are linear according to Eq. 4.

The Stern-Volmer quenching constant K_{SV} is determined in all the cases by least square fit method using Eq. 4 and these values are given in Table 1. Fluorescence lifetime τ of the solute was measured using Photophysics model of TCSPC fluorescence spectrometer available at R.S.I.C, I.I.T Mumbai, India and is given at the bottom of Table 1. Bimolecular quenching rate parameter k_q was determined from relation $k_q = K_{SV}/\tau$ and is shown in Table 1.

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. 4 the values of V and r are determined by least square fit method in all the solvent mixtures and are given in Table 1.

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 [6] and are given at the bottom of Table 1. From these values of R_Y and R_Q the sum of the molecular radii i.e. encounter distance R is determined. From Table 1, we see that the values of kinetic distance ‘ r ’ are greater than the encounter distance R . Therefore according to Zeng et al. [23] the static effect takes place irrespective of ground state complex formation provided the reactions are limited by diffusion indicating the sphere of action model holds well. Further it may also be noted that a positive deviation in S-V plot is expected when both static and dynamic quenching occur simultaneously. In order to find whether the reactions are diffusion limited we invoked finite sink approximation model.

Finite sink approximation model

Finite Sink Approximation model provides the well-known expression [24–27]

$$1/k_q = (1/k_d) + (1/k_a) \quad (5)$$

Table 1 Quenching parameters of 5,6 benzo-4-azidomethyl coumarin + aniline system in different solvent mixtures of benzene (BN) + acetonitrile (AN) at room temperature

Solvent mixture	Dielectric constant, D	$K_{SV} (mol^{-1})$	$k_q \times 10^{-10} (mol^{-1} s^{-1})$	$V (mol^{-1} dm^3)$	$r (\text{Å})$
100%BN	2.28	5.374	0.773	4.740	12.339
80%BN	10.67	5.823	0.837	8.050	14.722
60%BN	15.18	8.647	1.244	10.800	16.240
40%BN	24.30	10.231	1.472	8.940	15.250
20%BN	30.46	10.905	1.569	9.785	15.710
100%AN	37.12	12.855	1.849	9.240	15.415

$R_Y = 3.771\text{Å}$, $R_Q = 2.840\text{Å}$, $R (= R_Y + R_Q) = 6.611\text{Å}$, $\tau = 0.695$ ns

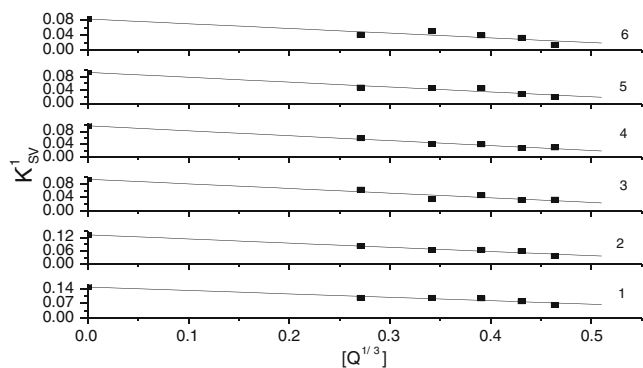


Fig. 4 Plots of K_{sv}^{-1} versus $[Q]^{1/3}$ for 5,6, benzo-4-azidomethyl coumarin + aniline system in different mixtures of benzene (BN) and acetonitrile (AN) solvents; 1) 100%BN + 0%AN 2) 80%BN + 20% AN 3) 60% BN + 40% AN 4) 40% BN + 60% AN 5) 20% BN +80% AN and 6) 0% BN + 100% AN

