

# Effect of solvent polarity on the fluorescence quenching of TMC molecule by aniline in benzene–acetonitrile mixtures

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**Abstract:** The fluorescence quenching of coumarin derivative, 6-methoxy-4-p-tolyloxymethyl-chromen-2-one by aniline is carried out in different solvent mixtures of benzene and acetonitrile at room temperature. The quenching is found to be appreciable and a positive deviation from linearity is observed in the Stern–Volmer plot in all the solvent mixtures. Various rate constants for fluorescence quenching processes have been estimated using a sphere of action static quenching model and a finite sink approximation model. From the positive deviation of linear Stern–Volmer plots and dependence of rate constants on the polarity of the solvents, it has been inferred that the quenching process is diffusion-limited, and static as well as dynamic quenching processes are responsible for the observed positive deviation in the Stern–Volmer plot. Further, both models have been found to agree well with transient state also in pure acetonitrile and benzene solvents.

**Key words:** coumarin, finite sink approximation model, fluorescence quenching, sphere of action static quenching model, Stern–Volmer plot.

**Résumé :** À l'aide d'aniline, nous provoquons l'atténuation de la fluorescence dans un dérivé de coumarine, 6-méthoxy-4-p-tolyloxyméthyle-chromène-2-un, dans différents mélanges de benzène et d'acétronitrile et à la température de la pièce. L'atténuation est appréciable et nous observons une déviation de linéarité dans le graphique de Stern–Volmer (S-V) pour tous les mélanges de solvant. Nous évaluons différentes constantes de taux pour le processus d'atténuation de fluorescence en utilisant un modèle de la sphère d'atténuation statique et une approximation de puits fini. À partir de la déviation positive de la linéarité du graphique de S-V et de la dépendance des constantes de taux sur la polarité des solvants, nous concluons que le mécanisme d'atténuation est statique et limité à la diffusion, alors même que les mécanismes dynamiques d'atténuation peuvent aussi être responsables de la déviation observée de linéarité. De plus, les deux modèles sont en bon accord sur l'état transitoire dans les solvants d'acétronitrile et de benzène purs. [Traduit par la Rédaction]

**Mots-clés :** coumarine, approximation du puits fini, atténuation de fluorescence, modèle de la sphère d'atténuation statique, graphique de Stern–Volmer.

## 1. Introduction

Coumarins are well-known oxygen heterocycles, which have been extensively explored as fluorescence probes [1, 2], optical brighteners [3], and as emitter layers in organic light emitting diodes (OLEDs) [4, 5]. They have also been successfully incorporated into energy- and electron-transfer arrays [6–8]. Dandriyal et al. [9] reviewed the synthetic strategy for the preparation of C-4 substituted coumarin derivatives as anti-cancer agents based on their mechanism of action and also discussed the structure activity relationship of the most active compound. Coumarin derivatives containing aryloxy group display potent biological properties including anti-histamine, anti-mycobacterial, vasodilator, anti-hypertensive, and monoamine oxidase-A and -B inhibitory activities. The aryloxy group at the C-4 position of coumarins (i.e., 4-aryloxymethylcoumarins) displays diverse biological activities, such as antimicrobial, anti-inflammatory, analgesic properties, which have been reviewed [10]. Basanagouda et al. [11, 12] reported that the 4-aryloxymethylcoumarins exhibit potent antimicrobial activity and it has been reported that the 6-methoxy-4-p-tolyloxymethyl-chromen-2-one (TMC) molecule was rated as a moderate to good

antibacterial agent against *K. pneumonia* and *S. faecalis*, and anti-fungal against *P. notatum* and *Rhizopus* [11]. Recently, our group reported on the iodinated-4-aryloxymethylcoumarins, which are similar to TMC as potent anti-cancer and anti-mycobacterial agents [13]. The effects of pure solvent, and solvent mixture, on absorption and fluorescence characteristics of 4-aryloxymethylcoumarins are well documented in the literature [14–17]. The fluorescence quenching of organic molecules in a solution using various quenchers like aniline, carbon tetrachloride, halide ions, ethylene tri thiocarbonate, CdS nanoparticle, oxygen, etc., has been studied by several investigators [18–23].

