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Solvent effect study on fluorescence quenching of 5-methyl-2-phenylindole by CCl_4

S.V.Nishti[1], R.S.Kunabenchi[1]*, B.G.Evale[1], J.S.Biradar[2], J.R.Mannekutla[3]
[1]Department of Physics, Gulbarga University, Gulbarga-585 106, Karnataka, (INDIA)
[2]Department of Chemistry, Gulbarga University, Gulbarga-585 106, Karnataka, (INDIA)
[3]Department of Physics, Karnatak University, Dharwad-580 003, Karnataka, (INDIA)
Tel: +91-8472-263298; Fax: +91-8472-263206
E-mail : kunabenchi@yahoo.co.in

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ABSTRACT

The effect of solvent polarity on fluorescence quenching of 5-methyl-2-phenylindole by carbon tetrachloride in different solvent mixtures of dioxane-acetonitrile has been studied at room temperature. The quenching is found to be appreciable and a positive deviation from linearity was observed in the Stern-Volmer plot in all the solvent mixtures. Analysis of the results indicates that the quenching is due to both static and dynamic processes. Further, it is found that the Stern-Volmer constant depends on the dielectric constant of the solvent mixtures, which indicates the charge transfer character in the excited complex.

KEYWORDS

Indole;
Fluorescence quenching;
Sphere of action static quenching model;
Charge transfer.

INTRODUCTION

Investigations on the photophysical properties of indole and its derivatives are gaining increasing interest and the photophysics of indole still remains a useful subject to understand the different quenching mechanisms. The role of fluorescence quenching can be studied experimentally by determining quenching parameters using Stern-Volmer plots. In most of the cases of quenching by CCl_4, the S-V plots were found to be linear, in which, the quenching mechanism is mainly due to dynamic process, where diffusion process is a dominant one. In a few cases, the experimental results show positive deviation from linear S-V relation. This positive deviation was attributed to various processes, like singlet-triplet excitation, formation of charge transfer complex both at ground and excited states, static and dynamic quenching, etc. The quenching of fluorescence of organic molecules by carbon tetrachloride (CCl_4) has been a subject of continued investigation for the last couple of decades to understand the nature of bimolecular reactions taking place both under steady state and transient methods[1-9]. Carbon tetrachloride is known to be a good quencher for several fluorescent molecules. The fluorescence properties of indole and its derivatives have been extensively studied[4,5,10]. This is in part due to the occurrence of the indole moiety in the majority of protein. The quenching studies are able to provide valuable information concerning the exposure of tryptophanyl residues and dynamics of proteins matrix surrounding such residues.

In the present study, the effect of solvent polarity on the fluorescence quenching of 5-methyl-2-phenyl indole has been studied at room temperature using CCl_4 quencher in different mixtures of dioxane and acetonitrile. The change of composition of the present solvents provides a good range of solvent polarity ranging from 2.1 to 36.0. Also, it has been studied the dependence of fluorescence quenching on solvent polarity at various quencher concentrations, which cover the non-linear range of the quenching curve.

EXPERIMENTAL

The indole derivative 5-methyle-2-phenylindole has been synthesized in our laboratory and characterized by using IR, NMR, and Massbaur techniques for its purity. The spectroscopic grade solvents viz., dioxane and acetonitrile (s.d. Fine Chemicals Ltd.) were used without further purification. However, the purity of the solvents was checked by the background fluorescence. Spectroscopic grade CCl_4 solvent has been used as quencher and it was double distilled before use. The solutions were prepared keeping the concentration of solute fixed at 1×10^{-5} M/L and varying the concentration of quencher from 0.00 to 0.10 M/L. Fluorescence intensities were recorded by exciting the solute at 320nm corresponding to longer wavelength absorption band by varying the quencher concentration from 0.00 to 0.10 M/L using fluorescence spectrophotometer (Hitachi Model F-2000).

RESULTS AND DISCUSSION

The Stern-Volmer plots for the quenching of fluorescence intensities of the solute by CCl_4 quencher in different solvent mixtures were plotted according to the Stern-Volmer equation[11]

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

where, I_0 and I are the fluorescence intensities of the solute in the absence and presence of the quencher respectively and K_{SV} is the S-V quenching constant and [Q] is the quencher concentration. The S-V plot (I_0/I against [Q]) as shown in figure 1 was found to be non-linear, and showing positive deviation. It may be concluded that the quenching is not purely collisional and may be due to the formation of either the ground state complex or static quenching process. But the formation of the ground sate complex for this system is ruled out[9]. Thus, the analysis of data for the positive deviation in S-V plots were made using ‘sphere of action’ static quenching model.

