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Interaction of Triton X-100 with cyclodextrins. A fluorescence study

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The interaction of Triton X-100 (TX) with α- and *β*-cyclodextrins (CD) has been studied, using 2,6-*p*-toluidinonaphthalene sulfonate (TNS) as a fluorescent probe, by steady-state and time-resolved emission spectroscopy. The critical micellar concentration (c.m.c.) is indicated by the point of abrupt increase of emission intensity and lifetime of TNS. The apparent c.m.c. increases significantly in the presence of *β*-CD by as much as 28 ± 1 times at 10 mM β-CD but remains more or less unaffected in the presence of α-CD at similar concentrations. This is attributed to the very strong binding of TX with the large β-CD cavity and negligible binding to the small α-CD. At concentrations below the c.m.c., on addition of TX to aqueous TNS solution containing β-CD the emission intensity decreases. This is ascribed to the competitive binding of TNS and TX with β-CD. This causes displacement of TNS from the CD cavity by the TX surfactant molecules. The binding constant of TX with β-CD is found to be *ca.* 9400 ± 1300 L mol^{-1}.

Due to the potential application of CD in targeted drug delivery, the study of the interaction of surfactants with CD has attracted much attention recently, particularly in order to understand how CD affects cell membrane surfactants. The important issues are whether CD preserves the structure of the membranes and releases the drug encapsulated inside its cavity. Thus different groups have used fluorescence, NMR, conductivity and ultrasonic absorption techniques to study such interactions.[1–9] The c.m.c. of several ionic surfactants (alkyl sulfates, sulfonates and tetra-alkyl ammonium halides)[1–7] as well as neutral surfactants (TX, Igepal, *etc.*),[8,9] have been reported to increase on addition of CD, while their aggregation numbers remain more or less unchanged. For fluorescence studies, the most popular probes are pyrene, TNS or in the case of TX the intrinsic probe TX itself. Amongst these probes TNS is most sensitive to the solvent polarity. TNS is almost non-fluorescent in water (quantum yield, ϕ_f = 0.001), with a very short fluorescence lifetime (τ_f = 60 ps). Compared to water, in 15 mM β-CD the emission intensity of TNS increases *ca.* 60-fold and the lifetime increases *ca.* 40-fold.[10–12] As we will discuss, in the TX micelles the emission intensity of TNS increases 570-fold compared to water and the lifetime increases *ca.* 160-fold. Other probes, such as pyrene, are far less sensitive. For instance, the fluoresence lifetime of pyrene increases from 143 ns in water to 258 ns in TX above its c.m.c. and to 215 ns in β-CD *i.e.* only *ca.* two-fold.[8] The remarkable sensitivity of TNS is due to the non-radiative twisted intramolecular charge transfer (TICT) process whose rate increases very rapidly with the polarity of the medium.[13–15] The TICT process is greatly retarded when the TNS molecules are transferred from the bulk water to the relatively non-polar interior of the CD cavity or the micellar aggregates.[11,12] This causes dramatic enhancement of the fluorescence intensity, lifetime and emission energy of TNS. The fluorescence enhancement of TNS caused by the anionic surfactants is, however, far less than that caused by TX or other neutral micelles, since the local environment of TNS in such anionic micelles (alkyl sulfates or sulfonates) is rather polar.[1] For the cationic surfactants, TNS is not suitable as it forms association complexes and often precipitates out of solution. In the present work, we report on the interaction of α- and β-CD with TX using TNS as a fluorescent probe. TNS is a particularly suitable fluorescent probe since the emission intensity and lifetime of TNS molecules bound to TX micelles are 10 and 4.5 times larger, respectively, than those of the TNS molecules bound to β-CD and, as a result, one would expect a large variation in the emission intensity and lifetime of TNS when it is transferred from the β-CD cavity to the TX micelles. Warner *et al.*, earlier, studied the interaction of β, and γ-CD with TX using surface tension, NMR and a fluorescence technique using pyrene as an extrinsic probe and TX as an intrinsic probe.[8–9] However, they did not study the interaction of the surfactants with the small α-CD. α-CD has been found to increase the c.m.c. of the linear alkyl surfactants in the same manner as β-CD. TX, however, contains, apart from long alkyl chains, a phenyl ring. Though the α-CD cavity can accommodate a phenyl ring, the two bulky substituents at the *para*-positions of TX make it difficult for it to be inserted in the α-CD cavity. Thus, as will be seen, there is a dramatic difference between the interaction of TX with α- and β-CD.