In almost all cases the experimental result follows the linear Stern–Volmer (S-V) equation and is given by for steady state method

$$\frac{F_0}{F} = 1 + K_{SV}[Q] \quad (1a)$$

and for the transient state method

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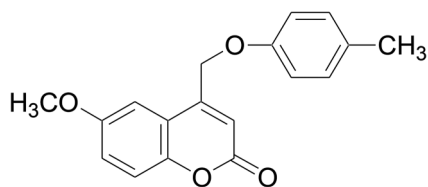
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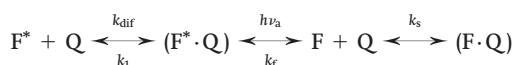
Fig. 1. The molecular structure of TMC molecule.



$$\frac{\tau_0}{\tau} = 1 + K'_{SV}[Q] \quad (1b)$$

where  $F_0$  and  $F$  are the fluorescence intensities in the absence and presence of the quencher, respectively;  $K_{SV} = k_q\tau_0$  or  $K'_{SV} = k'_q\tau_0$  is called S-V constant,  $k_q$  ( $k'_q$ ),  $\tau_0$ , and  $[Q]$  are the bimolecular quenching rate parameter, the lifetime of the excited solute in the absence of quencher, and concentration of quencher, respectively. But in a few cases, it has been observed that the experimental results show positive deviation from a linear S-V relation [24, 25]. This positive deviation was attributed to various processes like singlet to triplet excitation, static and dynamic quenching, and the formation of charge transfer complexes both at the ground and excited states. Apart from this, the polarity of the solvent medium and the range of quencher concentrations also play a part in this mechanism.

The charge transformation scheme in fluorescence quenching process is presented as



where  $F$  is a fluorophore,  $Q$  is a quencher concentration,  $F^*$  is an excited fluorophore, and  $k_{dif}$ ,  $k_1$ ,  $k_f$ , and  $k_s$  are the rate constants for the diffusion, back diffusion with breakup exciplex, radiative decay, and non-emissive quenching, respectively.

Prompted by the preceding observations and in continuation of our research work [26], the present paper reports the investigation of the steady-state fluorescence quenching of the TMC molecule at room temperature using aniline as a quencher in different mixtures of benzene (BN) and acetonitrile (AN) and transient state method as well in pure AN and BN solvents. The various rate constants responsible for fluorescence quenching mechanisms have been determined using a sphere of action static quenching model and a finite sink approximation model.

## 2. Materials and methods

The TMC molecule was synthesized according to the literature [11]. The molecular structure of the TMC molecule is shown in Fig. 1. The solvents BN and AN were obtained from SD-Fine Chemical Limited, India. The quencher aniline was double-distilled and tested for its purity before use. The solutions were prepared by taking BN and AN in different proportions by volume with a fixed solute concentration of  $5 \times 10^{-5}$  mol/L and quencher concentrations were varied from 0.02 to 0.10 mol/L. The absorption spectra were recorded using a UV-visible spectrophotometer (Hitachi U-3310, Japan) and the fluorescence spectra of the solutes were recorded in the absence and presence of the quencher using a fluorescence spectrophotometer (Hitachi F-7000, Japan). The fluorescence lifetime of the solute was recorded using the time-correlated single photon counting technique (model ISS-90021). All these spectroscopic measurements were carried out at room temperature (300 K).

## 3. Results and discussion

Initially, the fluorescence intensity  $F_0$  was measured without quencher concentration and then the fluorescence intensity  $F$  was measured for different quencher concentrations at a fixed solute concentration for all the solvent mixtures. The experimental values are reproducible within 5% of the experimental errors. The plots of  $F_0/F$  versus  $[Q]$  for TMC molecule for different proportions of AN and BN by volume were nonlinear and are shown in Fig. 2. Further, the lifetime of the TMC molecule without quencher ( $\tau_0$ ) and for different quencher concentrations ( $\tau$ ) in fixed solute concentration for pure AN and pure BN were recorded and plots of  $\tau_0/\tau$  versus  $[Q]$  are shown in Fig. 3 and deviate from linearity. It may be concluded that the quenching is not purely collisional and may be due to the formation of either the ground-state complex formation or static quenching process.

The ground-state complex formation is ruled out because there is no shift in absorption and emission spectra of TMC molecule in different solvent mixtures at room temperature, as observed in Fig. 4. Thus, the positive deviation was explained using a sphere of action static quenching model. According to this model, the deviation from the expected linear S-V plot was explained by the fact that only a certain fraction  $W$  ( $W'$ ) of the excited state is actually quenched by the collisional mechanism. In such cases, some molecules in the excited state, the fraction of which  $1 - W$  ( $1 - W'$ ) is deactivated almost instantaneously after being formed, because a quencher molecule happens to be randomly positioned in the vicinity at the time the molecules are excited and interacts strongly with them. This static quenching was explained by introducing an additional factor  $W$  ( $W'$ ) in the linear S-V equations (1a) and (1b).

