

Fluorescence studies on mixed micelles of ionic-nonionic surfactants

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Fluorescence properties of ionic dyes using oppositely charged non-fluorescent dyes as quenchers in mixed surfactant of ionic-nonionic systems have been investigated. Cationic dye safranin O and anionic dye eosin Y are used as probes; bromocresol green, thymol blue and bromothymol blue are used as quenchers for safranin O, and crystal violet, toluidine blue and methylene blue are used as quenchers for eosin Y. The fluorescence characteristics in mixed micellar media of ionic and non-ionic surfactants CTAB, CpCl, SDS, AOT, Triton X-100, Tween 20, and Brij 35 have been studied. The variations in the physico-chemical parameters, viz., cmc, aggregation number(n), binding constant between the quencher and the probe (K_{sv}), occupancy number of the quencher in the micelles ($\bar{n}$), the binding constant of the quencher with the micelle (K) in such interactions have been evaluated using suitable equations and the properties have been explained in terms of the micellar structures.

Surfactants possess unique property of self-aggregation in solvents due to the amphiphilic nature of their molecular structures¹. These are the vital components in biological systems. Cell-membranes owe their structures to the aggregation of surfactants known as lipids. There are many other examples of naturally occurring surfactants that play a vital role in living organisms^{2,3}. Surfactants also form key ingredients in consumer products and play an important role in many industrial processes⁴. All these are possible only because they can self-aggregate into species namely, micelles, vesicles, bilayers and liquid crystals, etc.

In most of the practical applications, well-chosen mixtures of different surfactants are made to yield better performances which are unattainable by the use of a single surfactant. Critical micelle concentration, surface charge density, aggregation number and several other physico-chemical parameters can be varied over a wide range by changing the composition of the two surfactants (particularly, ionic and nonionic). Worldwide consumption of mixed surfactant systems in different formulated products like cleansing detergents, dye-stuff, mineral processors, pharmaceuticals etc., are ever increasing⁴. This has encouraged us to study surfactant aggregation of mixed surfactant systems, particularly of ionic and nonionic surfactants.

Materials and Methods

The following surfactants were used in the

present investigation: Cationic: cetyltrimethylammonium bromide (CTAB), cetylpyridinium chloride (CpCl); anionic: sodium dodecylsulphate (SDS), sodium dioctylsulphosuccinate (AOT); and nonionic: polyoxyethyleneglycol-*tert*-octylphenylether (Triton X-100, TX-100), polyoxyethylenesorbitan monolaurate (Tween 20, T 20) and polyoxyethylene (23)lauryl ether (Brij 35, B 35)

The following cationic and anionic dyes were used during the present work: Cationic:safranin O (SO, fluorescent), crystal violet (CV), toluidine blue (TB), and methylene blue (MB) (all nonfluorescent); and anionic:eosin Y (EY, fluorescent), bromocresol green (BG), thymol blue (ThyB) and bromothymol blue(BThyB)(all nonfluorescent).

All the surfactants were purchased from E. Merck, Germany and the dyes were Sigma products.

All the ionic surfactants were purified by recrystallization from appropriate water/methanol mixtures after which they have been dried under vacuum. Purity was finally checked by their conductivity in aqueous medium. Nonionic surfactants were purified by partitioning them between water and chloroform. Thin layer chromatography of the purified samples with water-saturated butanol as the mobile phase indicated the absence of free polyethylene glycols⁵. Dyes were used as such without further purification.

Fluorescent dyes were used as probes in micellar media. Anionic dye eosin Y was used as a

probe for cationic-nonionic surfactant systems because this dye interacts well with cationic-nonionic surfactant systems. For a similar reason, safranin O was used as a probe for anionic-nonionic surfactant mixtures.

