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Fluorescence quenching of 2-aminofluorene by cetylpyridinium chloride, iodide ion and acrylamide in non-ionic micelles: Tweens

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Abstract

The fluorescence quenching of 2-aminofluorene by cetylpyridinium chloride (CPy), iodide ion (I^-) and acrylamide (AC) has been studied in different concentrations of Tween-20, Tween-40, Tween-60 and Tween-80 surfactants. The results show that CPy and I^- ion bind as well as get partitioned, whereas AC only gets partitioned. The high values of binding constants for CPy in comparison to I^- ion are due to the long hydrocarbon chain length. The binding and partitioning coefficients of CPy increase, whereas those of I^- ion decrease with the increase in the Tween number. The values of partitioning coefficient of AC also decrease with increase in the Tween number. On the other hand, quenching rate constants for each quencher in each Tween decrease in going from Tween-20 to Tween-80. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Fluorescence quenching; Tweens; 2-Aminofluorene; Non-ionic micelles

1. Introduction

Fluorescence quenching by neutral or charged species in micelles or lipids has been used extensively [1–13] to study the characteristic of these macromolecules, e.g., critical micelle concentration (CMC), aggregation number, location of the probe molecule and the penetration of water molecules into the micelles etc. [14–23]. Sawyer and Blatt group has made a lot of contribution in this field. They have used the fluorescence quenching of anthracene, 9-methylanthracence and *n*-(9-anthroxyl) fatty acids by dimethylaniline and I^- ions to study the association parameters of the quenchers and the location of the probe molecules in cetyltrimethylammonium bromide (CTAB), TritonX-100 (TX-100) and different lipid bilayers. The similar studies in Tweens or Brijs, which are also very useful detergents from academic and industrial points of view, are very scarce [2,24]. In the micellar medium, there are two factors that affect the mutual encounter of the probe and the quencher molecules. These are: (a) the partitioning of the fluorescent probe and the quenchers between the micellar and the aqueous phases and (b) the dynamic distribution of these species during the fluorescence quenching.

Recently, we have studied the photophysical properties of 2-aminofluorene (2-AF) in homogeneous [25] and non-ionic micelles (Tweens) [26]. The absorption and fluorescence spectra of 2-AF in aqueous medium and in different Tweens at 0.02 M concentration in aqueous medium are depicted in Fig. 1. These studies indicate that the fluorescence quantum yield and lifetime of 2-AF are very sensitive to the environments. The former in Tweens increases by a factor of 3 to 4.5, whereas the latter one by a factor of 3, when compared with these in water. Binding constants of 2-AF are quite large with Tweens. The large values of binding constants and observation of single exponential decay in the fluorescence clearly establish that 2-AF is completely solubilized and is present only at one site of the micelles. These studies also indicate that the hydrophobicity of these micelles also increases with the increase in the Tween number. Thus, 2-AF can be used as a suitable probe molecule to measure the association parameters of different quenchers with Tweens.

The present study involves the fluorescence quenching of 2-AF in different Tweens (Tween-20, 40, 60 and Tween-80) by three quenchers such as CPy, I^- and AC. As mentioned above, very little is known about Tweens, this study is aimed at finding the distribution coefficients of these quenchers among the aqueous and micellar phases, mechanism of quenching and permeability of water molecules towards the core of micelles. The chemical structure of each Tween is shown in Scheme 1.

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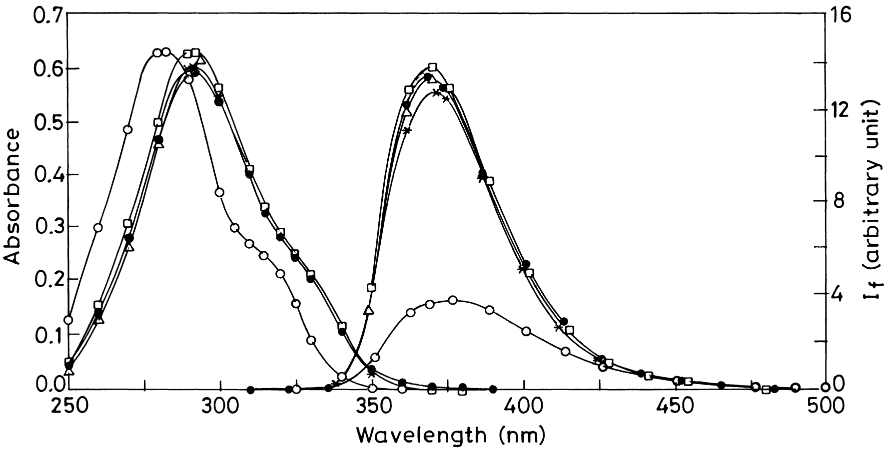
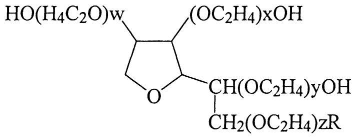


Fig. 1. Absorption and fluorescence spectra of [2-AF] in aqueous and 0.02 M aqueous surfactant solutions. 2-AF = 2×10^{-5} M. ○–○–○, water; ∗–∗–∗, Tween-20; □–□–□, Tween-40; △–△–△, Tween-60; ●–●–●, Tween-80.