Experimental

TNS (potassium salt), α- and β-cyclodextrins and Triton X-100 (Aldrich) were used as received. Steady-state absorption and emission spectra were recorded on JASCO 7850 and Perkin Elmer MPF 44B instruments, respectively. 2.5×10^{-5} M aqueous solutions of TNS were used for all the measurements. For lifetime measurement, the solutions were excited at 300 nm by the second harmonic of a synchronously pumped dual jet dye laser (Coherent 702-1) pumped by Antares 76s cw mode locked Nd : YAG laser. The emissions were detected at magic-angle polarization by a Hamamatsu MCP photomultiplier (2809U). The fluorescence decays were deconvoluted using a global lifetime analysis software (PTI).[16] Concentrations of the micelles [M] were calculated using the relation,[17] [M] = ([TX]-c.m.c.)/N_{av}, where [TX] denotes total surfactant concentration and N_{av}, the aggregation number, which is *ca.* 100 for TX.[18]

Results

Steady-state emission

On addition of TX to an aqueous solution of TNS the emission intensity remains more or less the same up to the re-

ported c.m.c.[13] Above c.m.c. the emission intensity of TNS increases abruptly and thus, the break in the plot of emission intensity against surfactant concentration corresponds to the c.m.c. of TX.[19] On the other hand, addition of 20 mM α- or 15 mM β-CD to an aqueous solution of TNS causes the ϕ_f to increase respectively *ca.* 30- and 60-fold.[11] The emission maximum of TNS exhibits a marked blue shift from 480 nm in water to 445 nm in the presence of TX above its c.m.c. and to 450 nm for the CDs. On addition of TX to aqueous TNS solution in the presence of β-CD, the emission intensity, instead of increasing, initially decreases and then, at a concentration much higher than the reported c.m.c. of TX in water, (0.26 mM[17]) the emission intensity of TNS increases abruptly and the emission maximum shifts to *ca.* 445 nm [Fig. 1 and 2(a)]. Obviously, if any micellar aggregates are formed at a surfactant concentration, below the break point in Fig. 2(a), the emission intensities would have increased over and above that caused by β-CD, as the emission quantum yield (ϕ_f^M) of TNS molecules bound to TX micelles is nearly 10 times larger than that in β-CD. Thus, in the presence of β-CD, the micellar aggregates of TX appear to form at a concentration corresponding to the break in Fig. 2(a). This concentration is the apparent c.m.c. (c.m.c.*) of TX in the presence of β-CD. Table 1 summarizes the c.m.c.* of TX at various β-CD concentrations. It is readily seen that the c.m.c. increases from 0.26 mM in the absence of β-CD to 7.25 mM *i.e. ca.* 28 times in 10 mM β-CD. Warner et al.[9] earlier reported that the c.m.c.* of TX-100 increases to 3 mM at 4 mM β-CD so that c.m.c.*/[β-CD] is 0.75 which is similar to the values (0.7–1.4) reported here. For TX-100 reduced (in which the phenyl ring is reduced) Warner *et al.* found that c.m.c.*/[β-CD] is *ca.* 0.2.[8]

In contrast to the rather dramatic increase in c.m.c.* of TX caused by β-CD, for α-CD even at a high concentration of 10 mM the break in the emission quantum yield *vs.* surfactant concentration [Fig. 2(b)] occurs at a concentration of *ca.* 0.3 mM TX *i.e.* very close to the reported c.m.c. (0.26 mM) of TX. It may be reiterated that the fluorescence quantum yield of TNS at infinite α-CD concentration is 0.040, which is similar to that for β-CD (0.053 for 1:1 and 0.074 for 1:2