Where $k_d = 4\pi N'RD$ and k_a is the activation energy controlled rate constant describing the reaction of encountered pairs at a reactive distance R . D is the sum of the diffusion coefficients of solute and quencher molecules. Following Eq. 5, 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 to static quenching of solute molecule in the vicinity of $[Q]$ and 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 $R \leq r \leq r_0$ such that all subsequent encounters are eliminated. This interpretation leads to the modification of expression (5) as

$$\frac{1}{k_q} = \frac{1 - (R/r_0)}{k_d} + \frac{1}{k_a} \tag{6}$$

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

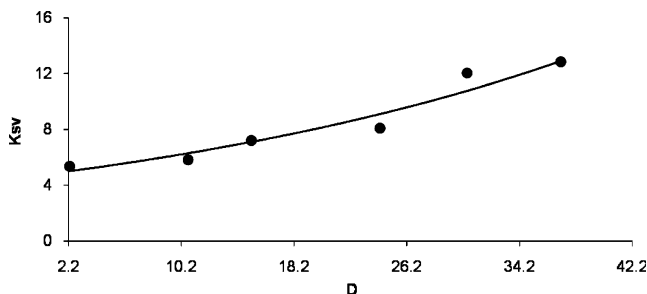


Fig. 5 Variation of K_{sv} as a function of Dielectric constant ϵ for the solute 5BAMC

pure quenching solvents where $R = r_0$. In the diffusion controlled limit ($k_a \gg k_d$) Eq. 6 reduces to

$$k_q = \frac{k_d}{1 - R/r_0}$$

and k_q depends on the quenching concentration through r_0 . Since the sink radius (r_0) is identified with the most probable nearest neighbour initial separation, the appropriate distribution requires that $r_0 = (2\pi N'[Q]^{-1/3})$. Replacing r_0 in Eq. 6 by $(2\pi N'[Q]^{-1/3})$ and k_d by its value (i.e. $4\pi N'RD$) and dividing everywhere by the fluorescence lifetime of solute in the absence of quencher (τ) one obtains the modified S-V relationship as [8, 16, 17, 23, 28].

$$K_{SV}^{-1} = (K_{SV}^O)^{-1} - \frac{(2\pi N')^{1/3}}{4\pi N'D\tau} [Q]^{1/3} \tag{7}$$

Where $K_{SV}^O = \frac{4\pi N'DR\tau k_a}{4\pi N'DR + k_a}$ 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. 7 and K_{SV}^O is obtained at $[Q] = 0$ regardless of the relative magnitudes of k_a and $k_d = 4\pi N'RD$, whether quenching is diffusion limited or not. K_{SV}^O can also be written as

$$K_{SV}^O = 4\pi N'DR'\tau \tag{8}$$

Table 2 The values of K_{sv}^o (steady state quenching constant at $[Q] = 0$), mutual diffusion coefficient D , distance parameter R' , $4\pi N'R'D$ for 5,6 benzo-4-azidomethyl coumarin + aniline system in different solvent mixtures of benzene (BN) + acetonitrile (AN) at room temperature

Solvent mixture	$K_{sv}^o (mol^{-1} dm^3)$	$D \times 10^5 (cm^2 s^{-1})$	$R' (\text{\AA})$	$4\pi N'R'D \times 10^{-10} (mol^{-1} s^{-1})$
100%BN	6.756	1.817	7.071	0.972
80%BN	7.575	1.576	9.140	1.089
60%BN	10.638	2.880	9.898	1.530
40%BN	10.204	2.581	9.495	1.468
20%BN	10.752	2.741	11.000	1.547
100%AN	12.048	2.351	9.745	1.733

$$R (= R_V + R_Q) = 6.611\text{\AA}$$

Where R' is the distance parameter and has the same meaning as in the long time SCK model [24–27] and is given as

$$R' = R[1 + 4\pi N'DR/k_a]^{-1} \quad (9)$$

Then according to the theory discussed above if k_a is greater than k_d [i.e. Eq. 5] then the reactions are said to be diffusion limited [23, 28] (i.e. for $R' < R$). But for $R' > R$, the bimolecular fluorescence quenching reactions are said to be diffusion limited [28] if the values of k_q determined from Eq. 4 are greater than $4\pi N'R'D$.