Where w + x + y + z = 20

When $R = CH_3(CH_2)_{10}COO$ → TWEEN-20
$R = CH_3(CH_2)_{14}COO$ → TWEEN-40
$R = CH_3(CH_2)_{16}COO$ → TWEEN-60
$R = CH_3(CH_2)_7CH{=}CH(CH_2)_7COO$ → TWEEN-80

Scheme 1.

2. Materials and methods

2-AF was procured from Aldrich and was purified [25] by repeated crystallization from 95% (V/V) ethanol-water mixture. All the Tweens (Aldrich), CPy (Aldrich), NaI (E-Merck) and NaOH (BDH) were of high purity grade and were used as such. AC (Qualigens) was purified by recrystallization from benzene. Triply distilled water was used to prepare the aqueous micellar solutions. A small amount of NaOH was used to adjust the pH of the solutions as 8 ± 0.5. At this pH 2-AF behaves as neutral species.

The instruments used to record the absorption and the fluorescence spectra, to measure the lifetimes and the procedure used to carry out this study are the same as described in our earlier paper [1]. The concentration of 2-AF used was 1×10^{-5} M, for CPy it varied between 1–7.5×10^{-4} M, between 0.1 to 0.6 M for I^- ion and 0.005–0.05 M for AC. The surfactant concentrations were in the range of 0.006 to 0.009 M. Under these conditions, the average number of probe molecules even in the smallest concentration of surfactant (0.006 M) and largest aggregation number of Tween-80 (124) [2] does not exceed 0.2. If Poission statistics [28] is assumed, this corresponds to a probability of 0.016 of there being more than one probe molecule per micelle in this case. This ensures that self quenching by the probe molecule is negligible. 10^{-3} M $Na_2S_2O_3$ was used to prevent the oxidation of I^- ion.

3. Methodology

The type of association of quencher with the micelles has been evaluated by the quenching method as proposed by Blatt et al. [16] and brief summary is given below. The basic assumption involved in this procedure is that the quenching efficiency at a particular concentration of quencher is determined by the average number of quencher per micelle $\langle Q \rangle$. This assumption is independent of the quenching mechanism or the type of quencher distribution in the micelles. The concentration of the quencher in the micellar phase $[M]$, is given by

$$[Q]_M = \langle Q \rangle [M] \quad (1)$$

where $[M]$ is the concentration of the micelles, given by $[M] = ([\mathrm{Det}] - \mathrm{CMC})/N$, CMC the criticle micelle concentration, N the aggregation number and [Det] is the concentration of the surfactants as monomer. When the volume of the micellar phase is small compared to the total volume, the total quencher concentration $[Q]_T$ can be defined as

$$[Q]_T = [Q]_M + [Q]_A \quad (2)$$

where $[Q]_A$ is the concentration of the quencher in the aqueous phase. Further, when the quencher is partitioning and/or binding to the micelles, a generalized parameter, K_{eq} can be defined in terms of a partitioning coefficient, K_p, and a binding constant, K_b by [14]

$$K_{eq} = \frac{\langle Q \rangle}{[Q]_A}$$

$$\langle Q \rangle = K_{eq}[Q]_A = \left\{ V_m K_p + \frac{p K_b}{1 + K_b [Q]_A} \right\} [Q]_A \quad (3)$$

where V_m is the molar volume of the micellar phase, p is the number of binding sites on or within the micelle. Combining Eqs. (1)–(3), we get

$$[Q]_T = \langle Q\rangle[M] + [Q]_A \tag{4}$$

$$= \langle Q\rangle[M] + \left(\frac{\langle Q\rangle}{K_{eq}}\right) \tag{5}$$

The values of $\langle Q\rangle$ and $[Q]_A$ can be obtained from the plots of $[Q]_T$ versus $[M]$ at the same quenching efficiency for a series of values of F_0/F, where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively. The value of K_{eq} can be determined from the plot of $K_{eq} = \langle Q\rangle/[Q]_A$ versus $\langle Q\rangle$ known as Scatchard plot [27] and following information can be obtained.

