Photochemistry in microemulsions: Compartmentalization of fluorophore and quenchers

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The fluorescence quenching of 2-naphthol (2-ROH) by some oil-soluble (CCl4) and water-soluble (copper ion, methyl viologen, methylene bis-(γ-picolinium bromide) ) quenchers in anionic and cationic microemulsions has been investigated. Both linear and nonlinear Stern-Volmer (SV) behaviour have been encountered for different solvent systems. Both dynamic and transient quenching models have been found to fit the experimental data. "Quenching Sphere of Action" model has been proposed for non-linearity of SV plots and the probable number of quenchers in the sphere has been determined from Poission distribution. The differential behaviour of 2-ROH for different quenchers has been attributed to compartmentalization of the quenchers at various solubilization pockets of microemulsions.

2-Naphthol (2-ROH) is a weak acid in the ground state (pK = 9.49) (ref.1). Being a photon initiated acid, it readily loses a proton at neutral pH (pK* = 2.8) in the excited state. The proton transfer from 2-ROH to the solvent requires a cluster formation involving at least 4±1 water molecules and thus is highly sensitive to its local environment. But in a rigid system like micelle, the deprotonation rate is decreased and only one band corresponding to the 2-ROH* species is obtained. In the excited state, 2-ROH dissociates to an extent of 33% in aqueous medium. However, in anionic microemulsions, the dissociation of 2-ROH is suppressed totally though the suppression takes place partially in the case of 1-naphthol. The total suppression of dissociation of 2-ROH* in microemulsions prompted the authors to choose it as the fluorophore to investigate the fluorescence quenching by some oil and water-soluble quenchers.

Materials and Methods
Sodium lauryl sulphate (Sigma) was crystallized twice from alcohol. Cetyltrimethylammonium bromide (Sisco-Chem) was crystallized twice from methanol. Isobutanol (Merck) was dried over K2CO3 and then distilled twice before use. Hexane was distilled twice in a fractionating column. Triply distilled water was used throughout the study. 2-Naphthol (Sisco Chem) was purified by subliming twice after crystallization from alcohol. Carbon tetrachloride (Merck) was purified by Takahashi method. Copper sulphate was crystallized twice from water. Methyl viologen was obtained from Sigma and was used as such. The microemulsions were prepared by stirring the various components in appropriate amount with a mechanical stirrer. The order of mixing of the components of microemulsion has no effect on the stability of the microemulsion. Solution of CuSO4 (0-0.2M) was taken instead of water for constructing the phase diagram.

Synthesis of methylene bis-(γ-picolinium bromide)
A mixture of -γ-picoline (0.93 g, 0.01 mol) and dibromomethane (0.87 g, 0.05 mol) was refluxed on a water bath for 6 h. A white solid separated which was washed with diethyl ether. The salt was crystallized thrice from water-alcohol mixture and was kept in the desiccator. Yield = 95 %, m.p. = 264-265°C (decomposed at 260°C). Found : C, 43.29; H, 4.40; N, 7.75; Br, 44.44 % ; methylene bis-(γ-picolinium bromide) requires C, 43.33; H, 4.44; N, 7.78; Br, 44.45%.

Phase diagram
The pseudo-ternary phase diagram constructed by visual titration of the mixtures of NaLS-
Table 1 - Formulation of A, B, C for the NaLS and D, E for CTAB microemulsions.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Isobutanol (w/w)</th>
<th>Hexane (w/w)</th>
<th>Water (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaLS</td>
<td>A 11.7</td>
<td>28.1</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>B 11.9</td>
<td>28.6</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>C 1.8</td>
<td>4.2</td>
<td>0.9</td>
</tr>
<tr>
<td>CTAB</td>
<td>D 11.7</td>
<td>28.1</td>
<td>44.6</td>
</tr>
<tr>
<td></td>
<td>E 11.7</td>
<td>28.2</td>
<td>5.3</td>
</tr>
</tbody>
</table>

isobutanol-hexane-water and CTAB-isobutanol-hexane-water have been described earlier. For the NaLS surfactant system, addition of CuSO₄ shrinks the Winsor IV domain of the phase diagram, where most of it concentrates at the W apex and there is no isotropic medium in the oil-rich region. For the CTAB surfactant system, no isotropic region is obtained in the phase diagram in the presence of CuSO₄. In the present study the formulations for NaLS and CTAB microemulsions are given in Table 1.

Spectral measurement

Absorption and fluorescence spectra were recorded by UV-Vis Shimadzu 160A spectrophotometer and Shimadzu RF-5000 spectrofluorimeter respectively. Narrow excitation and emission slit widths (half band width = 1.5 and 3nm) were chosen. The concentration of 2-ROH was maintained at 1x10⁻⁴ M. The quencher concentrations were varied in the range 0-0.2 M for CCl₄ and Cu++, 0-0.02 M for methyl viologen (MV++) and; methylene bis -(γ-picolinium) dication (BPM⁺⁺).