According to static quenching model, the instantaneous or static quenching occurs only when the quencher molecule is very near to, or in contact with the fluorescent molecule, just at the moment of its excitation. This model can be explained by the fact that only a certain fraction W of the excited state is quenched by the collisional mechanism. Some molecules in the excited state, the fraction of which is (1-W), are deactivated almost instantaneously after being excited because a quencher molecule happens to be randomly positioned in the proximity, at the time, when the molecules are excited and interacts very strongly with them. Thus, the fraction W decreases from unity in contrast to the linear S-V equation. Several models were employed to describe this static quenching process, all leading to the modified form of the S-V equation[12].

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

$$k_d = 4\pi N'DR + 4R^2 N' (\pi D)^{1/2} t^{-1/2} \qquad (3)$$

where N′ is the Avogadro’s number per millimole, R is the encounter distance, i.e. the sum of the radii of the solute (R_S) and quencher (R_Q) molecules, D is the sum of the diffusion co-efficient of solute (D_S) and quencher (D_Q) molecules and t is the time. The retention of the second term in the equation (3) leads to an additional factor W in equation (3). This additional factor is given by

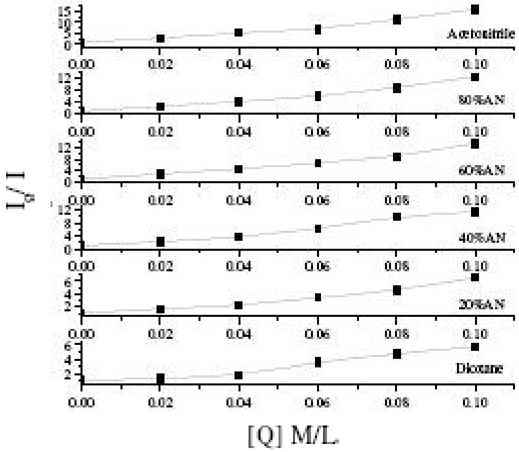

Figure 1: Stern-Volmer plots of I_0/I against [Q] in different solvent mixtures of dioxane and acetonitrile

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

i.e. $\ln W = -V[Q]$ (4)

where, V is the static quenching constant and it represents the active volume element surrounding the excited molecules.

According to Frank and Wawilow[13] instantaneous quenching occurs in a randomly distributed system, when a quencher happens to reside within a 'sphere of action' with volume of V/ N′ and is given by

$V/N' = (4/3)\pi r^3$ (5)

where r is the radius of sphere of action and also called as kinetic distance.

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, equation (2) can be rewritten as[4,6,10]

$[1-(I/I_0)]/[Q] = K_{SV}(I/I_0) + (1-W)/[Q]$ (6)

The modified S-V plot of $[1-(I/I_0)]/[Q]$ against I/I_0 is shown in figure 2 and was found to be linear. From this graph, it is evident that, the intercepts are non-zero and are large. The S-V quenching constant K_{SV} was determined in all the cases by least square fit method and the quenching rate parameter k_q was calculated using the equation $k_q = K_{SV}/\tau_0$, where τ_0 is the fluorescence lifetime in the absence of quencher. The values of K_{SV} and k_q are collated in TABLE 1. The intercepts of least square fit lines of figure 2 are equal to (1-W)/[Q]. From these intercepts, the values of W were determined for each concentration and the range of W are given in TABLE 1. Using the values of W, the static quenching constant V and the kinetic distance or radius of sphere of action 'r' were determined according to equations (4) and (5). All these data are collated in TABLE 1. It is observed that the values of K_{SV} are rather larger relative to V value in almost all the mixtures, which explains the lack of the absorption change on addition of the quencher[1]. Also, from TABLE 1 it is seen that the static quenching constant V is different for different solvent mixtures, this indicates that the static quenching constant V is independent of solvent polarity, which was also observed by others[1,4].