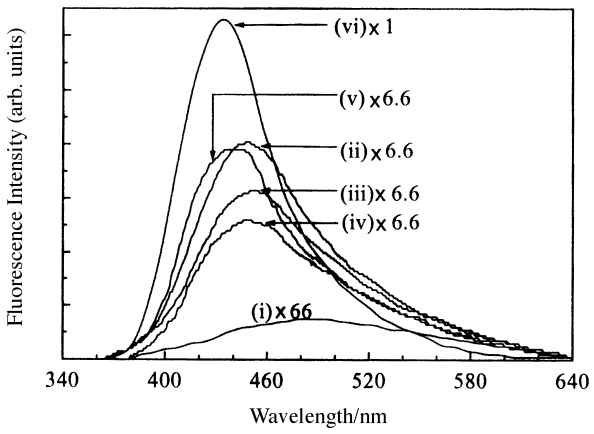

Fig. 1 Emission spectra of 2.5×10^{-5} M TNS, $\lambda_{ex} = 320$ nm, in (i) water, (ii) 10 mM β-CD, (iii) 10 mM β-CD and 5 mM TX, (iv) 10 mM β-CD and 6.75 mM TX, (v) 10 mM β-CD and 7.25 mM TX, (vi) 10 mM β-CD and 10 mM TX.

Table 1 Values of c.m.c. of TX at different β-CD*x* concentrations

[β-CD*x*] /mM	c.m.c.* /mM	c.m.c.*/[β-CD]
0.0	0.26 ± 0.05	—
0.5	0.70 ± 0.05	1.400 ± 0.10
1.0	1.00 ± 0.10	1.000 ± 0.10
2.0	2.25 ± 0.20	1.125 ± 0.10
10.0	7.25 ± 0.25	0.725 ± 0.25

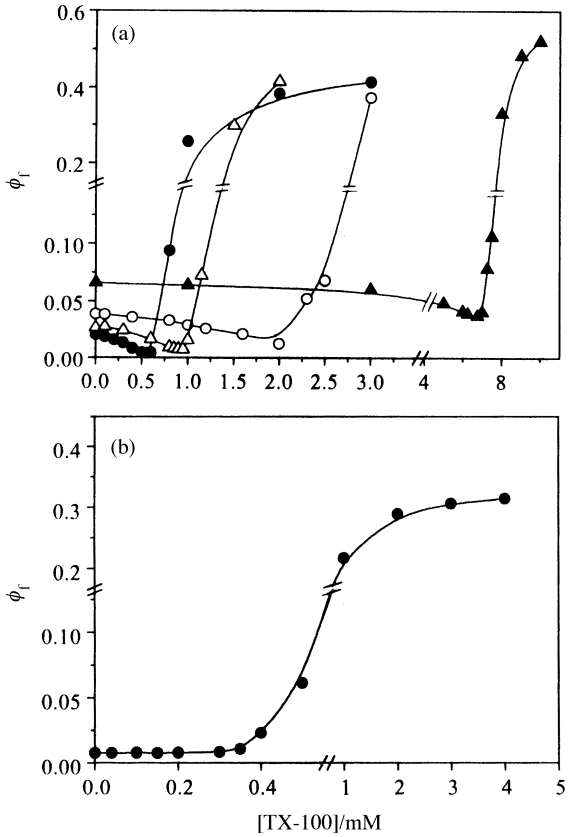

Fig. 2 (a) Fluorescence quantum yield of 2.5×10^{-5} M TNS, $\lambda_{ex} = 320$ nm, at different TX concentrations in (i) 0.5 mM (●) (ii) 1 mM (△) (iii) 2mM (○) and (iv) 10 mM (▲) β-CD. (b) Fluorescence quantum yield of 2.5×10^{-5} M TNS in 10 mM α-CD at different concentrations of TX.

complexes).[10,11] The differences in the emission quantum yields of TNS molecules, bound to α- and β-CD, is too small to explain the remarkable observation that, while 10 mM β-CD increases the c.m.c.* of TX 28 times, in the presence of 10 mM α-CD, the c.m.c.* of TX remains the same. Thus, it appears that α-CD does not increase the c.m.c. of TX. The effect of α-CD on TX is strikingly different from that on the linear surfactant molecules without a phenyl ring (*e.g.* alkyl sulfates or sulfonates or tetra-alkyl ammonium salts), for whom α-CD increases the c.m.c.* in a manner very similar to β-CD.[4]