The fluorescence intensities of 2-ROH (λₑₓ = 330 nm, λₑₘ = 352, 416 nm in water; λₑₓ = 330 nm, λₑₘ = 346 nm in hexane; λₑₓ = 330 nm, λₑₘ = 354 nm in MEs) were monitored at various concentrations of the quenchers.

Results and Discussion

The absorption spectra of 2-ROH in all the environments in the absence and presence of the quencher show no observable change in spectral shape and maxima which indicate the absence of ground state complexation of the fluorophore with the quencher in the experimental condition. In aqueous medium the emission peak due to both the neutral (ROH⁺, λₑₘ = 352 nm) and dissociated (RO⁻*,λₑₘ = 416 nm) species are observed whereas

in hexane and microemulsion media the emission peaks are only due to the neutral species.

Stern-Volmer (SV) plots

The I/I values of 2-ROH in all the environments have been correlated with the quencher concentration, [Q], by using the Stern-Volmer (SV) equation (Eq.1)

\[ (I/I_0) - 1 = K_{SV} [Q] \]

where \( I_0 \) and \( I \) are the fluorescence intensities of the fluorophores in the absence and presence of the quencher respectively, \( K_{SV} \) is the SV constant.

Some representative SV plots are presented in Fig. 1. The SV equation is obeyed for the fluorophore when CCl₄ is used as the quencher in hexane and in the microemulsions. In the presence of Cu++, the SV equation is obeyed up to a limiting concentration of copper ion, the limiting concentration being different for aqueous and microemulsion media. Beyond this limiting concentration, deviation is observed. When BPM⁺⁺ is used as the quencher, the SV equation is obeyed in the NaLS microemulsions whereas in CTAB...
Scheme 1- Probable localization sites of the fluorophore and quenchers in NaLS/CTAB-isobutanol-hexane-water microemulsion.

When compared to W/O ME, the amount of oil is less in O/W ME and thus the effective concentration of CCl₄ in oil phase increases.

(b) The $K_{SV}$ values of 2-ROH in the presence of BPM⁺ in both the water-rich and oil-rich regions do not differ much and thus are independent of the medium. It has been reported that the counterions with divalent charges, for small charge separation, move radially over the charged micelle surface. Due to electrostatic interaction, the quenchers would prefer the anionic interface in NaLS ME. Here, the quenching phenomenon may be due to the collision of the fluorophore with the quencher attached to the microemulsion interface. In CTAB ME, the dipositive BPM⁺ resides in the free water region far away from the cationic interface where no fluorophore is available. Hence no quenching is observed in CTAB microemulsion.

Deviations in the SV plots

There may be two possible reasons for the deviation in the SV plots: (i) static quenching due to ground state complex formation and (ii) a transient component in the dynamic quenching due to the presence of the quencher within a quenching sphere of action. No change is observed in the longest wavelength region of the absorption spectra in the presence of quencher. Thus deviation due to ground state complex formation is ruled out. Hence quenching is due to the presence of a transient component in dynamic quenching. According to this model, the fluorophore assumes a sphere of action with varying dimensions due to change in quencher. Within the sphere of action the probability of quenching is assumed to be one and outside the sphere it is zero. Once the quencher comes into the sphere, quenching occurs before fluorophore and quencher diffuses apart. The modified SV equation is represented as given in Eq. (2)

\[
I_0/I = \exp\left( -K_T [Q] \right) 
\]

Equation (2) can be written as Eq. (3)

\[
\ln (I_0/I) = K_T [Q] = vN [Q] 
\]

where $K_T$ is the transient quenching constant, $v$ is the volume of the transient quenching sphere, $N$ is the Avogadro's number.
The plots of \( \ln (I_0/I) \) versus \([Q]\) are found to be linear \((r \geq 0.99)\) passing through the origin for 2-ROH, in water when BPM\(^{++}\) is the quencher; in water and the microemulsions when MV\(^{++}\) is the quencher; in water and O/W NaLS ME after a limiting concentration for Cu\(^{++}\) as the quencher.

Some representative plots of \( \ln (I_0/I) \) versus \([Q]\) are given in Fig. 2. For all the plots which obey the equation \( \ln (I_0/I) = -K_T [Q] \) the \( K_T \) values have been calculated, the radii of the quenching sphere of action \((\text{taking } K_T = vN)\) have been determined and are given in Tables 3 and 4.

