Steady state and time resolved methods of fluorescence quenching of three coumarin dyes using S-V plots


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Studies of steady state and time resolved methods of fluorescence quenching of excitation energy of three coumarin dyes by aniline were carried out in toluene solvent only to understand the role of diffusion in the quenching mechanism. The coumarin dyes (dye1, dye2 and dye3) were excited directly by UV radiation of 350 nm and probabilities of quenching per encounter \( p \) (or \( p' \)) were determined in the toluene solvent. Further, the activation energy \( E_a \) (or \( E'_a \)) of quenching was determined using the literature values of activation energy of diffusion \( E_d \) and the experimentally determined values of \( p \) (or \( p' \)). Magnitudes of \( p \) (or \( p' \)) as well as the values of \( E_a \) (or \( E'_a \)) suggest that the quenching reaction is predominantly controlled by material diffusion.

Keywords: Fluorescence quenching, Coumarin dyes, Material diffusion, Activation process static, Dynamic quenching

1 Introduction

The fluorescence quenching of aromatic hydrocarbons in solvents by various quenchers like carbon tetrachloride, bromobenzene, aniline, halide ions, ethylthiothiocarbonate, metal ions etc. has been a subject of continued investigation for the last couple of decades1-18. Our study is mainly to understand the nature of bimolecular reactions and has not only been of importance in physical sciences but also in chemical, biological and medical sciences. The fluorescence yield in bimolecular liquid systems is hindered due to several mechanisms such as static and dynamic quenching, excimer and exciplex formation, charge transfer processes, etc. One of the well known experimental techniques used to study the role of fluorescence quenching is to determine the quenching rate parameter using Stern-Volmer (S-V) plots9-13. If the quenching mechanism is mainly due to dynamic process, then it will be largely due to diffusion in which diffusion rate parameter \( k_d \) equals the quenching rate parameter \( k_f (=K_{SV}/\tau_0) \) where \( K_{SV} \) is the slope of the linear S-V plot and \( \tau_0 \) is the decay time of the solute in the absence of the quencher. On the other hand, if the experimental results do not simulate with the simple linear Stern-Volmer relationship, then it may be due to one of the above processes other than or along with diffusion process.

Families of coumarin dyes are applicable in different fields9-11 of science and technology. They exhibit strong fluorescence in the UV and VIS region that make them suitable to use as a colorants, laser dye and as non-linear 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 have been a subject of considerable interest in numerous fields. Due to the interesting properties associated with coumarins, we undertook the fluorescence quenching studies of newly synthesized coumarin dyes. In the present study, we have used both steady state and time resolved methods experimental set-up to investigate quenching of three coumarin dyes by aniline in toluene solvent to understand the nature of quenching mechanism involved in these systems.

2 Experimental Details

The coumarin dyes, namely Dye-1 (5,6-benzo-4-azidomethyl coumarin), Dye-2 (7,8-benzo-4-azidomethyl coumarin) and Dye-3 (6-methoxy-4-azidomethyl coumarin) were synthesized using standard methods19-21. Molecular structures of all the
three coumarin dyes (solutes) are as shown in Fig. 1. Fluorescence emission spectra of all the three coumarin dyes (solutes) are as shown in Fig. 2. HPLC grade solvent (S D Fine Chemicals Ltd) were used without further purification to prepare the solution for each solute. The quencher aniline was double distilled and tested for its purity before use. The solutions were prepared keeping the fixed concentration of all three solutes (1×10^{-4} M/L) and varying quencher concentration (0.00-0.10 M) in all the cases. 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 solutes were excited at 350 nm and the fluorescence spectrums were recorded. The maximum fluorescence emission corresponds to 440 nm for all the solutes. Fluorescence lifetimes $\tau$ (with the quencher) and $\tau_0$ (without the quencher) of the solutes were measured using Photophysics model of TCSPC nanosecond fluorescence spectrometer available at RSIC, I.I.T Mumbai, India. First, the fluorescence intensity $I_0$ or $\tau_0$ was measured without the quencher and then the fluorescence intensity $I$ or $\tau$ was measured at different quencher concentrations at a fixed solute concentration. The experimental values are reproducible within 5% of the experimental error.