For the steady-state method

$$\frac{F_0}{F} = \frac{1 + K_{SV}[Q]}{W} \quad (2a)$$

For the transient state method

$$\frac{\tau_0}{\tau} = \frac{1 + K'_{SV}[Q]}{W'} \quad (2b)$$

here

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

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

Because the fraction  $W$  ( $W'$ ) depends on the quencher concentration  $[Q]$ , the S-V plots for a quencher with high quenching ability will generally deviate from linearity. Thus, (2a) and (2b) can be modified as follows.

For steady-state method

$$\frac{1 - (F/F_0)}{[Q]} = K_{SV} \left( \frac{F}{F_0} \right) + \frac{1 - W}{[Q]} \quad (4a)$$

and for transient state method

$$\frac{1 - (\tau/\tau_0)}{[Q]} = K'_{SV} \left( \frac{\tau}{\tau_0} \right) + \frac{1 - W'}{[Q]} \quad (4b)$$

The plots of  $[1 - (F/F_0)]/[Q]$  versus  $(F/F_0)$  and  $[1 - (\tau/\tau_0)]/[Q]$  versus  $(\tau/\tau_0)$  should be linear, and these plots are shown in Figs. 5 and 6,

Fig. 2. The plots of  $F_0/F$  versus  $[Q]$  for TMC molecule + aniline system in different solvent mixtures [BN + AN]. [Colour online.]

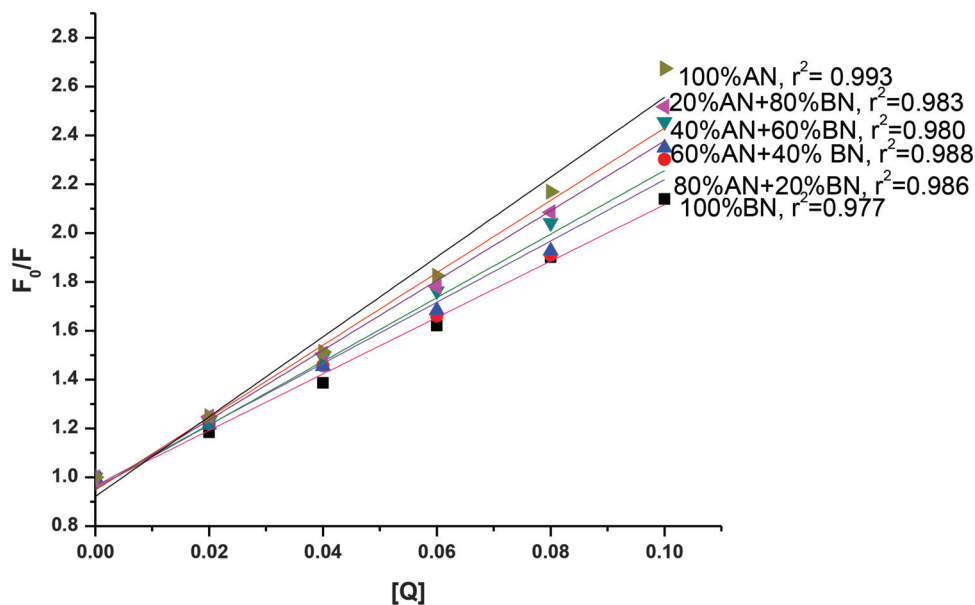
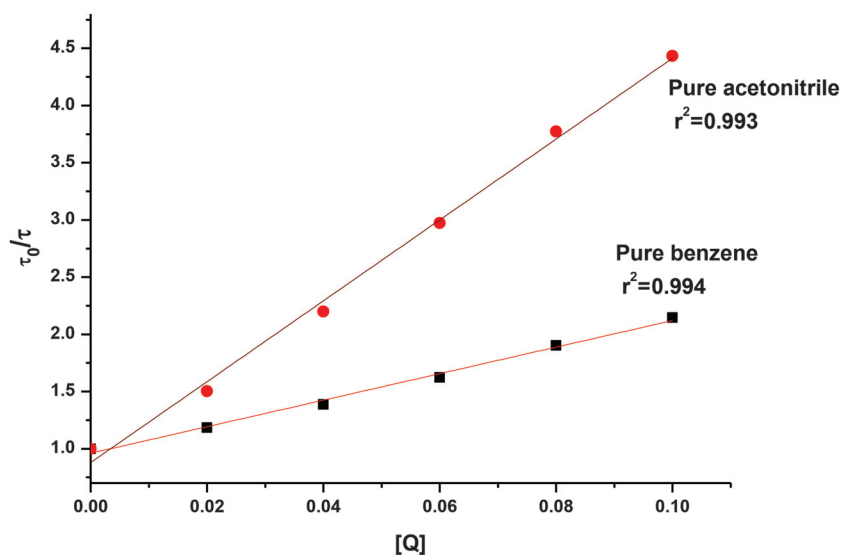


