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Role of Brij micelles in the quenching of fluorescence of Safranine T by inorganic ions

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Abstract

The results of quenching of fluorescence of the dye Safranine T (ST) by the inorganic ions $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$, Cu^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} in aqueous micellar solutions of the surfactants diethylene glycol hexadecyl ether (Brij 52), decaethylene glycol hexadecyl ether (Brij 56) and cicosaethylene glycol hexadecyl ether (Brij 58) are presented. The quenching results have been processed in the light of Stern–Volmer (SV) equation and its modified forms to evaluate the extents of interaction between the fluorophore (ST) and the quencher. The magnitudes of the Stern–Volmer constant (K_{SV}) in quencher micelles follow the trend $[Fe(CN)_6]^{3-} > [Fe(CN)_6]^{4-} > Co^{2+} > Cu^2 > Ni^{2+} > Mn^{2+}$ in all the Brij media; for each ion the trend in terms of the surfactants is Brij 52 > Brij 56 > Brij 58, which is the order of the polarity of the Brij environment. The ion Co^{2+} appears to partly quench the emission process by the static mode. The solvent parameters of the Brij micellar media like the Kosower *Z* value, the transition energy for the intramolecular charge transfer, $E_T{}^{30}$ and the dielectric constant, *D*, have been estimated from the shift between the absorption and emission frequencies. A comparison of the quenching behaviours of inorganic ions on ST fluorescence in different media viz., aqueous, aqueous polyethylene glycol and aqueous micellar solutions of Tweens and Brijs has been presented.

Keywords: Brij micelles; Safranine T; Quenching

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1. Introduction

The physical–chemical properties of many dyes are influenced by surfactant micelles and polymers. Studies of such systems are important for the understanding of the photophysical photochemical activities of dyes (model drugs) in organised environments either in vitro or in vivo. Although the field has been emphatically cultivated [1–11], it is still competitively open for exploration for in-depth understanding and experimental detailing.

In our programme of investigating the ground and excited state spectral properties of the dye Safranine T (ST) in compartmentalized liquids viz., micelles and reverse micelles, and in polymeric solutions as well, we have attempted for a comprehensive understanding of the complexing behaviour of ST with ionic and nonionic micelles and the quenching of the dye by various inorganic ions. Additional information regarding the aggregation number of the micelle and the polarity of the micellar environment have been also gathered [5,6,12].

The nonionic micelles Tweens are compounds of a fixed length of twenty polyethylene oxide polar head group, a sorbitan moiety and a variable length of polymethylene chain in the molecule (monolaurate, monopalmitate, monostearate and monooleate in Tween 20, Tween 40, Tween 60 and Tween 80, respectively). Their interaction extents on ST are different [12], which has prompted us to examine the contribution of the polyethylene oxide head group on the process. For this, we have examined the photochemical–photophysical properties of ST in polyethylene glycols (PEG) of graded molar masses wherein [1] we have found that the PEG residues have significant say on the ground and excited states spectral phenomena of ST compared to the bound residues (as in Tween micelles). It has been found that the free residues (as in PEG) are more reactive. As the reactivity of the Tween micelles have been observed [12] to be also dependent on the length of the nonpolar tail (increased length has an increasing effect on the interaction), it is equally interesting to study the contributions of the increased number of the bound ethylene oxide (EO) residues in micelle forming agents as in Brij's having a fixed (hexadecyl) nonpolar polymethylene chain; where the polar heads are directly attached via an ether linkage. Here, a sorbitan moiety occurring in between the polar and nonpolar sections in Tweens is absent. The EO numbers of 2, 10 and 20 correspond to the detergent names Brij 52, Brij 56 and Brij 58, respectively.

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In the present study, we have examined in detail the spectral properties of ST and the fluorescence quenching characteristic of it by inorganic ions viz., $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$, Cu^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} in Brij 52, Brij 56 and Brij 58 micellar environments to explore the general behaviour of the dye in relation to its interaction with the head groups as well as the polar–nonpolar characteristics of nonionic micelles.