### 3 Theory

Quenching process is one, which competes with the spontaneous emission process and thereby shortens the lifetime of the emitting molecule. Basically these
quenching reactions are energy transfer or electron transfer processes. Under steady illumination, the rate of formation of an excited molecule \([A^*]\) is equal to its rates of deactivation and the concentration \([A^*]\) remains constant.

\[
\frac{d[A^*]}{dt} = 0 \quad \ldots(1)
\]

The concentration of \(A^*\) in the absence of any bimolecular step is given as:

\[
[A^*] = \frac{I_a}{k_f + k_{IC} + k_{ISC}} = \frac{I_a}{k_f + \sum k_i} \quad \ldots(2)
\]

where \(I_a\) is the rate of formation of the activated molecule \(k_f\) the rate constant for fluorescence and \(\sum k_i\) is the sum of rate constants for all the unimolecular deactivation steps such as internal conversion \(k_{IC}\) and intersystem crossing \(k_{ISC}\) which originate from this state.

If another molecule \(Q\) is added to the solution which quenches the fluorescence by bimolecular quenching step, then:

\[
A^* + Q \rightarrow A + Q, \quad \text{Rate} = k_q[A^*][Q] \quad \ldots(3)
\]

then concentration of the fluorescor \([A^*]\) in the presence of the quencher is given as

\[
[A^*] = \frac{I_a}{k_f + \sum k_i + k_q}[Q] \quad \ldots(4)
\]

If \([A^*]^0\) and \([A^*]\) are fluorescor concentrations in the absence and presence of the quencher, the respective quantum yields are:

\[
I_0 = \frac{k_f[A^*]^0}{I_a} = \frac{k_f}{(k_f + \sum k_i)} \quad \ldots(5)
\]

\[
I = \frac{k_f[A^*]}{I_a} = \frac{k_f}{(k_f + \sum k_i + k_q[Q])} \quad \ldots(6)
\]

then the ratio of the two yields gives:

\[
\frac{I_0}{I} = 1 + k_q \tau_0[Q] \quad \ldots(7)
\]

where \(\tau_0 = 1/(k_f + \sum k_i)\) and \(k_q\) is quenching rate parameter. This equation is known as the Stern-Volmer equation. It can also be shown that \(S-V\) equation for transient state is:

\[
\frac{\tau_0}{\tau} = 1 + k_q \tau_0[Q] \quad \ldots(8)
\]

The quenching rate parameter \(k_q\) (or \(k'_q\)) can be determined experimentally by measuring the fluorescence intensity (or fluorescence lifetime) of the solute \(I\) (or \(\tau\)) and \(I_0\) (or \(\tau_0\)) with or without the addition of quencher. In fact, the slope of the Stern-Volmer plot \(\tau_0/\tau\) or \(I_0/I\) against \(Q\) gives the product \(k_q\tau_0\) (or \(k'_q\tau_0\)) where \(\tau_0\) is the fluorescence lifetime of the solute in the absence of the quencher.

The phenomenon of quenching is understood due to short-range collision between the excited solute molecules and the quencher molecules. If close collision between the excited solute molecules and the quencher should occurs, the two molecules should occupy the contiguous positions in the liquid and separate due to diffusion after the collisional encounter. In liquids, the molecules before separating under the action of diffusion, will undergo numerous repeated mutual collisions of which each series represent an encounter. The probability of quenching per encounter is represented by \(p\) (or \(p'\)) and it is always less than unity for the quenching process, the frequency of encounters by \(k_d\). The quenching rate parameter \(k_q\) (or \(k'_q\)) is then represented by the product of \(k_d\) and \(p\) (or \(p'\)) as:

\[
k_q = k_d p \quad \ldots(9)
\]

The value of \(k_d\) can be calculated from the theoretical expression giving the bimolecular reactions controlled by material diffusion. Comparison between them makes it possible to estimate the extent to which material diffusion controls inhibition, and also, the efficiency of the intimate mechanism of quenching. The rate constant \(k_d\) of the bimolecular reactions controlled by material diffusion \([p \text{ or } p'] = 1\) can be calculated from the following equation.

\[
k_d = 4\pi N'DR \left[ 1 + \frac{R}{(2D\tau_0)^{1/2}} \right] \quad \ldots(10)
\]

where \(N'\) is Avogadro's number per milimole and \(D\) and \(R\) represent the sum of the diffusion coefficients \((D_f + D_Q)\) and the sum of the molecular radii \((R_f, R_Q)\)
of the solute and quencher molecules, respectively. \( \tau_0 \) is the fluorescence lifetime of the solute in the absence of the quencher.