Scatchard plot is linear (i.e., K_{eq} independent of $\langle Q\rangle$) if only partitioning of the quencher takes place. Thus, the partition constant can be related to K_{eq} as

$$K_p = \frac{K_{eq}}{V_M} \tag{6}$$

The value of K_p and p can also be calculated from the slope and intercept of the linear region of a plot of $\langle Q\rangle$ versus $[Q]_A$. Using Eq. (7) derived by Haigh et al. [14]

$$\langle Q\rangle = p + V_M K_p [Q]_A \tag{7}$$

Scatchard plot is linear with negative slope if there is a binding to a single class of independent binding sites. K_b and p can be evaluated from the slope and intercept, respectively.

If partitioning and binding are taking place simultaneously, plot of K_{eq} versus $\langle Q\rangle$ will exhibit a decreasing dependence of K_{eq} with increasing $\langle Q\rangle$ asymptoting to a constant value of $V_M K_p$. The values of K_b can be calculated from equation Eq. (8), obtained after rearranging equation Eq. (3)

$$K_b = \frac{(K_{eq} - V_M K_p)}{\{p - [Q]_A(K_{eq} - V_M K_p)\}} \tag{8}$$

Care should be taken that the values of K_{eq} used, should correspond to the same values of $[Q]_A$. Thus, the above mentioned methodology has been used to calculate the association parameters of each quencher with the respective micelles.

4. Results and discussion

4.1. Fluorescence quenching of 2-AF by CPy

F_0/F and/or τ_0/τ (where τ and τ_0 are the lifetimes of 2-AF in a given surfactant concentration with and without the quencher, respectively) for the quenching of 2-AF by CPy in Tween-20 was plotted against the total quencher concentration $[Q]_T$ according to the Stern–Volmer equation Eq. (9) in Fig. 2, where k_q is the second order quenching rate constant. The Stern–Volmer plots are non-linear

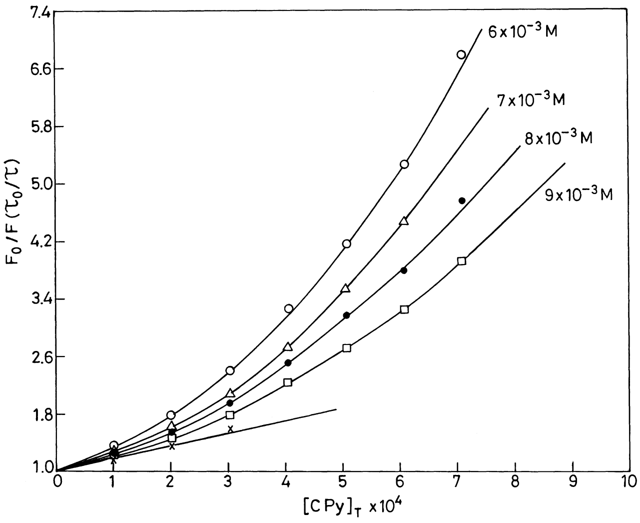


Fig. 2. Stern–Volmer plots for the quenching of 2-AF by CPy at different Tween-20 surfactant concentrations. The crosses indicate τ_0/τ vs. $[CPy]_T$ plot for 0.008 M Tween-20.

$$\frac{F_o}{F} = \frac{\tau_o}{\tau} = 1 + k_q\tau_o[Q]_T \tag{9}$$

after a certain concentration (above 8×10^{-5} M) of CPy. The Stern–Volmer plots for CPy quenching of 2-AF in other Tweens were also of similar type and are not shown. The excited singlet state lifetimes of 2-AF at a particular concentration of each Tween were measured in the absence and presence of CPy. In all the cases the fluorescence decay is a single exponential indicating that the fluorophore is present at only one equivalent site in the micelles. Further, the similarity of the lifetimes in different micelles and at different concentration of each micelles suggests that the location of the 2-AF in each micelle is nearly at the similar environments. The lifetimes of 2-AF decrease with increase in the quencher concentration. τ_o/τ versus quencher concentration $[Q]_T$ plot is linear (Fig. 2) in each surfactant at 0.008 M concentration, suggesting that the quenching is of dynamic in nature. The behaviour of the steady state and dynamic Stern–Volmer plots are similar, except at higher concentration of CPy. It is evident from the Fig. 2 that the fluorescence quenching efficiency of the fluorophore decreases with the increase in the concentration of Tweens. This behaviour is consistent with the fact that the effective quencher concentration decreases with the increase of surfactant concentration. A similar behaviour has also been observed in other Tweens as well as by other workers in other micelles and lipid bilayers [20]. The above observations clearly suggest that all the quencher molecules associate with the Tweens.