Eosin Y has an emission peak (λ_{em}) around 550 nm. Any nonfluorescent dye, having its absorption maxima (λ_{max}) around the emission peak of EY(λ_{em}), if mixed with EY, there will be a quenching of EY induced by the latter dye⁶. This principle was employed in fluorescence quenching studies. Successively, three cationic dyes, crystal violet, (λ_{max} , 578 nm; ϵ_{max} , 5800 mol⁻¹.m²), toluidine blue (λ_{max} , 625 nm; ϵ_{max} , 4200 mol⁻¹.m²) and methylene blue (λ_{max} , 660 nm; ϵ_{max} , 4200 mol⁻¹.m²) were used as quenchers for the dye EY. Similar reason was there in using bromocresol green (λ_{max} , 612 nm; ϵ_{max} , 4560 mol⁻¹.m²), thymol blue (λ_{max} , 595 nm; ϵ_{max} , 5600 mol⁻¹.m²) and bromothymol blue (λ_{max} , 615 nm; ϵ_{max} , 3300 mol⁻¹.m²) as quenchers for the cationic dye SO. Pure EY has the value of molar absorption coefficient ϵ_{max} , 7800 mol⁻¹.m² while that of SO is 3700 mol⁻¹.m² in water.

Stock solutions of the surfactants and dyes were prepared in doubly distilled water and kept at 4°C in the dark. Dye stock solutions were always prepared afresh although they could be used for a week.

Surfactant concentration was varied within 10⁻⁵-10⁻² (M) while the concentration of the probe was kept fixed at 10⁻⁵(M). Quencher concentrations (i.e., nonfluorescent dye concentration) were varied within 10⁻⁵–10⁻⁴ (M).

Absorbance of the solutions were measured at 400-700 nm employing a Milton Roy Spectronic 21D spectrophotometer.

Fluorescence spectra were taken at 400-700 nm in a Shimadzu-RF 5000 spectrofluorophotometer. Eosin Y was excited at 455 nm (λ_{ex}), while safranin O was excited at 490 nm. Fluorescence intensities of the solutions containing the probes were taken only at the emission peak of the probe in micellar media (surfactant concentra-

tion 10⁻² M).

All experiments were performed at 25°C.

Results and Discussion

Critical micelle concentration (cmc) for pure as well as mixed surfactant systems were determined by dye incorporation technique using fluorescence spectroscopy. For cationic-nonionic surfactants the anionic dye EY was used while the cationic dye SO was used for anionic-nonionic surfactant systems. The fluorescence intensity (FI) of the surfactant-dye solution at different surfactant concentration, [S], was measured and cmc was determined from the break point of the plot of 1/FI versus 1/[S]. The cmc values were also calculated for the mixed surfactant systems using Clint equation⁷:

$$\frac{1}{cmc_1} = \frac{(1-x_2)}{cmc} + \frac{x_2}{cmc_2} \quad \dots (1)$$

where, cmc is cmc of mixed surfactant system, cmc₁, that of ionic surfactant, cmc₂ that of non-ionic surfactant and x₂ is molefraction of the non-ionic surfactant

Some of the observed and calculated values of cmc are given in Table 1. In all the cases, they were found to be quite comparable. Effect of addition of nonionic surfactants on the cmc of mixed surfactant systems is shown in Fig. 1. The value of cmc of the mixed surfactants decreased with increase in molefraction of the nonionic surfactants (x₂). The decrease is very sharp in the beginning, then it decreased gradually up to x₂ ≈ 0.5 beyond which the cmc values remained almost constant till it attained the values of pure nonionic surfactants.

Static fluorescence quenching studies were successfully performed using nonfluorescent dyes (oppositely charged compared to the probes used) as quenchers to study the quenching phenomena of fluorescent dyes in different micellar systems. It is expected that in the case of a mixture of two different dyes, if a good overlap exists between

Table 1—Variation of the critical micelle concentration with the mole fraction of nonionic surfactant (x₂)
cmc (nM)

Surf. Mixture x ₂ =	0.0	0.2	0.4	0.5	0.6	0.8	1.0
CTAB/TX-100	0.80(0.80)	0.39(0.50)	0.25(0.36)	0.23(0.32)	0.22(0.29)	0.20(0.24)	0.20(0.20)
CpCl/TX-100	0.90(0.90)	0.47(0.53)	0.32(0.38)	0.27(0.33)	0.25(0.29)	0.22(0.24)	
SDS/TX-100	8.0(8.2)	0.52(0.91)	0.28(0.49)	0.26(0.39)	0.25(0.33)	0.21(0.25)	
AOT/TX-100	2.4(2.4)	0.86(0.75)	0.38(0.44)	0.33(0.37)	0.27(0.32)	0.22(0.24)	