Time-resolved emission

The time-resolved studies lend further support to the contention that while addition of β-CD causes an increase in the c.m.c.* of TX, α-CD hardly affects it and that no micellar aggregates are formed at a surfactant concentration below that corresponding to the break in the steady-state emission curves. In 10 mM CD the fluorescence decay of TNS is found to be biexponential with average lifetime ($\langle\tau\rangle = \Sigma a_i \tau_i$) 1.3 ± 0.1 and 2.0 ± 0.1 ns, respectively, for α- and β-CD. The biexponential decay arises not because of the different stoichiometry of TNS-CD complex but due to the heterogeneity in the environment in different complexes of the same stoichiometry and, in addition, in the case of α-CD the non-negligible number of free TNS in bulk water.[11,16] The lifetime of TNS, at TX concentrations much above the c.m.c. in the absence of CD, is 9.3 ± 0.2 ns. On addition of TX to an aqueous solution of TNS containing 10 mM α- or β-CD, the lifetime remains more or less constant initially and then exhibits a sudden increase from 2.0 ns for β-CD (1.3 ns for α-CD) to 9.3 ns *i.e.* to the value typical of TNS bound to TX micelles. It is interesting to note, that the sigmoidal plot of the lifetime against the surfactant concentration of TX (Fig. 3) exhibits a sharp

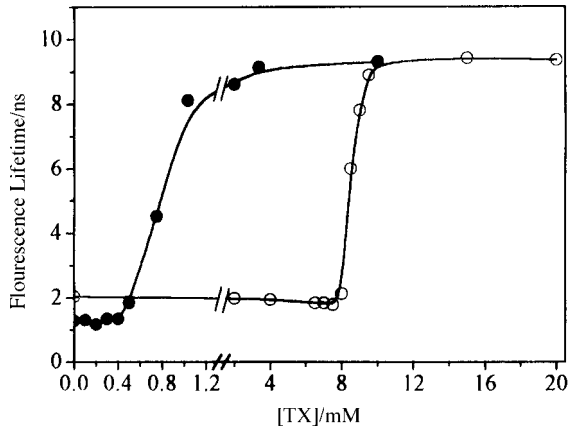

Fig. 3 Lifetime of TNS *vs.* surfactant (TX) concentration in the presence of 10 mM α-CD (●) and 10 mM β-CD (○).

break, at a surfactant concentration of 0.4 mM for 10 mM α-CD and 7.5 mM for β-CD, which are almost identical to that corresponding to the break in the steady-state emission intensities (Fig. 2). Thus, the striking difference between the effect of α- and β-CD, towards the micellisation of TX, is once again demonstrated in the time-resolved studies. A very long-lived fluorescence decay of TNS, 9.3 ns, is a clear signature of the presence of TNS molecules bound to the micellar aggregates. This indicates that the micellar aggregates of TX are formed above the break point. Two typical decay traces with weighted residuals are displayed in Fig. 4.

In summary, the abrupt increase in ϕ_f, much more than that caused by CDs, the blue shift in the emission spectra relative to those in CDs and the appearance of the very long-lived decay component, at a TX concentration corresponding to the break point, indicates that the micellar aggregates are formed at surfactant concentrations above that corresponding to the break. In the presence of these micellar aggregates, the probe TNS molecules are almost exclusively transferred from the CD cavity to the micelles, causing a dramatic change in ϕ_f, τ_f and λ_{max}^{em}. Thus the break point in the steady-state emission