(a) Cu\(^{++}\) as the quencher - Thulborn and Sawyer\(^1^9\) have proposed that the positive and negative deviations in the SV plots are due respectively to the similar and different environments of the fluorophore and quencher. In O/W-NaLS ME, the probable localization site of copper ion is the interface due to the Na\(^+\)/Cu\(^{++}\) exchange or the aqueous phase\(^6^2^0\). In aqueous medium the positive deviation in the SV plot, after a threshold concentration of the quencher indicates the similar environment of the fluorophore and quencher. For the negative deviation in the SV plot, different residential sites for the fluorophore and quencher have been reported.

(b) BPM\(^{++}\) as the quencher - The positive deviation in the SV plots in the aqueous medium is due to the similar environment of the fluorophore and the quencher. The higher \( K_T \) value for 2-RO\(^-\) as compared to 2-ROH\(^*\) may be attributed to the electrostatic effect of the fluorophore and quencher.

(c) MV\(^{++}\) as the quencher - The positive deviation in the SV plot in the NaLS MEs indicates the similar environment for both MV\(^{++}\) and 2-ROH. The appreciable quenching in CTAB ME (Fig. 2b) may be due to the percolation of MV\(^{++}\) into the microemulsion through the cationic interface. As in the case of BPM\(^{++}\), the higher \( K_T \) value for 2-RO\(^-\) as compared to the 2-ROH\(^*\) in aqueous medium (Fig. 2a) is due to the electrostatic effect. The order of decrease in \( K_T \) values of 2-ROH is found to be, CTAB (W/O) > CTAB (O/W) > water > NaLS (W/O) > NaLS (O/W). In NaLS ME, the effective concentration of methyl viologen dication is very high at the anionic interface. Thus there is no appreciable difference in the quenching efficiency in both the W/O and O/W-NaLS MEs. The nature of compartmentalization of the fluorophore and the quencher in the microemulsion depends on the charge of the surfactants. The CTAB ME has a cationic interface where the fluorophores are expected to be localized.
Table 5 - Probable number of quenchers in the quenching sphere of action at the moment of quenching.

<table>
<thead>
<tr>
<th>Quencher</th>
<th>Fluorophore</th>
<th>Medium</th>
<th>[Q] in mol dm$^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.20</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>2-ROH</td>
<td>Water</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>Water</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>O/W NaLS</td>
<td>&lt;1</td>
</tr>
<tr>
<td>BPM$^{2-}$</td>
<td>2-ROH</td>
<td>Water</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>Water</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>O/W NaLS</td>
<td>6</td>
</tr>
<tr>
<td>MV$^{+}$</td>
<td>2-ROH</td>
<td>Water</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>Water</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>O/W NaLS</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2-ROH</td>
<td>W/O NaLS</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>O/W CTAB</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td></td>
<td>W/O CTAB</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

Fluorophore is restricted whereas in NaLS ME the mobility of the quencher is restricted. In view of the very high concentration of the quencher as compared to the fluorophore in the ME, the quenchers are expected to partition to the interfacial region much more than the fluorophore. In view of this the $K_T$ values of 2-ROH in NaLS ME is less than that in CTAB ME. The higher $K_T$ value in the W/O-CTAB ME as compared to the O/W-CTAB ME may be attributed to the concentration effect.

Probability distribution of quencher in the quenching sphere

Assuming that the association of the quencher in forming a quenching sphere obeys Poisson statistics, the probability distribution ($P_i$) that a fluorophore is bound with $i$ number of the quenchers is given by

$$P_i = (m^i / i!) \exp(-m) \quad \ldots(4)$$

Here, $m(ROH) = [Q]_b/[ROH]_t$, where $[ROH]_t$ denotes the total concentration of naphthol and $[Q]_b$ is the concentration of quencher bound to the fluorophore.

The values of $m$ has been calculated from the equation $m = K_T [Q]$, where $K_T$ is the transient quenching constant. The values of $P_i$ are obtained for different $i$ values (1, 2, 3, ...) of the quencher at any $[Q]$ and then theoretical plots of $P_i$ versus the number of quencher molecules incorporated in the sphere at various quencher concentrations are made. Representative plots for 2-ROH in aqueous...
medium are shown in Fig. 3. The maximum of each curve is taken as the number of quenchers incorporated in the sphere at a specific $[Q]_t$.

The analysis of the data in Table 5 reveals the following salient features:

(i) In CTAB microemulsion the space can accommodate a larger number of quenchers than that in NaLS microemulsion; (ii) the number is larger in case of W/O-CTAB ME than in case of O/W-CTAB ME at $[Q] = 0.05M$; (iii) in NaLS surfactant system, O/W and W/O microemulsions have almost same number of quencher molecules; (iv) methyl viologen dications are found to be relatively more in number in the quenching sphere than BPM$^{2+}$ in water medium; and (v) the number of quenchers in the quenching sphere in case of Cu$^{2+}$ is the least.

All these results may be ascribed to the electrostatic and hydrophobic interactions of the quenchers with surfactant.

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