2. Experimental details

ST (E. Merck) was recrystallized twice from ethanol–water medium as reported earlier. [1] The surfactants diethylene glycol hexadecyl ether (Brij 52), decaethylene glycol hexadecyl ether (Brij 56) and cicosaethylene glycol hexadecyl ether (Brij 58) were Aldrich/BDH Products. Their light insensitive characteristics were checked by absorption and emission measurements. Doubly distilled conductivity water was used for solution preparation. AR (BDH) grade salts $K_4[Fe(CN)_6]$, $K_3[Fe(CN)_6]$, $NiSO_4$, $CoSO_4$, $CuSO_4$ and $MnSO_4$ were used as quenchers.

Fluorescence spectral measurements of ST were taken in a spectrofluorometer (Fluorolog F 111A Spectrofluorometer, Spex., NJ, USA) with a slit width of 2.0 nm. The excitation and emission wavelengths were 520 nm and 586 nm, respectively. Absorption spectra were recorded in a Shimadzu (160 A) UV–Visible spectrophotometer with a matched pair of silica cuvettes (path length, 1 cm). The concentration of ST used in aqueous and micellar solutions was of the order of 10^{-5} mol dm^{-3}. The quencher concentrations were varied in the range of 0.01, to 0.07 mol dm^{-3} for $[Fe(CN)_6]^{4-}$, $[Fe(CN)_6]^{3-}$, Cu^{2+} and 0.1 to 0.7 mol dm^{-3} for Co^{2+}, Cu^{2+}, Ni^{2+} and Mn^{2+}. The fluorescence spectra of the dye at a constant surfactant concentration (1 m mol dm^{-3} for each surfactant) in the presence of different quencher concentrations were recorded. All the measurements were taken at 25 ± 0.1°C.

3. Results and discussions

3.1. Fluorescence quenching

The inorganic ions $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$, Cu^{2+}, Co^{2+}, Ni^{2+} and Mn^{2+} have quenched the fluorescence of ST in the aqueous Brij 52, Brij 56 and Brij 58 micellar media, a phenomenon qualitatively similar to that observed in the micellar solutions of Tweens [5,6]. For $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$ and Cu^{2+}, the concentrations of quencher ions were varied in the range of 0.01–0.07 mol dm^{-3}; in the cases of Co^{2+}, Ni^{2+} and Mn^{2+}, concentrations in the range of 0.1–0.7 mol dm^{-3} were used. For Cu^{2+} and $[Fe(CN)_6]^{3-}$ (a strong quencher), the concentration ranges of 0.1–0.7 and 0.001–0.007 mol dm^{-3}, respectively, were also used.

The Stern–Volmer (SV) Eq. (1) is normally used to process fluorescence data

$$F_o/F = 1 + K_{SV}\,[Q] \qquad (1)$$

where F_o and F are the fluorescence intensities without and with quencher, $[Q]$ is the concentration of the quencher, and K_{SV} is the Stern–Volmer constant (it is the binding constant of the 1:1 complex that forms between the fluorescent molecule and the quencher).

The Eq. (1) written in the logarithmic form can be conveniently used and diagnostic for the departure of the results from it, which reflects on the slope value which is expected to be unity. Thus,

$$\log[(F_o - F)/F] = \log K_{SV} + \log\,[Q] \qquad (2)$$

The results of the present study show (Fig. 1) that Eq. (2) is obeyed by most of the quencher ions but the slopes are less than unity for $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$, Ni^{2+} and Mn^{2+}, greater than unity for Co^{2+}, and unity for Cu^{2+}. Nonequivalent quenching manifestations of the inorganic ions are envisaged. The K_{SV} and the slope values obtained according to Eq. (2) are presented in Table 1. The deviations follow the sequence $Cu^{2+} < Ni^{2+} [Fe(CN)_6]^{3-} [Fe(CN)_6]^{4-} <$