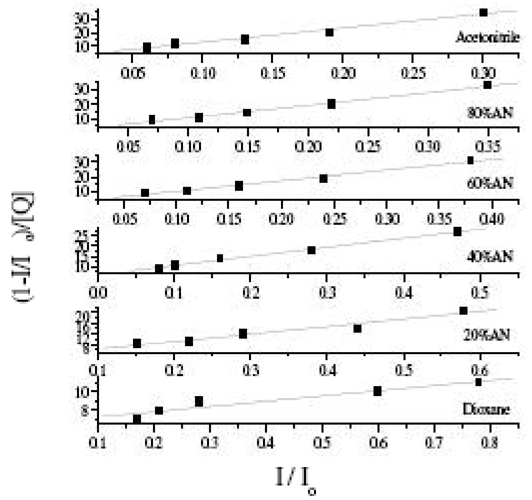

Figure 2: Modified Stern-Volmer plot of $(1-I/I_0)/[Q]$ against I/I_0 in different solvent mixtures of dioxane and acetonitrile

TABLE 1. The Stern-Volmer constant K_{SV}, quenching rate parameter k_q, range of W, static quenching constant V and kinetic distance 'r'

Solvent mixture (% v/v)	Dielectric constant ε	K_{SV} (M^{-1})	$k_q \times 10^{-9}$ ($M^{-1}s^{-1}$)	Range of W	V (M^{-1})	r (Å)
Dioxane	2.10	5.62	3.64	0.33-0.86	11.95	16.80
20% AN	8.10	28.24	18.34	0.66-0.93	4.3	11.95
40% AN	15.3	43.65	28.34	0.37-0.87	10.60	16.14
60% AN	22.0	70.49	45.77	0.66-0.93	4.35	12.00
80% AN	28.6	86.59	56.23	0.81-0.96	2.10	9.41
Acetonitrile	36.0	103.55	67.24	0.75-0.95	3.00	10.60

$R_S = 3.64$ Å, $R_Q = 2.79$ Å, $R = R_S + R_Q = 6.43$ Å, $\tau_0 = 1.54$ ns

TABLE 2: The values of K_{SV}^0 (steady state quenching constant at [Q] = 0), mutual diffusion co-efficient D, distance parameter R′, 4πN′DR′.

Solvent mixture(% v/v)	K_{SV}^0 (M^{-1})	$D \times 10^5$ (cm^2s^{-1})	R′ (Å)	$4\pi N'DR' \times 10^{-9}$ ($M^{-1}s^{-1}$)
Dioxane	6.67	0.89	6.43	4.33
20% AN	25.64	3.26	6.75	16.64
40% AN	31.25	2.62	10.24	20.30
60% AN	50.00	5.56	7.72	32.48
80% AN	66.66	7.86	7.28	43.29
Acetonitrile	76.92	9.03	7.31	49.94

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 solute (R_S) and quencher molecules (R_Q), the radii of the solute and quencher were determined and are given at the bottom of the TABLE 1 and from TABLE 1, it is evident that, the values of r are greater than the encounter distance R. Similar results were also obtained for other molecules[4,14,15].

According to Andre et al.[16], if the distance between the quencher molecule and excited molecule lies between the encounter distance R and the kinetic distance r, the static effect takes place especially in the case of steady state experiments, irrespective of ground state complex formation provided the reactions are limited by diffusion. To find out whether the reactions are diffusion limited, we considered the finite sink approximation model, which helps to estimate independently the mutual diffusion coefficient D, distance parameter R′ and the activation energy controlled rate constant k_a.

Finite sink approximation model

The modified Smoluchowski model known as the SCK model proposed by Keizer [17] has been found to be adequate to explain the experimental data for several fluorescence quenching reactions. In this model, time dependent rate co-efficient k(t) for diffusion limited reaction of initially randomly distributed reactants is given by[12,18]

$$k(t) = a + b\exp(c^2 t)\,\mathrm{erfc}(c\,t^{1/2}) \qquad (7)$$

$$\text{where } a = k_a\left[1 + \frac{k_a}{4\pi N'RD}\right]^{-1} \qquad (8)$$

$$b = k_a\left[1 + \frac{4\pi N'RD}{k_a}\right]^{-1} \qquad (9)$$

$$c = \left[1 + \frac{k_a}{4\pi N'RD}\right]\frac{D^{1/2}}{R} \qquad (10)$$

Integration of equation (10) between the limits [Q] (∞) at r→∞ and [Q] (R) at r = R provides well known expression

$$\frac{1}{k_q} = \frac{1}{k_d} + \frac{1}{k_a} \qquad (11)$$

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 co-efficient of solute and quencher molecules. In the equation (11), 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 these[12].