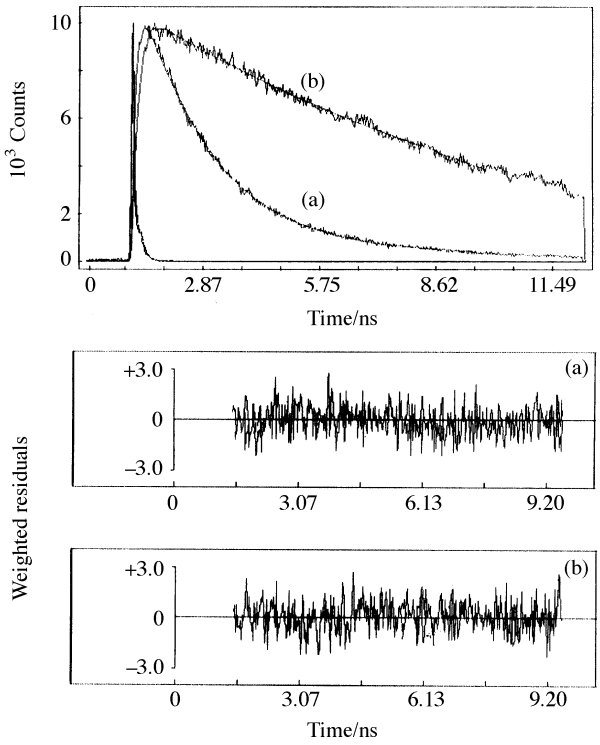

Fig. 4 Fluorescence decays of 2.5×10^{-5} M TNS in 10 mM β-CD containing (a) 6.5 mM TX and (b) 10 mM TX at 450 nm.

intensity curves gives the c.m.c.* of TX in the presence of CDs. The most interesting observation, however, is the fact that while the c.m.c. of TX corresponding to this break, is very much increased in the presence of β-CD, there is little or no change in the c.m.c.* of TX, in the case of α-CD.

Discussion

Our most important finding is the sharp contrast in the effect of α- and β-CD on the c.m.c. of TX. As stated earlier, the very dramatic differences in modifying c.m.c. by α- and β-CD cannot be due to the very slight differences in the emission quantum yields of TNS molecules bound to α- and β-CD. The inability of α-CD to increase the c.m.c. of TX may be attributed to the smaller size of the α-CD cavity which cannot accommodate the large TX molecule containing a phenyl ring with two bulky substituents. Note that, even for TNS, the binding constant for the smaller α-CD (120 L mol^{-1}) is much smaller than that for β-CD (2000 L mol^{-1} for the 1:1 complex).[10,11] The larger β-CD easily accommodates and "siphons away" TX molecules and renders them unavailable for micelle formation. As a result, the formation of the micellar aggregates of TX is disrupted in the presence of β-CD causing an increase in c.m.c. The inability of α-CD to accommodate the TX molecule, on the other hand, leaves the micellation of TX unaffected.

Obviously, in the presence of β-CD, there is the possibility of competitive binding between (a) TNS and β-CD, (b) TNS and micelles and (c) free TX surfactant molecules and β-CD. The decrease in the emission intensity of TNS on addition of TX, in the presence of β-CD, appears to be due to the very strong binding of β-CD with TX molecules, causing displacement of the TNS molecules from the β-CD cavities by the TX molecules. Consequently, in the presence of TX the amount of free β-CD molecules accessible to TNS decreases. The *ca.* two-fold decrease in the emission intensity of TNS, in 10 mM β-CD, on addition of 6.75 mM TX, indicates that the amount of free β-CD decreases. The amount of β-CD bound to TX and the free amount can be estimated from the observed steady-state emission intensities. One needs to know the binding constant of TNS with β-CD and that with TX micelles. The former is very well studied.[10,11] The latter is not reported in the literature but can be obtained using the relation suggested by Almgren *et al.*[17]