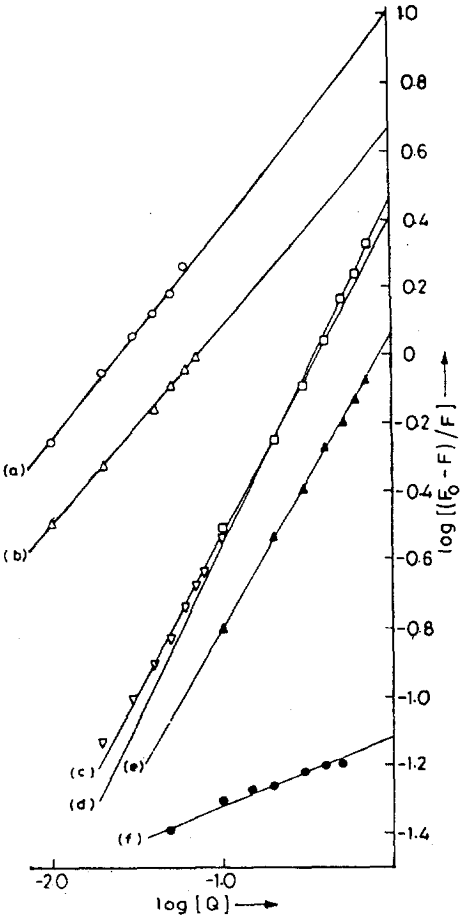

Fig. 1. Plot of log $[(F_o - F)/F]$ vs. log $[Q]$ in micellar solution of Brij 52 and 300 K. (a) $[Fe(CN)_6]^{3-}$ (b) $[Fe(CN)_6]^{4-}$ (c) Cu^{2+}, (d) Co^{2+}, (e) Ni^{2+}, (f) Mn^{2+}.

Table 1
Fluorescence quenching results analyzed according to Eqs. (2)–(4) for the ST–Brij systems at 300 K

(*a*)

Surfactant		$[Fe(CN)_6]^{3-}$	$[Fe(CN)_6]^{4-}$	Cu^{2+}	Ni^{2+}	Mn^{2+}
Brij 52	(i)	9.33(0.60)	4.68(0.55)	2.69(0.95)	1.16(0.87)	0.076(0.21)
	(ii)	62.0(0.80)	52.5(0.63)	3.12(1.0)	2.0(0.77)	7.5(0.11)
Brij 56	(i)	5.75(0.60)	2.69(0.53)	1.82(1.0)	0.53(0.67)	0.066(0.21)
	(ii)	55.4(0.69)	50.0(0.50)	2.5(0.83)	1.90(0.53)	5.3(0.08)
Brij 58	(i)	4.68(0.55)	1.91(0.52)	1.32(1.0)	0.39(0.62)	0.048(0.20)
	(ii)	52.5(0.68)	48.0(0.42)	2.19(0.71)	1.77(0.44)	4.21(0.05)

(*b*)

Quencher		Brij 52	Brij 56	Brij 58
	(i)	2.95(1.3)	2.40(1.2)	2.04(0.699)
	(ii)	2.86(1.0)	2.35(1.0)	2.10(1.0)
Co^{2+}	K_{SV}	0.456	0.275	0.243
	K_o	1.974	1.815	1.647
(Eq. (4))				

(i) K_{SV} (slope): Eq. (2) (ii) K_{SV} (f_α): Eq. (3).

Mn^{2+}; in comparison, the slope value for Mn^{2+} is much lower than that for the others. The efficiencies of the quenchers follow the order $[Fe(CN)_6]^{3-} > [Fe(CN)_6]^{4-} > Co^{2+} > Cu^{2+} > Ni^{2+} > Mn^{2+}$.