4 Results and Discussion

The Stern-Volmer (S-V) plots of \( \tau_0/\tau \) against \([Q]\) and \( I_0/I \) against \([Q]\) for all the solutes in toluene solvents are shown in Figs 3 and 4, respectively. The solid lines are least square fit lines and the solid circles show the experimental points. It is observed from Figs 3 and 4 that the S-V plots for all the solutes in toluene are linear with intercept nearly equal to unity. This indicates that the phenomenon of fluorescence quenching both in steady state and transient methods, follow the Stern-Volmer relation.

Using least square fit method, the slopes of the Figs 3 and 4 give dynamic quenching constants \( (K_{SV} = k_q/\tau_0, \text{ steady state and } K'_{SV} = k'_q \tau_0) \). The transient state taking Eqs. 7 and 8) were determined for all the solutes in toluene solvent. The quenching rate parameters, \( k_q = K_{SV}/\tau_0 \) and \( k'_q = K'_{SV}/\tau_0 \) were determined using the experimentally determined value of \( \tau_0 \). The values of \( K_{SV}, K'_{SV}, k_q \) and \( k'_q \) are presented in Table 1 for all the solutes.

In order to estimate the probability of quenching per encounter \( p (=k_q/k_d) \) represented by Eq. (9), it is necessary to determine \( k_d \) (diffusion rate parameter). The diffusion rate parameter \( k_d \) of the bimolecular quenching reactions controlled by material diffusion can be calculated by Eq. (10), using the values of sum of the diffusion coefficients \( D (= D_Y + D_Q) \) and the sum of the molecular radii \( R (= R_Y + R_Q) \) of the solute and quencher, respectively.

\[
D = \frac{kT}{a\pi R} \quad \ldots (11)
\]

The diffusion coefficients of the solute \( D_Y \) and the quencher \( D_Q \) can be calculated using Stoke’s-Einstein Eq. (11). In Eq. (11), Stokes’-Einstein number \( ^{13,15} a = 3 \) for quencher, and \( a = 6 \) for the solute considered, since the radii of the solutes have larger size than that of solvent molecules and the radius of the quencher (aniline) molecule is approximately close to the radius of solvent molecules. The radii of the solute \( R_Y \) and the quencher \( R_Q \) were calculated as suggested by Edward\textsuperscript{22} and these values are given at the bottom of the Table 1. Using the literature value of the viscosity of the toluene solvent\textsuperscript{13,15} and calculated values of radii \( R_Y \) and \( R_Q \), the diffusion coefficients \( D_Y \) and \( D_Q \) were determined according to Eq. (11). In this case, the viscosity of the solute and the quencher was taken as that of viscosity of the medium since their concentrations are very small. The values \( D_Y \) and \( D_Q \) are given in the Table 1 for all the solutes. Thus, using the values of \( D \) and \( R \), the values of \( k_d \) were determined according to Eq. (10) as given in Table 2.

Further, the probability of quenching per encounter \( p (=k_q/k_d) \) (steady state) and \( p' (=k'_q/k_d) \) (transient State) were determined according to Eq. 9 using the experimentally determined values of \( k_q \) (and \( k'_q \)) and the theoretically estimated values of \( k_d \) for all the solutes in toluene and these values of \( p \) and \( p' \) are given in the Table 2. From Table 1, it is observed from both steady and transient methods that the values of \( K_{SV} \) and \( K'_{SV} \), \( k_q \) and \( k'_q \) are comparable in all the solutes. That is the results obtained by two experimental techniques correlate each other. Therefore, it may be inferred that phenomenon of quenching is dynamic in nature that is quenching of
excitation energy of solute molecules is only due to close collision between the solute and the quencher molecules. Also from the Table 2 it is found that the values of $p$ and $p'$ are less than unity in all the solutes, indicating that the probability of quenching per encounter is always less than unity a fact hitherto observed by others. This fact may indicate that the quenching reaction may also govern by activation process other than the material diffusion.