Values within parentheses indicate calculated values according to Clint. *et al.*

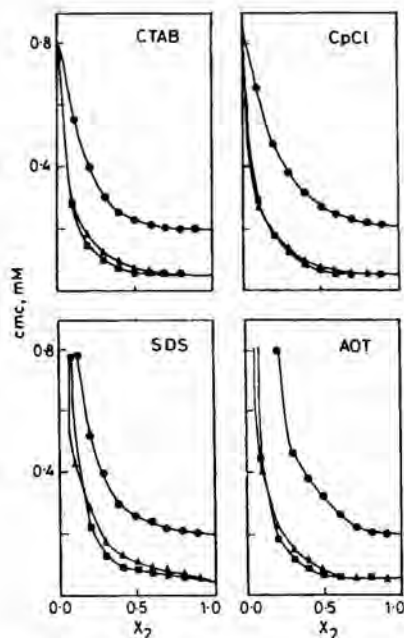


Fig. 1—Variation of cmc of mixed surfactants with the mole-fraction of nonionic surfactant (x_2). ●, Ionic-TX-100; ▲, Ionic-T 20; ■, Ionic-B 35

the emission spectrum of one dye (probe) with the absorption spectrum of another dye (quencher), then different energy transfers from the probe to the quencher would usually occur leading to a very efficient quenching⁶. Accordingly, three non-fluorescent cationic dyes, crystal violet, toluidine blue and methylene blue, were chosen as quenchers for the probe eosin Y; three anionic dyes bromocresol green, thymol blue and bromothymol blue were used as quenchers for the probe safranin O. These were selected from the absorbance and fluorescence spectra of the dyes in the micellar media. Quenching efficiency was found to decrease with increasing surfactant concentration, [S]. This was due to the fact that the probability of finding the quencher and the probe in the same micelle was reduced with increasing [S]. This led to the decreasing value of quenching efficiency as revealed from Stern-Volmer plots of F_0/F versus [Q], where F_0 and F are fluorescence intensity of the probe in absence and presence of the quencher, [Q] being the quencher concentration. Figure 2 shows the plots of F_0/F versus [Q] at different concentrations of the surfactant. There were two linear portions of the curve in Fig. 2 of which the initial one indicated Stern-Volmer type quenching making unit intercept on the F_0/F axis. The values of K_{sv} were, therefore, evaluated from the

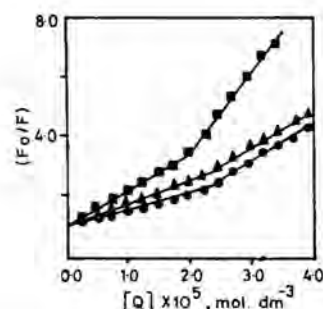


Fig. 2—Stern-Volmer plots for the quenching of safranin O by bromocresol green in different concentrations of SDS. [SDS] = ■, 10.0 mM; ▲, 15.0 mM and ●, 20.0 mM

slope of initial linear line. The deviations observed at higher [Q] values were not unexpected. They might have occurred due to either association of the quencher with the fluorescer or the self association of the quencher molecules. Fluorescence intensity was measured for the probe SO in the presence of the quencher BG at 10.0 mM, 15.0 mM, and 20.0 mM SDS surfactant solution. The calculated value of K_{sv} decreased from $11.00 \times 10^4 \text{ mol}^{-1} \cdot \text{dm}^3$ to $5.50 \times 10^4 \text{ mol}^{-1} \cdot \text{dm}^3$ when SDS concentration was increased from 10.0 mM to 20.0 mM. Similar effects were observed in all the other cases when different surfactants, quenchers and probes were used.