Fig. 3. The plot of  $\tau_0/\tau$  versus  $[Q]$  for TMC molecule + aniline system for pure AN and pure BN. [Colour online.]



respectively. Using a least-square fit method we can easily estimate the values of  $K_{SV}$  ( $K'_{SV}$ ) and  $W$  ( $W'$ ) for each quencher concentration and these values are shown in Table 1. The values of  $k_q$  ( $k'_q$ ) are calculated using the relations  $K_q = K_{SV}/\tau_0$  and  $K'_q = K'_{SV}\tau_0$ , and these values are shown in Table 1.

The values of static quenching constant  $V$  ( $V'$ ) are estimated using (3a) and (3b) and these values are also shown in Table 1.

Frank and Wawilow [27] suggested that the instantaneous quenching occurs in a randomly distributed system whenever a quencher happens to reside within a sphere of action with a volume of  $V/N'$  and kinetic distance  $r$  ( $r'$ ) and the volume of the sphere of action is given as follows.

For steady state method

$$\frac{V}{N'} = \frac{4}{3}\pi r^3 \quad (5a)$$

and for transient state method

$$\frac{V'}{N'} = \frac{4}{3}\pi r'^3 \quad (5b)$$

where  $N'$  is Avogadro's number per millimole. Using the values of static quenching constant  $V$  ( $V'$ ) the kinetic distance  $r$  ( $r'$ ) were determined and are shown in Table 1. The literature values [28] of dielectric constant of solvent mixtures are shown in Table 1. The radii of the solute ( $R_s$ ) and a quencher ( $R_Q$ ) were determined as suggested by the Edward method [29] and are given at the bottom of Table 1, from these values the sum of the molecular radii  $R$  ( $R_s + R_Q$ ) is determined and it is referred to as the encounter distance. Then this value of  $R$  is compared with the values of  $r$  ( $r'$ ) to verify whether the reaction is due to the sphere of action static quenching

Fig. 4. The fluorescence spectra of TMC molecule for different proportions of AN and BN by volume at different quencher concentrations. [Colour online.]