$$\frac{I_\infty - I_0}{I_t - I_0} = 1 + \frac{1}{K_M[M]}$$

where I_∞, I_t and I_0, denote, respectively, the emission intensities at infinite micellar concentration, at an intermediate micellar concentration and in the absence of micelles, [M], the micellar concentration and K_M, the binding constant. From the slope of the plot of $(I_\infty - I_0)/(I_0)$ *vs.* inverse micellar concentration (Fig. 5) the binding constant of TNS with TX micelles is estimated to be 3.5×10^5 L mol^{-1}. It should, however, be pointed out that, in this case, the concentration of the probe, TNS, (2.5×10^{-5} M) is quite close to the concentration of the micelle and, hence, it is not fully justified to assume single occupancy of the micelles. Unfortunately, since the emission intensity of TNS quickly saturates at *ca.* 2 mM TX, it is not possible to work under conditions where [M] ≫ probe (TNS) concentration. However, since there is no report on the variation of emission quantum yield of TNS with its own concentration due to formation of excimer or self quenching or other reasons, we believe that the equation used is perfectly valid. I_∞ is obtained from the emission spectrum of TNS at a very high surfactant concentration (10 mM) when >97% of the TNS molecules are bound to the micelle. The very few TNS molecules remaining free contribute very little to the total emission as their ϕ_f is very small (0.001). I_∞ so

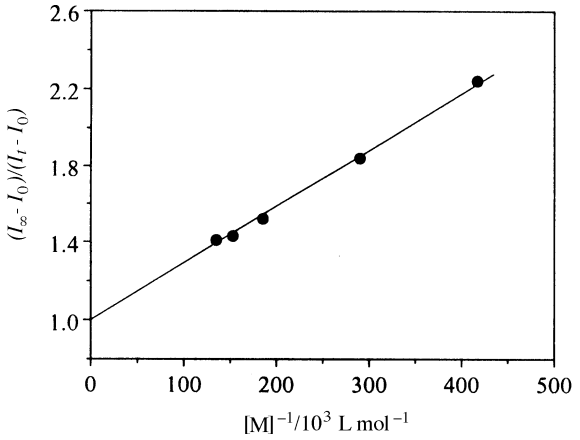

Fig. 5 $(I_\infty - I_0)/(I_t - I_0)$ of 2.5×10^{-5} M TNS *vs.* inverse TX micellar concentration.

obtained is 570 times that of TNS in water so that the emission quantum yield of micelle bound TNS, ϕ_f^M is 0.57, which is nearly 10 times larger than those of TNS at infinite β-CD concentration.[10–12]

In an aqueous solution containing TX above the apparent c.m.c., in the presence of β-CD, obviously the overall emission of the TNS molecules may originate from various sources, such as those bound to the TX micelles, β-CD (both as 1:1 and 1:2 complexes) and the free TNS molecules in bulk water. Note that, in 10 mM β-CD, in the presence of 10 mM TX, the observed emission parameters (observed quantum yield, $\phi_{obs} = 0.52$, emission maximum at 445 nm and $\tau_f = 9.3$ ns) are very different from that of TNS in 10 mM β-CD, in the absence of TX. Therefore, one may assume that most of the TNS molecules are bound to the micellar aggregates and the number of the TNS molecules bound to β-CD is negligible and that the emission originates very predominantly from those TNS molecules bound to the TX micelles. Thus, the observed emission quantum yield (ϕ_{obs}) in 10 mM β-CD, in the presence of 10 mM TX, is approximately equal to $\chi^M \times 0.57$, where, χ^M is the fraction of total TNS molecules bound to the TX micelles with $\phi_f^M = 0.57$. Under such a simplified model, the binding constant of β-CD with TX can be calculated as follows. In the mixture of β-CD and TX, total concentration of each being 10 mM, let the concentration of β-CD molecules bound to TX be x mM. Then, assuming a 1:1 complex between β-CD and TX, the concentration of β-CD accessible to TNS is $(10 - x)$ mM while the micellar concentration, [M] is $(10 - x - \text{c.m.c.})/N_{av}$. If P_f and P_b denote the concentration of free TNS molecules and those bound to micelles, respectively, then $\chi^M = P_f/(P_f + P_b)$ is $\phi_{obs}/0.57$ *i.e.* (0.52/0.57) and $P_b/P_f = K_M[M]$. From this, x is calculated to be 6.77 mM. This leads to a free β-CD concentration of 3.23 mM. The concentration of free TX is obviously the c.m.c. *i.e.* 0.26 mM. From these, the binding constant (K_b) of TX with β-CD is estimated to be 8.1×10^3 M^{-1}. Such an analysis is repeated for various other concentrations of TX. The results summarised in Table 2 indicate that, notwithstanding the complications associated with the given situation, one gets a fairly good estimate of the binding constant as $(9.4 \pm 1.3) \times 10^3$ L mol^{-1}. Evidently, such a binding constant of TX with β-CD is about an order of magnitude higher than that of β-CD with the linear alkyl surfactants.[1–7]

Table 2 Binding constant (K_b) of TX with β-CDx

[β-CDx] /mM	[TX] /mM	ϕ_f[a]	K_b /10^3 L mol^{-1}
10	7.25	0.078	8.70
10	7.50	0.106	9.77
10	8.00	0.329	10.67
10	10	0.52	8.1

[a] ±5%.