The variations of K_{sv} in mixed micellar media at different molefractions of the nonionic surfactant were studied using different sets of cationic-nonionic and anionic-nonionic surfactants; different probes and quenchers were used in this set of experiments. Some of the results are shown in Table 2. It was observed that the values of K_{sv} did not change significantly up to $x_2 \approx 0.5$ in the case of cationic-nonionic mixed surfactant systems. However, beyond this value of x_2 , K_{sv} increased to a large extent. At initial stages ($x_2 < 0.5$), addition of nonionic surfactant to the ionic one made non-ideal mixing and destabilized the mixed micellar structures. In such systems the average aggregation number of the mixed micelles did not change significantly. Therefore, the values of K_{sv} also did not change appreciably. Almost similar effects were observed also in the case of anionic-nonionic systems except that K_{sv} decreased significantly at initial stages when surfactant B 35 was used.

Binding constant of the quencher with the micelle (K) can be expressed as:

$$K = \frac{[Q]_{\text{mic}}}{[Q]_{\text{water}} \times [\text{mic}]} \quad \dots (2)$$

Table 2—Variation of K_{av} with the mole fraction of nonionic surfactant (x_2)

$[S] = 10^{-2} (M)$, exception $[S] = 2.0 \times 10^{-2} (M)$							
Surf. mixture	$x_2 = 0.0$	0.2	0.4	0.5	0.6	0.8	1.0
$K_{av} \times 10^{-4} \text{ mol}^{-1} \text{ dm}^3$							
CTAB/TX-100	7.45	7.41	7.58	7.64	7.50	8.53	14.25
CTAB/T 20		8.82	9.11	9.75	9.75	9.89	13.73
CTAB/B 35		8.36	9.46	9.11	8.14	10.17	10.40
CpCl/TX-100	9.28	9.86	10.09	9.62	8.60	10.23	
CpCl/T 20		8.96	8.59	10.86	9.98	9.98	
CpCl/B 35	*	7.03	8.49	7.97	9.06	9.52	
SDS/TX-100	5.85	7.00	6.50	5.25	6.50	5.75	8.87
SDS/T 20		6.62	5.77	7.39	5.66	5.95	5.19
SDS/B 35		1.74	1.53	1.98	2.66	3.82	3.75
AOT/TX-100	6.23	5.29	5.32	5.40	6.02	5.67	
AOT/T 20		5.58	5.88	5.04	6.35	7.73	
AOT/B 35		1.48	1.61	1.89	1.69	4.29	

For cationic/nonionic surfactant : probe-eosin Y, quencher-crystal violet; anionic/nonionic surfactant: probe-safranin O, quencher-bromocresol green.

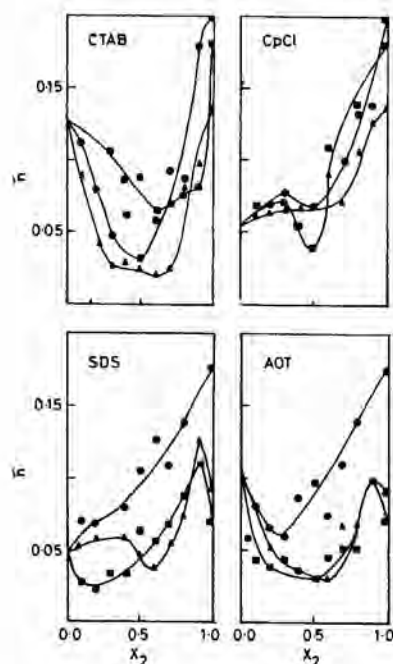


Fig. 3—Variation of the occupancy number ($\bar{n}$) of the quencher per micelle with the mole fraction of nonionic surfactant (x_2). For CTAB and CpCl: quencher, CV and probe, EY. For SDS and AOT: quencher, BG and probe, SO ●, Ionic-TX-100; ▲, IonicT 20; ■, Ionic-B 35

where, $[Q]_{mic}$ = quencher concentration in micellar media,
 $[Q]_{water}$ = quencher concentration in water, and
 $[mic]$ = micellar concentration.