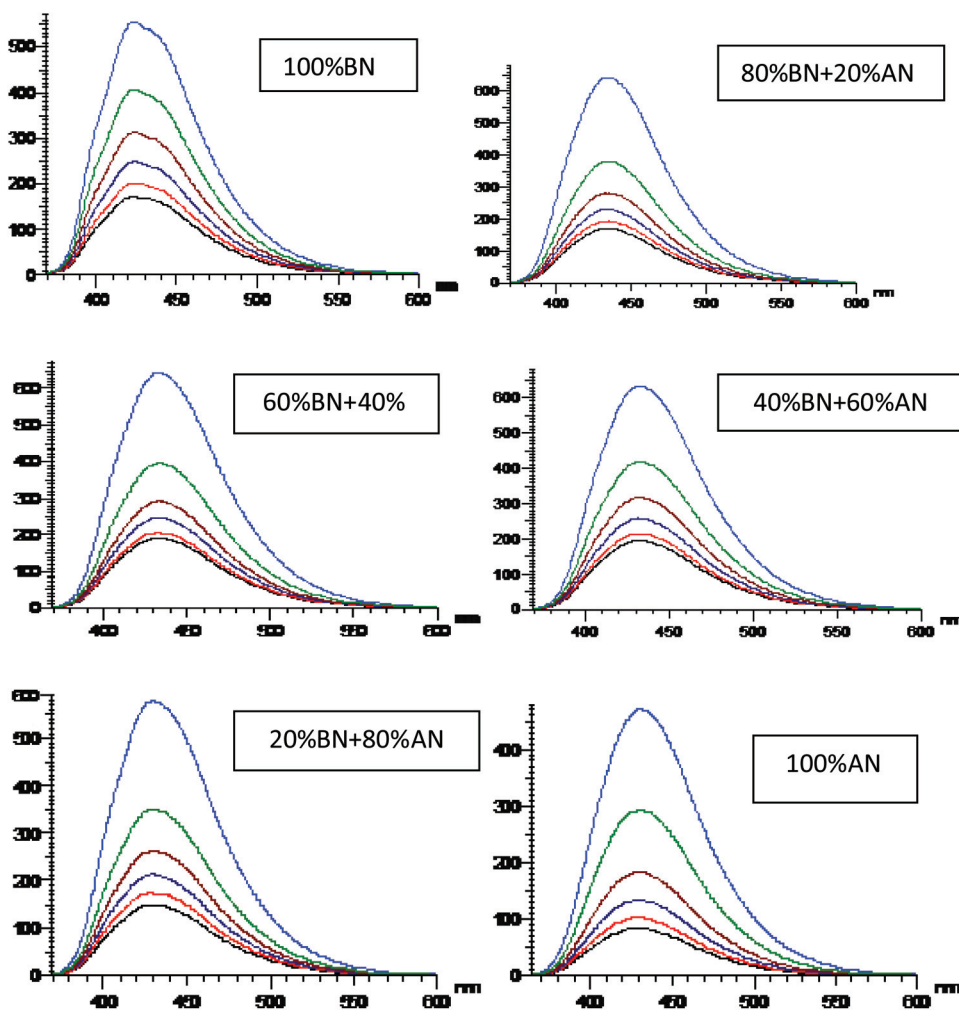
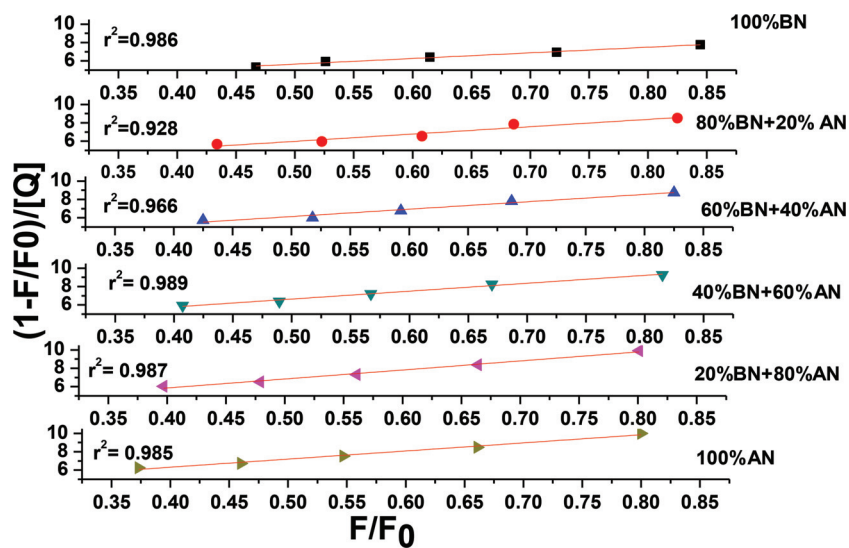
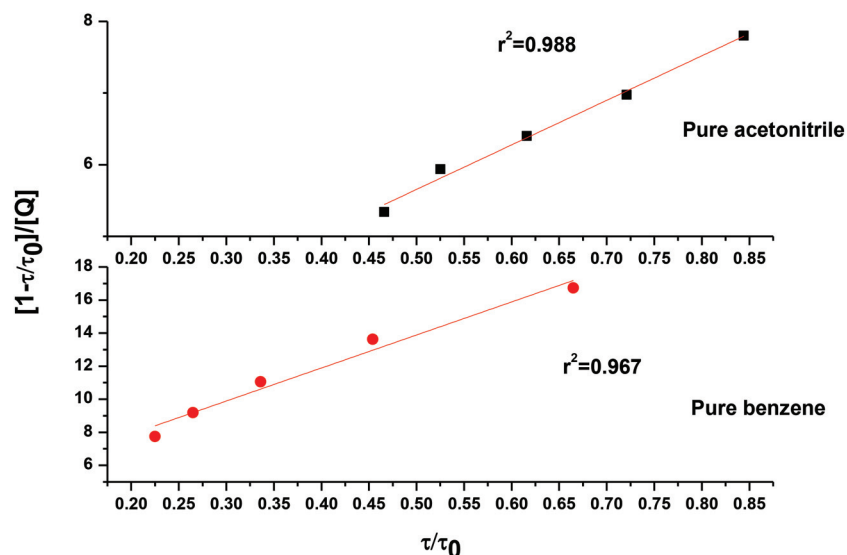


Fig. 5. The plots of  $[1 - (F/F_0)]/[Q]$  versus  $F/F_0$  for TMC molecule + aniline system in different solvent mixtures [BN + AN]. [Colour online.]



**Fig. 6.** The plot of  $[1 - (\tau/\tau_0)]/[Q]$  versus  $[\tau/\tau_0]$  for TMC molecule + aniline system for pure AN and pure BN. [Colour online.]**Table 1.** The dielectric constant of solvent mixtures and various fluorescence quenching rate parameters for TMC molecule + aniline system in solvent mixtures of BN + AN estimated using sphere of action static quenching model at room temperature.