Therefore according to Eq. 7 we need to determine the values of K_{SV}^{-1} and $[Q]^{1/3}$. Where $K_{SV} = [I_0/I - 1]/[Q]$ and $[Q]$ the quencher concentration from 0.00 to 0.10 M. For efficient quenching processes (concentration dependent) the value K_{SV} is often observed to increase with $[Q]$. Hence the values of K_{SV} were determined at each quencher concentration in all the solvent mixtures and the values of K_{SV}^{-1} are also accordingly determined. Figure 4 shows the plots of K_{SV}^{-1} against $[Q]^{1/3}$. From this figure we see that all the plots in BN - AN solvent mixtures are almost linear and small deviation may be due to experimental uncertainties. Hence, the linear dependence of K_{SV}^{-1} on the one-third power of quencher concentration $[Q]^{1/3}$ within the error limits is confirmed [23]. Then the values of K_{SV}^0 (i.e. S-V constant at $[Q] = 0$) and mutual diffusion coefficient D were obtained from the intercept and slope of the plot of K_{SV}^{-1} against $[Q]^{1/3}$ respectively. Using the values of K_{SV}^0 and D , the distance parameter R' was determined from Eq. 8 and is given in Table 2 for all the solvent mixtures. It has been observed from Table 2 that $R' > R$ in all the BN - AN mixtures and hence the values of k_a cannot be determined. According to Joshi et al. [28] the bimolecular quenching reactions are said to be diffusion limited if the values of k_q 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 both are given in Table 2. We see from Table 2 that in 20%BN + 80%AN solvent mixture and 100%AN solvent, the values of k_q are greater than $4\pi N'R'D$, which is an expected result for diffusion limited reaction [8, 16, 17, 28]. But for the remaining 80%BN + 20%AN, 60%BN + 40%AN, 40%BN + 60%AN solvent mixture and 100%BN solvent the values of k_q are not greater than $4\pi N'R'D$, such low value of k_q is due to low value of diffusion coefficients (D) [28]. It appears that in the 80%BN + 20%AN, 60%BN + 40%AN, 40%BN + 60%AN solvent mixture and 100%BN solvent, efficiency of quenching is less when compared with other solvent mixtures and solvent. Hence, reduced quenching efficiency is due to decrease in diffusion coefficients (D) of given bimolecular quenching reaction. However it may also be noted that the values of R' and 'r' kinetic distance do not change drastically. Further, we observe from Fig. 5 that the

values of K_{SV} increases as the dielectric constant of the solvent mixture increases. This effect of dielectric constant as function of K_{SV} suggests the charge transfer character in excited complex. The high value of K_{SV} in polar solvent acetonitrile compared to non-polar solvent benzene may also be explained by the greater charge transfer character of the excited complex in the polar solvent. Similar trend was observed by others in some fluorophore quencher systems [8, 16, 17, 23, 28].

Conclusion

From the above discussion and magnitude of rate parameters determined, we may conclude that:

- (1) The S-V plots show positive deviation, indicating efficient fluorescence quenching.
- (2) The value k_q is greater than $4\pi N'R'D$ in 20%BN + 80%AN solvent mixture and 100%AN solvent, indicating efficient fluorescence quenching. But for the remaining 80%BN + 20%AN, 60%BN + 40%AN, 40%BN + 60%AN solvent mixture and 100%BN solvent the values of k_q are not greater than $4\pi N'R'D$, such low value of k_q is due to low value of diffusion coefficients (D) in these solvent mixtures. In 80%BN + 20%AN, 60%BN + 40%AN, 40%BN + 60%AN solvent mixture and 100%BN solvent, efficiency of quenching is less when compared with other solvent mixtures and pure solvent.
- (3) The value of K_{SV} increases with increase in dielectric constant of the solvent mixture suggesting the charge transfer character of the excited complex.
- (4) Finite sink approximation model helps to recover R' and D directly from the plots K_{SV}^{-1} versus $[Q]^{1/3}$.

In view of the above facts, we conclude that the positive deviation in the S-V plots is due to the simultaneous presence of both static and dynamic quenching processes. The major one is a dynamic component which is of a charge transfer character. The other minor process is static quenching component contributes to the nonlinearity of the S-V plots and the quenching reaction is diffusion limited in this system.

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