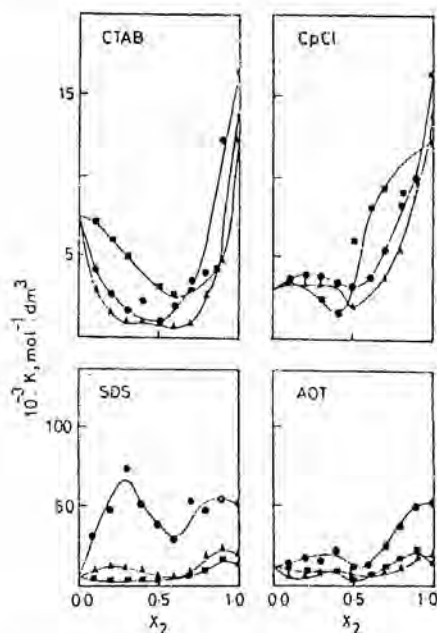


Fig. 4—Variation of the binding constant between quencher and micelle (K) with the mole fraction of the nonionic surfactant (x_2). For CTAB and CpCl: quencher, CV and probe, EY. For SDS and AOT: quencher, BG and probe, SO ●, Ionic-TX-100; ▲, IonicT 20; ■, Ionic-B 35

K can be related to the average occupancy number ($\bar{n}$) of the quencher per micelle by the following equation of Encinas and Lissi⁸, modified by Gehlen *et al.*⁹.

$$[Q]_T = \bar{n} / K + \bar{n} [Mic] \quad \dots (3)$$

$[Q]_T$ is the value of $[Q]$ required to attain a fixed value of $[F_0/F]$ at different micellar concentrations. By plotting $[Q]_T$ versus $[mic]$, values of both $\bar{n}$ and K were obtained from the slope and intercept of the linear lines. Effect of the addition of nonionic surfactants to ionic surfactants on these parameters are shown in Figs 3 and 4. It was observed that the variations in the values of $\bar{n}$ and K were not so regular up to the value of $x_2 \approx 0.5$. However, after this value of x_2 , the values of $\bar{n}$ and K increased in all the cases.

Fluorescence data were treated according to expression (4) to determine the micellar aggregation number¹⁰ (n)

$$\ln (F_0/F) = n[Q]/[S] \quad \dots (4)$$

Keeping $[S]$ at $10^{-2} (M)$, the values of F were measured at different quencher concentrations. This procedure of determining micellar aggregation number (from the plots of $\ln (F_0/F)$ versus $[Q]$), however, yielded much higher values compared to the values obtained by other techniques for pure

Table 3—Aggregation number of surfactants using different quenchers

Surfactants	Probe	λ_{em} (nm)	Cationic quenchers			Anionic quenchers			Reported values ^(10,11)
			CV	TB	MB	ThvB	BG	BlhvB	
CTAB	EY	547	A	480	258	145			56
			B	55	54	44			
CpCl	EY	552	A	611	344	164			
			B	60	68	48			
SDS	SO	561	A	—	—	—	305	257	50
			B	—	—	—	53	53	
AOT	SO	549	A	—	—	—	198	156	
			B	—	—	—	47	40	
	EY	554	A	712	311	224	—	—	
			B	127	119	128	—	—	
TX 100	SO	564	A	—	—	—	439	383	140
			B	—	—	—	140	149	
	EY	557	A	582	282	164	—	—	
			B	90	103	91	—	—	
T 20	SO	536	A	—	—	—	271	159	90
			B	—	—	—	94	107	
	EY	554	A	577	222	161	—	—	
			B	103	85	92	—	—	
B 35	SO	565	A	—	—	—	286	—	
			B	—	—	—	110	—	

A: Values of n obtained using Eq. (4)B: Values of n obtained using Eq. (5)

surfactants¹⁰. Interestingly in the present investigation, observed values of n were found to depend on: (i) wavelength difference ($\Delta\lambda$) between the absorption peak of the quencher and the emission peak of the probe in the micellar media; (ii) ratio of the molar absorption coefficients of the anionic and cationic dyes (ϵ_a/ϵ_c); and (iii) ratio of molar charges (numerical) on surfactants (q_s) and the probe molecules (q_p).