The nature of association of CPy with the Tweens has been determined with the help of a model suggested by Blatt and Sawyer [16] as described in Section 3. In the present case, to calculate micelle concentration the values of CMC for various Tweens are taken from earlier work and are 8×10^{-5}, 5×10^{-5}, 4.4×10^{-5} and 1.5×10^{-5} M for Tween-20, 40, 60 and Tween-80, respectively [2,26]. Similarly, the N used are 86, 92, 112, 124, respectively [2]. Fig. 3 shows the plot of CPy_T versus $(Tween\text{-}20)_T$ Eq. (4) in case of CPy quenching at the same quenching efficiency for a series of values with same F_o/F. $\langle Q \rangle$ and $[Q]_A$ were determined from the slope and intercept, respectively. The values so obtained were used in a Scatchard plot (i.e., $\langle Q \rangle / [Q]_A = K_{eq}$ versus $\langle Q \rangle$). Fig. 4 depicts such a plot for CPy quenching in Tween-20.

The Scatchard plots for 2-AF-CPy quenching in all the Tweens are non-linear and show a decreasing dependence of K_{eq} with increasing $\langle Q \rangle$, asymptotically approaching a constant value (Fig. 4). Since the fluorescence quenching observed in the present case is of dynamic in nature, it may be concluded that CPy partitions as well as binds to the Tween micelles. This behaviour can be described by Eq. (3). The asymptotic behaviour indicates that at this value of $\langle Q \rangle$ (i.e., at the asymptotic point), the binding sites are saturated and only the partitioning contributions remain. The values of $V_m K_p$ can also be obtained from both the plots of $\langle Q \rangle$ versus Q_A (Fig. 5) for 2-AF-CPy quenching in Tween-20 and Eq. (7) [18] and linear portion of K_{eq} versus $\langle Q \rangle$ plot (Fig. 4). The values of $V_m K_p$ calculated from both these methods agree well with each other.

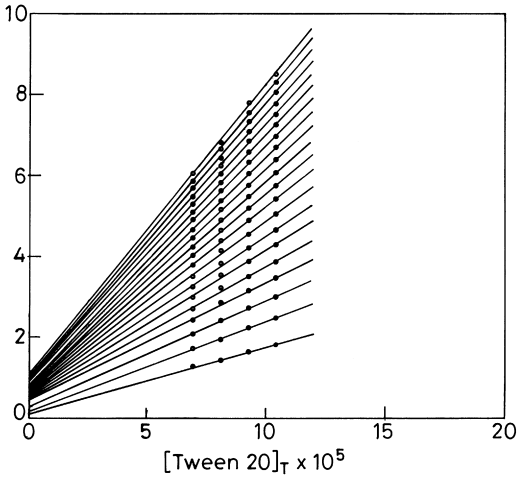


Fig. 3. Plot of $[CPy]_T$ vs. $[Tween\text{-}20]_T$ at the same quenching efficiency for a series of values of the F_o/F. $[2\text{-}AF] = 2 \times 10^{-5}$ M.

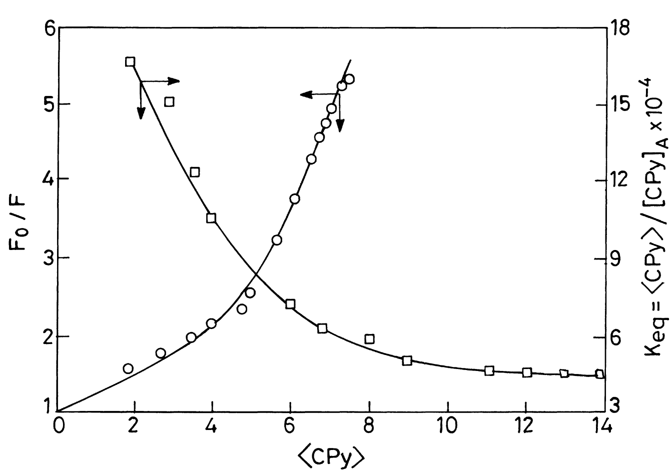


Fig. 4. $\langle CPy \rangle / [CPy]_A$ (obtained from Fig. 3, see text) vs. $\langle CPy \rangle$ plot with CPy as quencher in Tween-20 and F_o/F vs. $\langle CPy \rangle$ plot with CPy as quencher in Tween-20. $[2\text{-}AF] = 2 \times 10^{-5}$ M.