Solvent mixture (%)		Dielectric constant	$K_{SV} (K'_{SV}) (M^{-1})$	$k_q (k'_q) \times 10^{-9} (M^{-1}s^{-1})$	$V (V') (M^{-1}dm^3)$	$r (r') (\text{\AA})$
BN	AN					
100	00	2.284	6.181	5.42	2.809	10.364
100	00	2.284	*5.832	*5.30	*2.613	*9.135
80	20	10.67	7.956	6.97	2.281	9.670
60	40	15.18	8.052	7.06	2.417	9.858
40	60	24.30	8.603	7.54	2.727	10.263
20	80	30.46	9.785	8.58	2.202	9.557
00	100	37.12	10.25	8.99	2.009	9.268
00	100	37.12	*6.215	*8.96	*2.767	*10.957

Note:  $R_S = 5.07 \text{ \AA}$ ;  $R_Q = 2.840 \text{ \AA}$ ;  $\tau_0 = 1.140 \text{ ns}$ .

\*Lifetime measurement.

**Table 2.** The various quenching rate parameters for TMC molecule+aniline system in solvent mixtures of BN+AN estimated using finite sink approximation model at room temperature.

Solvent mixture (%)		$K_{SV}^0 (K'_{SV})' (dm^3M^{-1})$	$D (D') \times 10^5 (cm^2s^{-1})$	$R' (R'') (\text{\AA})$	$\frac{4\pi N'R'D}{(4\pi N'D'R'')} \times 10^{-9} (M^{-1}s^{-1})$	$k_q (k'_q) \times 10^{-9} (M^{-1}s^{-1})$
BN	AN					
100	00	13.947	3.059	5.524	12.79	13.39
100	00	*15.139	*2.013	*6.215	*13.10	*14.23
80	20	20.846	10.498	2.408	19.12	16.79
60	40	17.266	2.938	8.539	18.99	19.27
40	60	20.703	4.082	5.125	15.84	20.56
20	80	22.416	7.176	3.785	20.56	22.64
00	100	23.992	7.795	3.730	22.00	24.10
00	100	*20.123	*6.253	*2.956	*19.86	*23.21

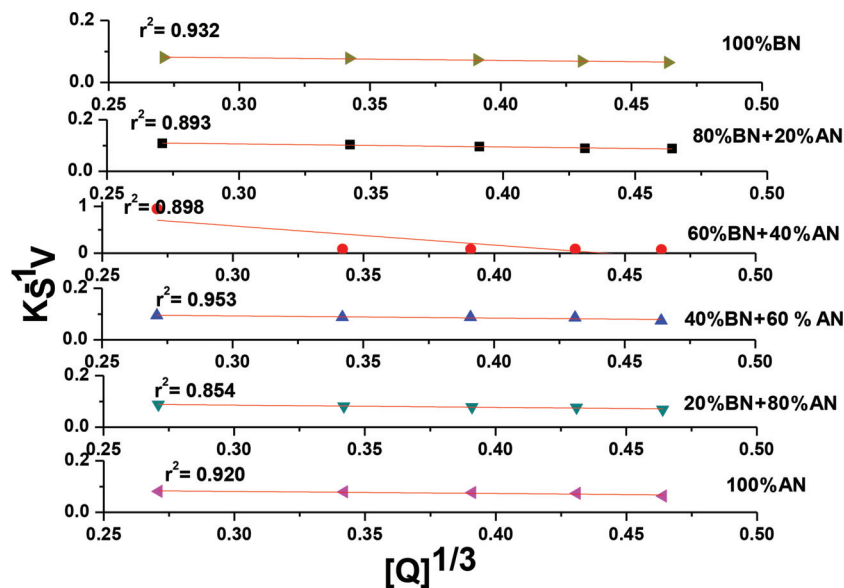
\*Lifetime measurement.

model or not. From Table 2 we see that values of kinetic distance  $r (r')$  are greater than the encounter distance  $R$ , suggesting that the sphere of action static quenching model may be playing the role outlined by Andre et al. [30] and Zeng and Durocher [31]. However,

the static effect takes place irrespective of ground-state complex formation, provided the reactions are limited by diffusion.