The lower slopes than unity may be rationalized on account of two effective classes of the fluorophore of which one is inaccessible to the quencher. To explain the results, the distribution of the dye between aqueous phase and micellar phase has been considered. The quantification of the distribution of the dye between the micellar and aqueous phases requires additional auxiliary physical measurements which will be in a future study. Considering inaccessibility of f_α fraction of the fluorophore, a modified SV equation of the following form may be useful [13].

$$\frac{F_o}{(F_o-F)}=\frac{1}{f_\alpha}K_{SV}[Q]+1/f_\alpha \tag{3}$$

The results obtained applying the Eq. (3) (Fig. 2) are also given in Table 1. The f_α values constitute the order $[Fe(CN)_6]^{3-} > Ni^{2+} > [Fe(CN)_6]^{4-} > Mn^{2+}$. The value for Mn^{2+} is very low, only 5–11% fluorophore is accessible to the quencher ion. The K_{SV} values are also significantly higher than that obtained from Eq. (2), the order is $[Fe(CN)_6]^{3-} > [Fe(CN)_6]^{4-} > Mn^{2+} > Ni^{2+}$. The quencher molecules interact with lower number of fluorophore with higher K_{SV} than otherwise. The normalization of K_{SV} (ii) in Table 1 with respect to $f_\alpha = 0$ does not make it comparable with K_{SV} (i). The K_{SV} (ii) f_α/K_{SV} (i) ratio depends on the quencher type.

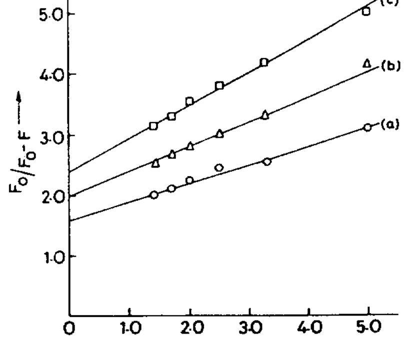

Fig. 2. Plot of $F_o/(F_o-F)$ vs. $[Q]$ in different Brij micellar solutions with $[Fe(CN)_6]^{4-}$ as quencher at 300 K. (a) Brij 52, (b) Brij 56 (c) Brij 58.

It is known that when quenching by collision of the fluorophores as well as complex formation of it with the quencher occur, the SV equation takes the form [14,15]

$$\frac{(F_o/F-1)}{[Q]}=(K_{SV}+K_o)+K_{SV}K_o[Q] \tag{4}$$

where K_o is the complexing constant (ground state binding constant).

The results with Co^{2+} as the quencher (where the slope of Eq. (2) is greater than unity) are processed according to Eq. (4) (Fig. 3). The K_{SV} and K_o obtained in the Brij media are also presented in Table 1. The K_{SV} values decrease in the order Brij 52 > Brij 56 > Brij 58. The K_o values also follow the same trend. The deviations from the normal SV plot (Eq. (2)) in the case of Co^{2+} producing slopes 1.3 and 1.2 in Brij 52 and Brij 56 micellar media respectively get a justification through complex formation. Nevertheless, the Co^{2+} in Brij 58 medium is a marginal case for which also data analysis according to Eq. (4) has been shown. It appears that for Co^{2+} ion along with dynamic quenching an element of static quenching is associated.

The bimolecular rate constant (k_q) for the quenching process has been found out from the K_{SV} and the known fluorescence lifetime (τ) of ST [4] ($k_{sv}/\tau = k_q$). The rate constants

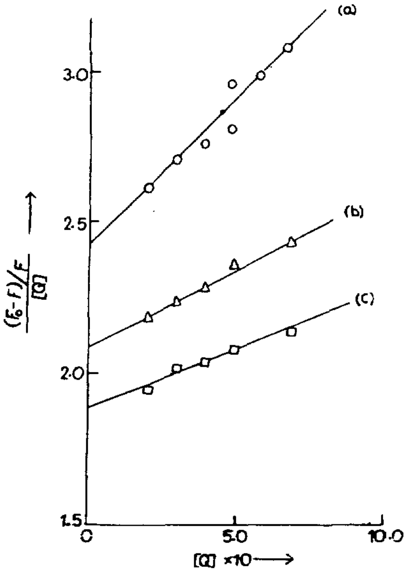

Fig. 3. Plot of $[(F_o-F)/F]/[Q]$ vs. $[Q]$ for Co^{2+} as quencher in different micellar solutions of Brijs. (a) Brij 52, (b) Brij 56, (c) Brij 58.