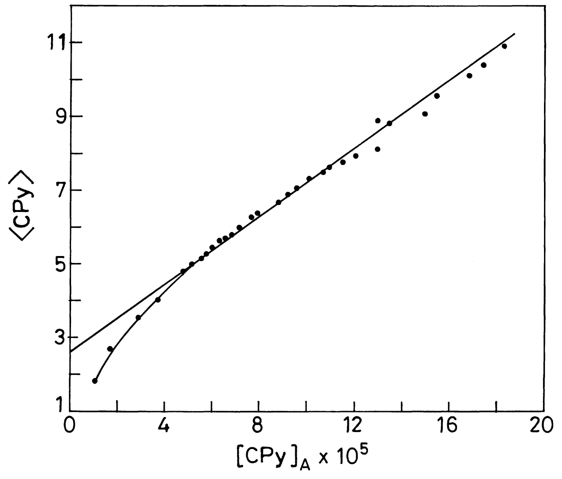


Fig. 5. Plot of $\langle CPy \rangle$ vs. $[CPy]_A$. $\langle CPy \rangle$ and $[CPy]_A$ are obtained from Fig. 3.

Since the molar volumes of these micelles are not available, these were calculated using the group additivity method [29–32]. These values are found to be 91, 100, 126 and 142 l mol^{-1} for Tween-20, Tween-40, Tween-60 and Tween-80, respectively. The accuracy of these methods can be judged from the following data, i.e., the molar volumes of SDS, CTAB and Triton X-100 calculated by the group additivity methods are found to be 15, 29 and 82 l mol^{-1} [35], respectively, whereas experimentally these values are found to be 15.3 [33], 28.6 [34] and 82 l mol^{-1} [35]. The agreement seems to be very good and thus one can rely on the results obtained by the additivity methods for the Tween micelles. The values of K_p obtained from the slope and molar volume of Tweens and p obtained from the intercept in case of $\langle Q \rangle$ versus $[Q]_A$ plot (Fig. 5) and K_p obtained from the linear portion of K_{eq} versus $\langle Q \rangle$ plot (Fig. 4) are compiled in Table 1. Under these conditions, the values of K_b can be calculated from Eq. (8). The values of K_b are very sensitive to the values of p and K_{eq}. The maximum error observed could be 30% in case of 2-AF-CPy quenching. The data compiled in Table 1 give the upper and lower limits of K_b.

The data of Table 1 show that both K_p and K_b of CPy with Tweens increase with increase in the Tween number. As it is well established that the hydrophobicity of the Tweens increases with increase in the Tween number [2,36,37], it is expected that the values of K_p and K_b of CPy will also increase with increase in Tween number, because CPy is having a long aliphatic hydrocarbon chain containing 16 carbon atoms with pyridinium ion at one end of the chain. The agreement between the values of p and K_b obtained by

Table 1
Different parameters obtained from the fluorescence quenching of 2-AF by CPy, I$^-$ ion and AC

Surfactant	p^a	$(K_p)^a$	$(K_b)^a$	$(K_p)^a$	τ_o (ns)^b	k_q (M^{-1} s^{-1})^b
Quencher: CPy			(10^5)			(10^9)
Tween-20	2.65 ± 0.4	500 ± 36	0.8–1.2	500	7.1	3.35
Tween-40	3.40 ± 0.1	540 ± 37	1.8–2.0	545	7.0	2.00
Tween-60	3.41 ± 0.1	491 ± 20	1.7–1.9	490	7.1	1.90
Tween-80	3.44 ± 0.1	570 ± 40	2.2–2.4	580	7.0	1.40
Quencher: I$^-$ ion			(10^2)			(10^5)
Tween-20	456 ± 6	313 ± 14	7.0–8.0	331	7.1	9.3
Tween-40	441 ± 7	200 ± 5	5.0–7.3	211	7.0	8.7
Tween-60	419 ± 10	152 ± 3	3.6–4.2	175	7.1	8.2
Tween-80	144 ± 3	109 ± 4	3.3–3.8	113	7.0	7.3
Quencher: AC						(10^7)
Tween-20		260 ± 15		260	7.1	1.75
Tween-40		170 ± 10		180	7.0	1.65
Tween-60		70 ± 4		70	7.1	1.55
Tween-80		82 ± 3		82	7.0	1.35

[a] From $\langle Q\rangle$ vs. $[Q]_A$ plot.
[b] From K_{eq} vs. $\langle Q\rangle$ plot.

this method and those found by Bhattacharyay et al. [2] is quite good.

The modified Stern–Volmer plot (i.e., F_o/F versus $\langle Q\rangle$ instead of $[Q]_T$) for 2-AF-CPy quenching is also non-linear after a certain value of $\langle Q\rangle$ (Fig. 4). The quenching rate constant (k_q) were calculated from the slope of the linear portion of the modified Stern–Volmer plots using the values of τ_o and V_m according to the Eq. (10)

$$\frac{F_o}{F} = 1 + k_q\tau_o[Q]_m = 1 + k_q\tau_o\frac{\langle Q\rangle}{V_m} \qquad (10)$$

where $[Q]_m$ is the concentration of the micellized quencher. The relevant data are listed in Table 1 and τ_o used were the average value of the lifetimes at three different concentrations of the surfactants. The values of k_q obtained for each micelle decrease with increase in Tween number and this trend is opposite to that noticed for the binding constant or the partitioning coefficients.