The binding constant of TNS with β-CD for the 1:1 complex is 2000 ± 200 L mol^{-1}.[7,10] Since the binding constant of TX with β-CD is four times larger than that with TNS the concentration of TX (5 mM) is 200 times larger than that of TNS (0.025 mM), TX readily displaces TNS from the β-CD cavity. In 10 mM β-CD and 5 mM TX, nearly all the TX molecules bind to β-CD, so that out of the total 10 mM β-CD, 5 mM β-CD remain free. Using the reported binding constants (1:1 and 1:2) of TNS with β-CD[7,10,11] on going from 10 mM β-CD to 5 mM β-CD one expects a (1.2 ± 0.15)-fold decrease in the emission intensity of TNS, which is consistent with the observed (1.3 ± 0.1)-fold decrease in the emission intensity of TNS on addition of 5 mM TX to 10 mM β-CD, reported here [Fig. 1 (iii)].

Warner *et al.* had earlier determined that the binding constant for the TX-100 (reduced) : β-CD system is 145 L mol^{-1}, using surface tensiometry[8] and for the TX-100 : β-CD system it is 3327 L mol^{-1}, by intrinsic fluorescence of TX.[9] The latter value is of the same order of magnitude of that reported here $(9400 \pm 1300$ L mol$^{-1})$. However, note that addition of TX-100 to a TNS solution containing 10 mM β-CD causes a 10-fold (1000%) increase in the emission intensity, while an aqueous TX-100 solution without TNS leads to only a 40% change in the emission intensity of TX, on addition of β-CD. Given the inherent error in the emission intensity measurement (±5%), TX is a much less sensitive probe than TNS. One might be tempted to attribute the discrepancy in the binding constant of TX-100 and β-CD between our value and that of Warner *et al.* to the possibility of formation of ternary complexes involving TNS, CD and TX. Such ternary complexes have been considered by several authors.[12,20] In such a ternary complex the alkyl chain of TX may wrap around the TNS : CD complex causing further decrease in the local polarity and, thereby, increasing the emission intensity and lifetime of TNS. However, the marked decrease in the emission intensity of TNS : β-CD on addition of TX is evidence against the formation of the ternary complex. Again, as noted earlier, the changes in the emission intensity of the TNS: β-CD system on addition of TX can be explained nearly quantitatively without assuming such ternary complexes. Finally, the ternary complexes cannot explain the striking difference between the effect of α- and β-CD on the c.m.c. of TX. Considering all this, we feel that the ternary complex plays a minor role, if any. We are more inclined to attribute the differences in the values of the binding constant to the inherent errors and assumptions involved in the two methods.

Conclusion

This work demonstrates that TNS is a very sensitive fluorescence probe for studying the interaction of α- and β-CD with TX micelles. β-CD disrupts the micellisation of TX by binding with the TX molecules very strongly, rendering them unavailable for the formation of micellar aggregates. An abrupt increase in the emission intensity and lifetime and a blue shift of the emission maximum, characteristic of TNS molecules bound to TX micelles, are observed in the presence of β-CD, at a concentration much above the reported c.m.c. of TX. However, for α-CD, such changes occur at a concentration similar to the reported c.m.c. of TX in water. This indicates that the apparent c.m.c. of TX increases, in the presence of β-CD, by as much as 28 times, at 10 mM β-CD. However, the smaller α-CD is found to have a negligible effect on the micellisation process and the c.m.c. of TX remains unaffected, even in the presence of 10 mM α-CD. It is proposed that this is due

to the fact that the large TX molecules cannot be encapsulated in the small α-CD cavity.

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