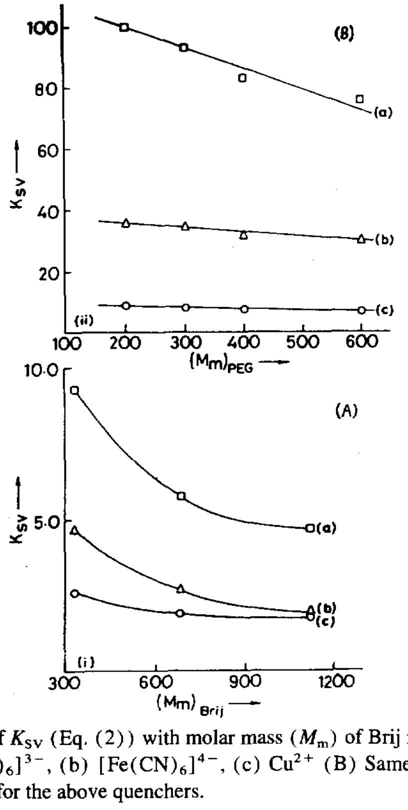

Fig. 4. Plot of K_{SV} (Eq. (2)) with molar mass (M_m) of Brij micelles. (A) (a) $[Fe(CN)_6]^{3-}$, (b) $[Fe(CN)_6]^{4-}$, (c) Cu^{2+} (B) Same plot for ST-PEG system for the above quenchers.

for the different quenchers follow the same order as K_{SV}: $[Fe(CN)_6]^{3-} > [Fe(CN)_6]^{4-} > Cu^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+}$. The order of the quenching efficiencies of the ions are the same in all the Brij media.

3.2. Solvent parameters

In addition to the above features the solvent parameters of the Brij micellar solution viz., the Kosower Z values, the transition energy for intramolecular charge transfer, E_T^{30}, and the dielectric constant, D, have been also estimated from the spectral shifts $\Delta\bar{\nu}$ based on the frequencies of absorption ($\bar{\nu}_\alpha$) and fluorescence ($\bar{\nu}_f$) i.e., $\Delta\bar{\nu}=\bar{\nu}_\alpha-\bar{\nu}_f$. The evaluation procedures are the same as described in our recent works [1,16]. The results are presented in Table 2. All the parameters decrease with increasing Eo residues in the Brij; the fall in D is rapid. Compared to the unbound polyoxyethylene molecules as in polyethylene glycols (PEG), the decrease is pronounced particularly for D.

3.3. General comprehension

For ST quenching by inorganic ions in PEG media, it has been found [17] that a relation like

$$(K_{SV})_{PEG}=(K_{SV})_{Aq}-\alpha(M_m)_{PEG}$$

(where α is a constant and M_m is the molar mass of PEG) holds. But in the present study a comparable relation,

$$(K_{SV})_{Brij}=(K_{SV})_{Aq}-\alpha(M_m)_{Brij}$$

does not hold, the initial drop in K_{SV} between aqueous to Brij 52 is large, thereafter among the Brij's the decline is of lesser extent. A similar effect of the molar masses of Tween micelles on K_{SV} has been also observed. A representation for the Brij's for three strong quencher ions, $[Fe(CN)_6]^{3-}$, $[Fe(CN)_6]^{4-}$ and Cu^{2+} are shown in Fig. 4A. In Fig. 4B, similar plots for ST-PEG systems are illustrated. The behavioural differences between the free and bound (as well as self organised) polyoxyethylene residues are evident. The results herein presented in the Brij media qualify a comprehensive comparison with the previous reports in the aqueous media of Tween and PEG [5,6,17]. With this end, the K_{SV} values for the ST fluorescence quenching by the inorganic ions studied in different solvent media along with micellar characteristics of the Tween and Brijs are presented in Table 3. The results show that the medium influence on the quenching