4.2. Fluorescence quenching of 2-AF by I$^-$ ion

The fluorescence quenching of 2-AF by I$^-$ ions was investigated in different concentration of each Tween (0.005–0.008 M). The concentration of I$^-$ ion used was in the range of 0.1 to 0.6 M. Becher [39] studied the effect of ionic strength on the micellar molecular weight and aggre-

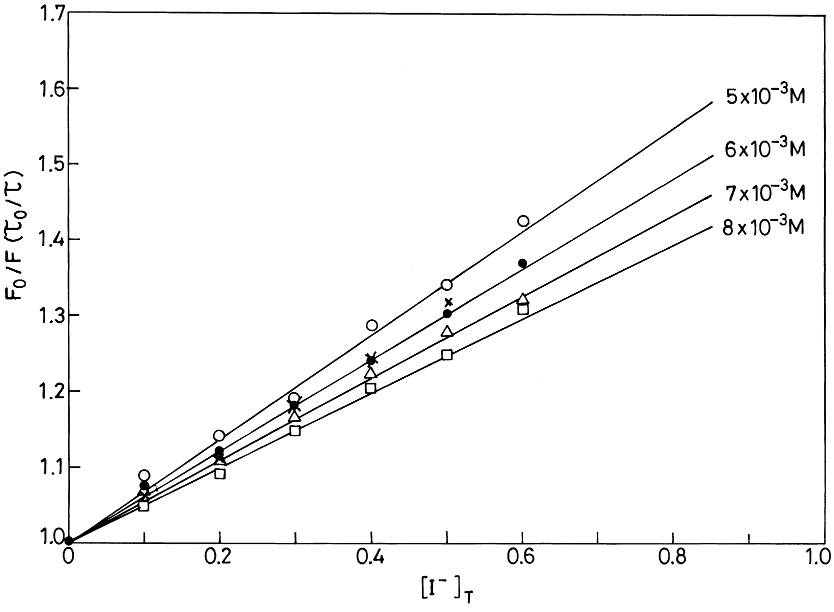


Fig. 6. Stern–Volmer plots for the quenching of 2-AF by I$^-$ ion at different Tween-20 surfactant concentrations. The crosses indicate τ_o/τ vs. [I$^-$] plot for 0.006 M Tween-20. [2-AF] = 2×10^{-5} M.

gation number of polyoxyethylated lauryl alcohols containing 8, 12, 18 and 23 molecules of ethylene oxide. It was observed that the effect was very small for the higher members of the series. Therefore, we can consider here that at high concentration of NaI (~0.6 M), the micellar molecular weight and aggregation number of Tweens (containing 20 ethylene oxide group) will not be changed.

F_0/F versus $[I^-]_T$ (Stern–Volmer plot), in case of Tween-20 is plotted as a representative one in Fig. 6. Unlike 2-AF-CPy quenching, these plots are linear for 2-AF-I^- combinations. The excited singlet state lifetimes in the presence of different concentration of I^- ion in a particular concentration of each Tween were determined and are compiled in Table 1. τ_0/τ versus $[I^-]_T$ is also plotted in Fig. 6 in case of 0.006 M Tween-20. The steady state and τ_0/τ plots are overlapping with each other indicating that the fluorescence quenching is of dynamic in nature.

The Scatchard plots obtained for 2-AF-I^- combination, as described for 2-AF-CPy combination, were non-linear in each Tweens. These plots indicate that like CPy, I^- ions also bind as well as partition to each Tween. The values of K_p obtained from the linear portion of the Scatchard plot and from the plot of $\langle Q\rangle$ versus $[Q]_A$ are nearly similar to each other. The values of p, K_b and K_p were calculated in the same manner as described earlier and are compiled in Table 1. The data of Table 1 show that both the values of K_p and K_b decrease with the increase in the Tween number. These trends are consistent with the hydrophobicity of the Tweens. As I^- ion is polar in nature, its solubility will decrease with the decrease in the polarity of the medium.