To study whether the bimolecular quenching reaction in TMC molecule is diffusion limited or not, the finite sink approximation

Fig. 7. The plots of  $K_{SV}^{-1}$  versus  $[Q]^{1/3}$  for TMC molecule + aniline system in different solvent mixtures [BN + AN]. [Colour online.]



model [32] is invoked. According to this model, for the steady state method,

$$K_{SV}^{-1} = (K_{SV}^0)^{-1} - \frac{(2\pi N')^{1/3}}{4\pi N' D \tau_0} [Q]^{1/3} \quad (6a)$$

where

$$K_{SV}^0 = \frac{4\pi N' DR' \tau_0 k_a}{4\pi N' DR' + k_a}$$

$$R' = R \left( \frac{1 + 4\pi R D N'}{k_a} \right)^{-1}$$

$$\frac{1}{k_a} = \frac{1}{k_d} + \frac{1}{k_q}$$

and

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

For the transient state method

$$(K_{SV}^{-1})' = [(K_{SV}^0)^{-1}]^{-1} \frac{(2\pi N')^{1/3}}{4\pi N' D' \tau_0} [Q]^{1/3} \quad (6b)$$

where

$$(K_{SV}^0)' = \frac{4\pi N' D' R'' \tau_0 k'_a}{4\pi N' D' R'' + k'_a}$$

$$R'' = R \left( \frac{1 + 4\pi R D' N'}{k'_a} \right)^{-1}$$

$$\frac{1}{k'_a} = \frac{1}{k'_d} + \frac{1}{k'_q}$$

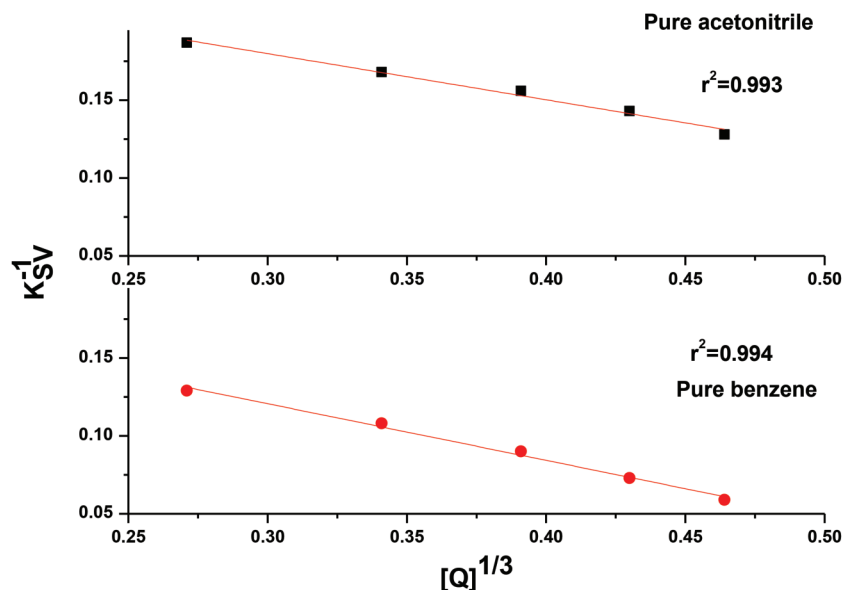
and

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

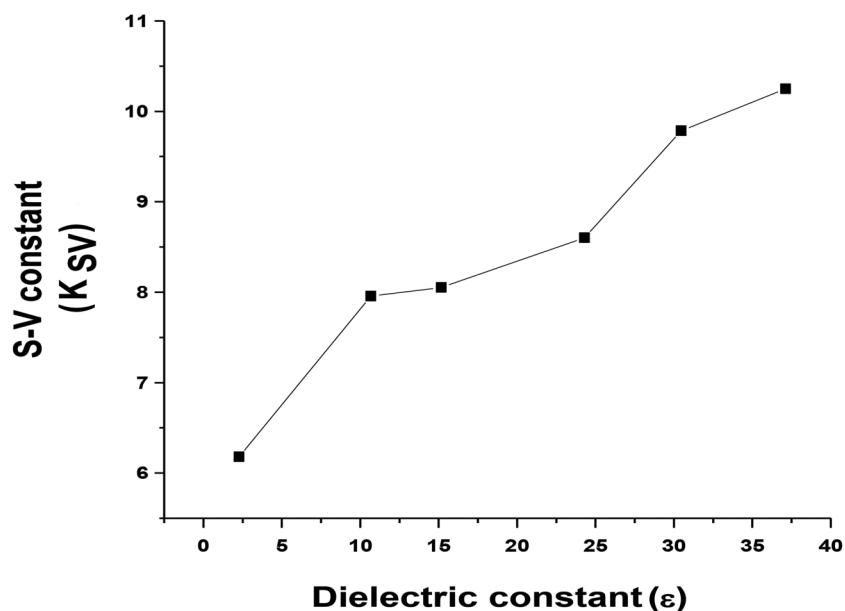
where  $N'$  is Avogadro's number per millimole,  $D$  ( $D'$ ) is the sum of the diffusion coefficients of reactants,  $R'$  ( $R''$ ) is distance parameter,  $k_a$  ( $k'_a$ ) is the activation energy controlled rate constant, and  $k_d$  ( $k'_d$ ) is the frequency of encounters. This model helps to calculate independently the mutual diffusion coefficient  $D$  ( $D'$ ) and a distance parameter  $R'$  ( $R''$ ). According to (6a) and (6b), the plots of  $K_{SV}^{-1}$  ( $(K_{SV}^{-1})'$ ) versus  $[Q]^{1/3}$  must be linear with negative slope. Thus the plots of  $K_{SV}^{-1}$  ( $(K_{SV}^{-1})'$ ) versus  $[Q]^{1/3}$  for steady-state and for transient-state methods are linear with a negative slope and are shown in Figs. 7 and 8, respectively. Then from the least square fit method, the values of mutual diffusion coefficient  $D$  ( $D'$ ),  $K_{SV}^0$  ( $(K_{SV}^0)'$ ) and distance parameter  $R'$  ( $R''$ ) were estimated. Using the values of  $D$  ( $D'$ ) and  $R'$  ( $R''$ ),  $4\pi N' DR'$  ( $4\pi N' D' R''$ ) are determined and all these values are shown in Table 2. To see whether the diffusion-limited reaction is taking place,  $k_q$  ( $k'_q$ ) is to be compared with  $4\pi N' DR'$  ( $4\pi N' D' R''$ ). According to Joshi et al. [33], if  $k_q$  ( $k'_q$ ) >  $4\pi N' DR'$  ( $4\pi N' D' R''$ ), then the bimolecular quenching reaction is said to be diffusion limited. Thus, from Table 2 it is observed that  $k_q$  ( $k'_q$ ) are greater than  $4\pi N' DR'$  ( $4\pi N' D' R''$ ), which indicates that the finite sink approximation model agrees well with the system studied.