Equation (4) was, therefore, suitably modified on the basis of the experimentally observed results as:

$$\ln(F_0/F) = n \frac{[Q]}{[S]} \left[\frac{100}{\Delta\lambda} \times \frac{\epsilon_a}{\epsilon_c} \left(1 + \frac{q_s}{q_p} \right) \right] \quad \dots (5)$$

The values of n of different surfactants using the original and modified equation are given in Table 3. The values obtained by the modified equation (5) were found to be in good agreement with the literature values^{10,11}. In a similar way, n

values for mixed surfactant systems were also determined. Results are shown in Fig. 5. The values of n increased gradually with x_2 in the case of cationic-nonionic surfactant systems. The effects were similar when TX-100, T 20 or B 35 were used. But in the case of anionic surfactants, the effect of B 35 was significantly different. The value of n initially decreased, reached to a minimum and then increased again sigmoidally. However, TX-100 or T 20 had similar effects as in the case of CTAB and CpCl.

From the results discussed above it was observed that in mixed micellar systems the values of K_{sv} , $\bar{n}$, K and n changed in an unusual way with increase in mole fraction (x_2) of nonionic surfactant up to $x_2 \approx 0.5$, and beyond this value of x_2 , they changed more systematically. Ionic surfactants like CTAB, CpCl, SDS, AOT etc. have smaller molecular size and lower aggregation number compared to those of nonionic surfactants. Hence, due to bigger head group and longer

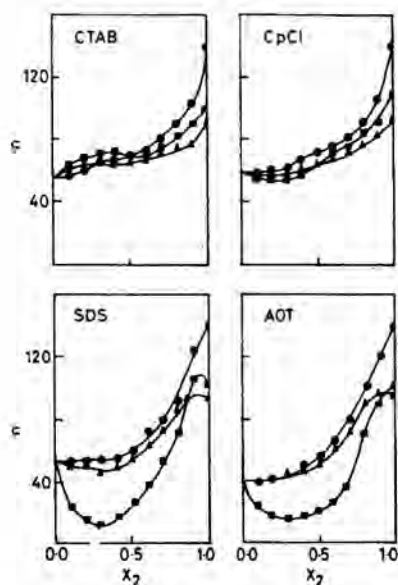


Fig. 5—Variation of aggregation number (n) of mixed micelles with the mole fraction of nonionic surfactant (x_2). ●, Ionic-TX-100; ▲, Ionic-T 20; ■, Ionic-B 35

hydrocarbon tail the nonionic surfactants cannot co-reside inside the micelle of ionic surfactants comfortably at initial stages. The addition of a nonionic surfactant was, therefore, expected to destabilize the mixed micellar structure indicating nonideal mixing. But after a certain stage ($x_2 \approx 0.5$) the system was reversed making it a mixture of ionic surfactant in a nonionic one where ionic surfactants could then co-reside with

the nonionic surfactant micelle in a favourable situation. In the present investigation, the observed results followed sigmoidal variation of the parameters with x_2 only beyond the value of $x_2 \approx 0.5$ in the mixed micellar system indicating ideal mixing of the surfactants as expected in the homogeneous micelles.

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References

- 1 Clint J H, in *Surfactant aggregation*, (Chapman and Hall, New York), (1990).
- 2 Linding B A & Rodger M A, *J Photochem, Photobiol*, 31 (1980) 47.
- 3 Calvin H, *J Photochem Photobiol*, 37 (1978) 849.
- 4 Gupta S, Moulik S P & Das A R, *Makromol Chem*, 192 (1991) 447.
- 5 Moulik S P, Gupta S & Das A R, *Can J Chem*, 67 (1989) 356.
- 6 Rohatgi K K & Mukhopadhyay A K, *Indian J Pure Appl Phys*, 41 (1976) 481.
- 7 Clint J H, *J Chem Soc (Faraday Trans I)* 71 (1975) 1327.
- 8 Encinas M V & Lissi E A, *Chem Phys Lett*, 91 (1982) 55.
- 9 Gehlen B F P & Neuman H G, *J Photochem Photobiol A: Chem*, 59 (1991) 335.
- 10 Yekta A, Aikwa M & Turro N J, *Chem Phys Lett*, 63 (1979) 543.
- 11 Bhattacharya S C, Das H T & Moulik S P, *J Photochem, Photobiol A: Chem*, 71 (1993) 257.