Table 2
Solvent parameters Z, E_T^{30} and D for Brij micellar media at 300 K

Solvent	λ_{ab} (nm)	λ_{em} (nm)	$\Delta\zeta$ (cm^{-1})	E_T^{30} (kcal mol^{-1})	Z (kcal mol^{-1})	D (by D vs. E/Z plot)
Broj 52	523	577	1771	54.5	85.0	70/69.5
Brij 56	525	574	1619	51.0	80.0	57/56.0
Brij 58	529	573	1444	46.5	73.5	40/40

Table 3
Comparison of K_{SV} from ST Fluorescence quenching in different studied media at 300 K

Solvent	$[Fe(CN)_6]^{3-}$	$[Fe(CN)_6]^{4-}$	Cu^{2+}	Co^{2+}	Ni^{2+}	Mn^{2+}
Aqueous	107.2	47.9	9.77	5.01	2.00	0.48
[PEG200]	100.0	39.8	8.51	4.78	1.40	0.22
[PEG 300]	93.32	34.7	7.94	4.42	1.35	0.17
[PEG 400]	91.20	31.6	7.69	4.17	1.12	0.08
[PEG 600]	83.12	25.1	6.76	3.89	1.02	0.01
[Tween 60]	11.60	3.20	1.50	1.30	0.90	0.30
[Brij 52]^a	09.33	4.68	2.63	2.95	1.16	0.08
[Brij 56]^a	05.75	2.69	1.95	2.40	0.53	0.07
[Brij 58]^a	04.68	1.91	1.82	2.04	0.39	0.05

[a] (CMC $\times 10^6$/mol dm^{-3}, Aggregation number); Tween 60 (21, 110)[10] [Brij 52 (0.96, 182); Brij 56 (1.1, 141); Brij 58 (1.1, 92)][15].

process follows the order Aqueous > Aqueous PEG > Aqueous Tween > Aqueous Brij. In each medium, the efficiencies of the quencher ions follow the sequence, $[Fe(CN)_6]^{3-} > [Fe(CN)_6]^{4-} > Co^{2+} > Cu^{2+} > Ni^{2+} > Mn^{2+}$. The data in Table 3 for aqueous medium suggest that the quenching process for $[Fe(CN)_6]^{3-}$ is likely to be diffusion controlled. The increased length of the polyoxyethylene chain in PEG and Brij has decreased the quenching efficiencies of the ions, which is greater in PEG than in Brij. The free polyoxyethylene chain (as in PEG) provides environment for higher quenching effect than the bound chain (as in Brij and Tween). On a comparative basis, Tween 60 and Brij 58 (both having identical nonpolar tails and equal number of polyoxyethylene residues in the head group) have unequal quenching regulating powers, quenching is more effective in the former. This indicates the role of the sorbitan moiety in the Tween which is absent in the Brij. The assistance of the quenching process by a carbohydrate moiety has been observed by us (unpublished results) in the aqueous solutions of glucose and galactose. This warrants extension of the study in aqueous carbohydrate media. The decrease of the quenching process with increasing Brij number may be a result of the polarity effect. The dielectric constant of the Brij solutions presented in Table 2 indicate a decrease in polarity with increasing Brij number. As a consequence quenching is reduced with a reduction in the K_{SV} values. Similar are the observations in PEG and Tween environments [5,6,17].

4. Conclusions

(1) The quenching of fluorescence of ST by inorganic ions is affected by Brij micelles in the order Brij 58 < Brij 56 < Brij 52.

(2) A fraction of the fluorophore (ST) may remain inaccessible to the quencher ions by way of partitioning between the aqueous and pallisade layer of the micelles.

(3) The quenching process is dynamic in nature for all the inorganic ions studied except Co^{2+}, where it may be partly static in nature.

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