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 $R \le r \le r_0$ 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 (11) as

$$\frac{1}{k_q} = \frac{1-\left(R/r_0\right)}{k_d} + \frac{1}{ka} \qquad (12)$$

This equation reduces to the reaction limited form ($k_q = k_a$), both for inefficient quenching ($k_a << k_d$) and for quenching in pure quenching solvents where $R = r_0$. In the diffusion controlled limit ($k_a >> k_d$), equation (12) reduces to

$$k_q = \frac{k_d}{1 - R/r_0} \qquad (13)$$

and k_q depends on the quenching concentration through r_0.

Since the sink radius (r_0) is identified with the most probable nearest neighbor initial separation, the appropriate distribution requires that, $r_0 = (2\pi N'[Q])^{-1/3}$. Replacing r_0 in equation (13) and k_d by its value ($4\pi N'DR$) and dividing the equation by the fluorescence lifetime of solute in the absence of quencher (τ_0), one obtains the modified Stern-Volmer relationship as[12,18]

$$K_{SV}^{-1} = \left(K_{SV}^{0}\right)^{-1} - \frac{(2\pi N')^{1/3}}{4\pi N' D\tau_0}[Q]^{1/3} \qquad (14)$$

$$\text{where } K_{SV}^{0} = \frac{4\pi N' DR\tau_0 k_a}{4\pi N' DR + k_a} \qquad (15)$$

A plot of K_{SV}^{-1} against $[Q]^{1/3}$ becomes linear with negative slope. Mutual diffusion co-efficient D is accessible directly from the slope of the graph exemplified in equation (14) and K_{SV}^{0} is obtained at [Q] = 0 regardless of the relative magnitudes of k_a and k_d ($=4\pi N'DR$), whether quenching is diffusion limited or not. From K_{SV}^{0}, R′ is estimated using[19]

$$K_{SV}^{0} = 4\pi N' DR'\tau_0 \qquad (16)$$

where R′ has the same meaning as in the long-time SCK model[12,18] and is given as,

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

Then according to the theory discussed above, if $k_a > k_d$, then the reactions are said to be diffusion limited[14,19]. i.e. for R′<R. But, if R′>R, the bimolecular quenching reactions of fluorescence quenching are said to be diffusion limited, if the values of k_q determined

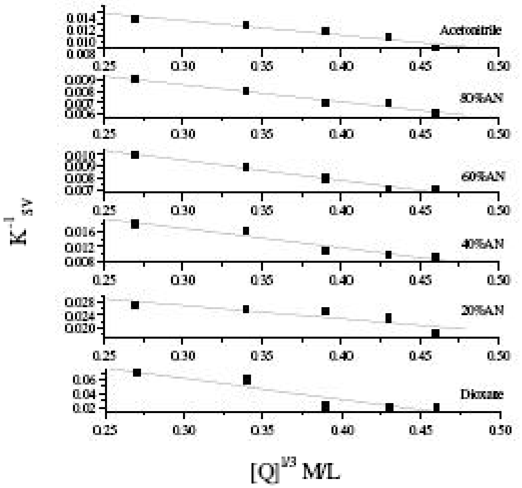

Figure 3: Modified Stern-Volmer plot of K_{SV}^{-1} against $[Q]^{1/3}$ in different solvent mixtures of dioxane and acetonitrile

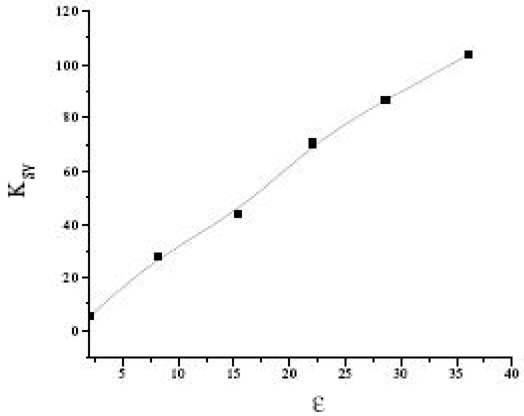

Figure 4: Plot of Stern-Volmer constant (K_{SV}) against solvent polarity (ε)

from the equation (6) are greater than 4πN′DR′[18].

For efficient quenching process, the values of K_{SV} are often observed to increase with [Q]. Hence, the values of K_{SV} were determined at each quencher concentrations ranging from 0.02 to 0.10 M/L in different binary mixtures using $K_{SV} = [(I_0/I)-1]/[Q]$. The graph K_{SV}^{-1} versus $[Q]^{1/3}$ was plotted using the equation (14) as shown in figure 3 and is found to be linear in all the mixtures. Using least square fit method, the values of D and K_{SV}^{0} (S-V constant at [Q] =0) were determined by measuring the slopes and intercepts. Then, distance parameter R′ was determined according to equation (17) using the values of K_{SV}^{0} and D. It is found that R′> R, which indicates that activation energy controlled rate parameter k_a cannot be determined. But, from TABLES 1 and 2 we can observe that the values of k_q are greater than 4πN′D R′, which indicates that the reactions are diffusion limited[18].