To see the variation of  $K_{SV}$  as a function of dielectric constant of the solvent mixtures for TMC molecule, a plot of  $K_{SV}$  versus dielectric constant is plotted and is shown in Fig. 9. From Fig. 9 we observed that the values of  $K_{SV}$  increase as the dielectric constant of the solvent mixture increases. This fact may suggest that the charge transfer character occurs in the excited state complex. From the higher value of  $K_{SV}$  for AN (polar solvent) as compared to BN (non-polar solvent) it may be inferred that the polar solvent has a greater charge transfer character for the excited state complex.

**Fig. 8.** The plots of  $K_{SV}^{-1}$  versus  $[Q]^{1/3}$  for TMC molecule + aniline system in pure AN and pure BN. [Colour online.]



**Fig. 9.** Variation of  $K_{SV}$  as a function of dielectric constant of solvent mixture (BN + AN) for TMC molecule + aniline system.



From the results of fluorescence quenching obtained for TMC solute molecule in binary mixtures for different proportions of AN and BN by volume and using aniline as a quencher it may be inferred that the static quenching effect plays a role and the reactions appear to be diffusion limited as is evident from sphere of action static quenching and finite sink approximation models. However, the dynamic effect also plays its role because, at lower quencher concentration of aniline, S-V plots appear to be linear. Further, various fluorescence quenching rate parameters for TMC molecule calculated using sphere of action static quenching and finite sink approximation models under both steady-state and transient-state methods in AN and BN solvents appear to be closely correlated. Based on this assumption, the fluorescence quenching rate parameters may also appear closely correlated for

different proportions of AN and BN if transient methods are recorded.

#### 4. Conclusion

From the ongoing discussions, we observed that:

1. The S-V plots show the positive deviation leading to a high value of  $k_q$ , suggesting the higher efficiency of fluorescence quenching.
2. Finite sink approximation model helps to verify diffusion-limited reactions based on the comparison of  $k_q$  and to determine  $R'$  and  $D$  independently.
3. The value of S-V constant  $K_{SV}$  increases with increasing dielectric constant of the binary solvent mixtures, suggesting charge

transfer character for the excited state complex. The higher value of  $K_{SV}$  for AN (polar solvent) as compared to BN (non-polar solvent), indicates that the polar solvent has a greater charge transfer character than non-polar solvent.

- There is a good correlation between the parameters determined from steady-state and transient-state methods.

In view of these facts, it may be inferred that for fluorescence quenching of TMC molecule in different proportions of AN and BN using aniline as a quencher, both static and dynamic quenching and diffusion-limited reactions play a role.

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