The modified Stern–Volmer plots (F_o/F versus $\langle I^-\rangle$), unlike CPy quenching, are linear for I^- ions. The quenching rate constants calculated in the same manner as discussed earlier for 2-AF-CPy combination are compiled in Table 1. The results indicate that the quenching rate constants are nearly three to four orders of magnitude less than that by CPy. Similar to CPy, the quenching rate constants decrease with the increase in the Tween number. A point worth considering is that the fluorescence quenching efficiency (k_q^T/k_q^D, where k_q^T and k_q^D are the quenching rate constants of 2-AF by I^- ions in Tweens and at diffusion controled limit in water, $\sim 10^{10}\,M^{-1}\,s^{-1}$, respectively) of 2-AF by I^- ion varies between 9.3×10^{-5} to 7.3×10^{-5} for Tween-20 to Tween-80. Since the fluorescence quenching is of dynamic in nature, it may be suggested that like hydrophobicity, the microviscosity of the Tweens also increases with the increase in Tween number.

4.3. Fluorescence quenching of 2-AF by AC

Fluorescence quenching was also observed in the presence of different concentration (0.005–0.05 M) of AC at different concentration (0.005–0.008 M) of each Tween. The Stern–Volmer plots for AC quenching in Tween-40 are plotted in Fig. 7 as a representative one. Similar plots were also observed for other Tweens and like I^- quenching, these are linear. τ_0/τ versus $[Q]_T$ plot shown in Fig. 7 for AC quenching in 0.006 M Tween-40 proves the dynamic nature of quenching.

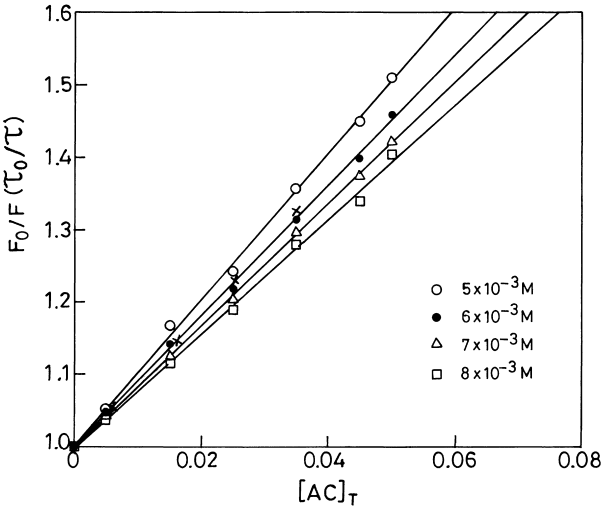


Fig. 7. Stern–Volmer plots for the quenching of 2-AF by AC at different Tween-40 surfactant concentrations. The crosses indicate τ_o/τ vs. $[AC]_T$ plot for 0.006 M Tween-40. $[2\text{-AF}] = 2 \times 10^{-5}$ M.

Unlike other quenchers (CPy and I^- ions), the Scatchard plots for 2-AF-AC quenching are linear in all the Tweens, which indicate that AC only partitions to each Tween. As a representative one, Fig. 8 depicts the Scatchard plot in Tween-40. The K_p values obtained from Scatchard plots and from the $\langle Q\rangle$ versus $[Q]_A$ plots are similar to each other. K_p values are also compiled in Table 1 which decrease with increase in the Tween number. This is consistent with the hydrophobicity of Tweens.

Like I^- ion quenching, the modified Stern–Volmer plots for 2-AF-AC combination in each Tween are linear (Fig. 8). The quenching efficiencies for a particular value of $\langle Q\rangle$ and thereby, quenching rate constants (k_q) for AC quenching decrease (Table 1) with increase in the Tween number which are consistent with the K_p values of AC with Tweens.

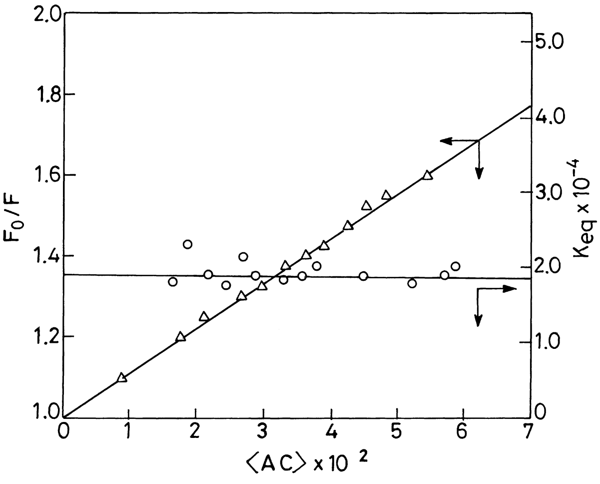


Fig. 8. Plot of K_{eq} vs. $\langle AC\rangle$ with AC as quencher in Tween-40 and F_o/F vs. $\langle AC\rangle$ plot with AC as quencher in Tween-40. $[2\text{-AF}] = 2 \times 10^{-5}$ M.