The sphere of action model holds well for this system and the reactions are diffusion limited. This confirms that both static and dynamic processes are partly playing a role in quenching[16,19]. All the determined values, i.e. K_{SV}^{0} , D,R′,4πN′DR′ are collated in TABLE 2.

Further, from figure 4 it is observed that there is a regular increase in quenching with solvent polarity i.e. dielectric constant. It is worth to note that the dielectric constant of the mixed solvent is in principle determined as an average, but fluorescence quenching is determined locally. This may be attributed to the fact that the solvent molecule composition around the solute is different from that of the bulk in different solvent mixtures[1,20]. This effect of dielectric constant suggests the charge transfer in the excited state. High value of K_{SV} in acetonitrile compared to dioxane may also be explained by the greater charge transfer of the excited complex in the polar solvent[12].

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) Sphere of action model holds good for this system indicating both static and dynamic components partly play a role in quenching, (3) The increase in the value of K_{SV} with respect to ε indicates the charge transfer character in the excited complex.

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REFERENCES

[1] P.K.Behera, A K.Mishra; J.Photochem.Photobiol. A., **71**, 115 **(1993)**.
[2] P.K.Behera, T.Mukherjee, A.K.Mishra; J.Lumin.,

65, 131 **(1995)**.

[3] J.Sujatha, A.K.Mishra; J.Lumin., **75**, 135 **(1997)**.

[4] T.P.Giraddi, J.S.Kadadevaramath, G.H.Malimath, G.C.Chikkur; Appl.Rad.Isot., **47**, 461 **(1996)**.

[5] H.M.Suresh Kumar, M.N.Ravikanth, R.S. Kunabenchi, S.M.Hanagodimath, J.S.Biradar; Ind. J.Pure Appl.Phys., **40**, 466 **(2002)**.

[6] T.P.Giraddi, J.S.Kadadevaramath, G.H.Malimath, G.C.Chikkur; Ind.J.Pure Appl.Phys., **34**, 244 **(1996)**.

[7] H.M.Suresh Kumar, R.S.Kunabenchi, J.S.Biradar, N.N.Math, J.S.Kadadevarmath, S.R.Inamdar; J. Lumin., **116**, 35 **(2006)**.

[8] S.V.Nishti, R.S.Kunabenchi, H.M.Suresh Kumar, S.M.Hanagodimath, J.S.Biradar; Indian J.Phys., **81**, 53 **(2007)**.

[9] S.V.Nishti, R .S.Kunabenchi, O.V.Mangalgi, H.M. Suresh Kumar, S.M.Praveen, J.S.Biradar; Macromolecules: An Indian Journal, **3(4)**, 163 **(2007)**.

[10] R.Roy, S.Mukherjee; Chem.Phys.Lett., **140**, 210 **(1987)**.

[11] K.K.Rohtagi-Mukherjee (Ed); 'Fundamentals of Photochemistry'.Wiley Eastern Limited, New Delhi **(1986)**.

[12] J.R.Lackowicz; 'Principles of fluorescence spectroscopy', Plenum Press, New York **(1983)**.

[13] J.M.Frank, S.J.Wawilow; Z.Physik., **69**, 100 **(1931)**.

[14] J.S.Kadadevarmath, T.P.Giraddi, G.C.Chikkur; J.Photosci., **4**, 105 **(1998)**.

[15] T.L.Namzek, W.R.Ware; J.Chem.Phys., **62**, 477 **(1975)**.

[16] J.C.Andre, M.Niclauese, W.R.Ware; Chem.Phys., **28**, 371 **(1978)**.

[17] J.Keizer; Chem.Rev., **87**, 167 **(1987)**.

[18] G.C.Joshi, R.Bhatnagar, S.Doraiswamy, N. Periasamy; J.Phys.Chem., **94**, 2908 **(1990)**.

[19] H.Zeng, G.Durocher; J.Lumin., **63**, 75 **(1995)**.

[20] C.C.Leiten, K.Razi Naqvi; Chem.Phys.Lett., **4**, 35 **(1969)**.