In liquid systems, in order to see the role of activation process, activation energies $E_a$ (for diffusion) and $E_q$ (for quenching reaction) are needed since probability of quenching per encounter $p$ (or $p'$) is related to activation energies according to Eq. 12 & 14 [13 & 15].

$$p = \frac{Pe^{-\frac{E_a}{RT}}}{Pe^{-\frac{E_d}{RT}} + Ce^{-\frac{E_q}{RT}}} \quad \cdots(12)$$

$$1/p = [1 + e^{(E_a-E_d)/RT}]$$

$$E_a = E_d + RT \ln\left(\frac{1}{p'} - 1\right) \quad \cdots(13)$$

$$p' = \frac{Pe^{-\frac{E_a}{RT}}}{Pe^{-\frac{E_d}{RT}} + Ce^{-\frac{E_q}{RT}}} \quad \cdots(14)$$

$$E_q = E_d + RT \ln\left(\frac{1}{p} - 1\right)$$

$$1/p = [1 + e^{(E_q-E_d)/RT}]$$

$$i.e. E_a = E_d + RT \ln\left(\frac{1}{p'} - 1\right) \quad \cdots(15)$$

$E_a$ is estimated according to Eq. 13 & 15 by using experimentally determined values of $p$ (or $p'$), the literature values of $E_d$ [13 & 15] and the gas constant $R$ and these values of $E_a$ (and $E_q$) are given in Table 2. From these tables, it is found that, the values of the activation energies for quenching reactions ($E_a$ and $E'_q$) are less than the activation energy for diffusion $E_d$ in all the solutes. This indicates that in the bimolecular quenching reactions influence of material diffusion is more significant than the activation process.

<table>
<thead>
<tr>
<th>Solutes</th>
<th>Solvent</th>
<th>$k_q \times 10^{-9}$ (M⁻¹s⁻¹)</th>
<th>$p$</th>
<th>$p'$</th>
<th>$E_d$ kcal/mole⁻¹</th>
<th>$E_a$ kcal/mole⁻¹</th>
<th>$E'_q$ kcal/mole⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye-1</td>
<td>toluene</td>
<td>2.479</td>
<td>0.934</td>
<td>0.923</td>
<td>2.662</td>
<td>1.082</td>
<td>1.181</td>
</tr>
<tr>
<td>Dye-2</td>
<td>toluene</td>
<td>2.380</td>
<td>0.908</td>
<td>0.715</td>
<td>2.662</td>
<td>1.297</td>
<td>2.114</td>
</tr>
<tr>
<td>Dye-3</td>
<td>toluene</td>
<td>2.591</td>
<td>0.757</td>
<td>0.570</td>
<td>2.662</td>
<td>1.985</td>
<td>2.494</td>
</tr>
</tbody>
</table>

5 Conclusions

From the above discussion, it is very interestingly investigated from both steady and transient methods that the values of $K_{SV}$ and $K'_{SV}$ (Stern-Volmer constant), $k_q$ and $k'_q$ (quenching rate parameter) are comparable in all the solutes. That is the results obtained by two experimental techniques correlate each other. As the S-V plots are linear, the phenomenon of quenching is dynamic in nature that is quenching of excitation energy of solute molecules is only due to close collision between the solute and the quencher molecules. Further, the probabilities of
quenching per encounter ($p$ and $p'$) are less than unity in all the solutes. This fact may indicate that the quenching reaction may also govern by activation process other than the material diffusion. Estimated values of activation energies for quenching reactions ($E_a$ and $E'_a$) are found to be less than the activation energy for diffusion $E_d$ in all the solutes. This suggests that the bimolecular quenching reactions are predominantly influenced by material diffusion than the activation process.

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