5. Conclusions

Based on the above study the following points are worth considering:

1. CPy and I^- ions bind as well as get partitioned, whereas AC only get partitioned. This could be because both the former species are charged and thus may be interacting more strongly with the Tweens, whereas AC is a neutral species and thus may be interacting through dispersive interactions only.
2. The values of K_b obtained for CPy are much larger (2–3 order) than those of I^- ion with the Tweens. This is because the former ion contains a non-polar long chain of 16 carbon atoms and thus plays a major role in the solubilization of CPy. This agrees with the fact that the solubilization of non-polar solute increases in the non-polar solvent. The values of K_b and K_p obtained for CPy suggest that hydrophobicity of the Tweens increases with the Tween number, as observed earlier [2,26].
3. The values of K_b for I^- ion to these micelles are nearly two order of magnitude less than that observed for CTAB ($5–6 \times 10^4$) [1]. This is because CTAB is a cationic micelle and Br^- ions are counter ions. Since Br^- ions are more hydrophilic than I^- ion, the latter will replace the former from the Stern layer and thus Br^- ion will be present in the aqueous phase. In other words the CTAB micelles will present stronger interactions to I^- ion than Cl^- or Br^- ions.
4. Although I^- ion is an ionic species, its partitioning coefficient is larger than AC in each Tween. It is well known that I^- ion, being large ion, can be easily polarized. Thus, quite hydrophobic in nature and can be solubilized in less polar environment. From our data it is evident that I^- ion is less polar than AC but we cannot offer any explanation.
5. The values of k_q decrease for each quencher with the increase of Tween number. Our earlier results [26] have clearly shown that the D_{eff} (effective dielectric constant), where 2-AF binds to Tweens, decreases with increase in Tween number. In other words 2-AF is located more towards the core of the Tweens and may offer higher viscous environments. Since the quenching observed is of dynamic in nature, the value of k_q observed explains the nature of environments.
6. Stern–Volmer plot between F_o/F versus the effective concentration of CPy in Tweens is non-linear at higher CPy concentration, whereas for other quenchers these plots are linear throughout the effective concentration range. The non-linear behaviour of the Stern–Volmer plot in case of 2-AF-CPy combination can be explained as follows: (a) CPy is completely solubilized in the micelles and thus offers greater effective concentration. (b) Pyridinium ion is the polar head group of the CPy. The long non-polar hydrocarbon chain will be present in the core of the micelles. Due to this the pyridinium ion will be either at the interface of polar and non-polar moiety of the micelle or in polar part. Thus, it will be present at a closer distant to 2-AF than I^- ion to 2-AF. (c) If the singlet state energy of the quencher is greater than that of the fluorophore, the fluorescence quenching of the fluorophore has been explained by involving a charge transfer complex as an intermediate [38]. The charge can be donated either by the fluorophore or by the quencher. In our earlier study [38], we could not measure the reduction potential of 2-AF in acetonitrile, being larger than 2.8 V, whereas the oxidation potential of 2-AF is 0.61 V. In other words, it will be easier for the 2-AF to act as charge donor rather than charge acceptor. This has been substantiated by the fact that k_q for 2-AF-CCl_4 combination (where 2-AF is donor and CCl_4 is an acceptor) is greater than the diffusion controlled limit ($\sim 10^{10}\ M^{-1}\ s^{-1}$) in all the non-aqueous solvents and non-linear Stern–Volmer plot was observed. On the other hand, for 2-AF-I^- combination (where 2-AF is an acceptor and I^- ion is a donor), the Stern–Volmer plot was linear in aqueous medium and k_q ($4 \times 10^8\ M^{-1}\ s^{-1}$) is much less than the diffusion limit. Based on the above discussion and the pyridinium ion being a positively charged ion (which is effective in the fluorescence quenching of 2-AF), intermediate charge transfer complex (where 2-AF acts as a donor and pyridinium ion as an acceptor) will possess stronger interactions and will be effective over a larger distances. This explains the non-linear behaviour in the Stern–Volmer plot. Since no change is observed in the absorption spectra of 2-AF when CPy is added to the Tweens, it can be suggested that active sphere model may be playing an active role. On the other hand, for 2-AF-I^- combination the intermediate charge transfer complex will involve weaker interactions and thus will be effective only over the collisional distances. Since quenching of 2-AF by I^- ion is of dynamic in nature, the decrease in the value of k_q with increase in Tween number suggests that microviscosity of the Tweens increase with increase in the Tween number.
7. Similar to our earlier study [1] and the results of other studies, regarding the penetration of water soluble ions or neutral species into the interior of the micelles (Tweens), we agree with the model proposed by Menger and coworkers [40–43], i.e., the water soluble quenchers diffuse to the fluorophore rather than the fluorophore diffusing to the quencher in the aqueous phase.

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