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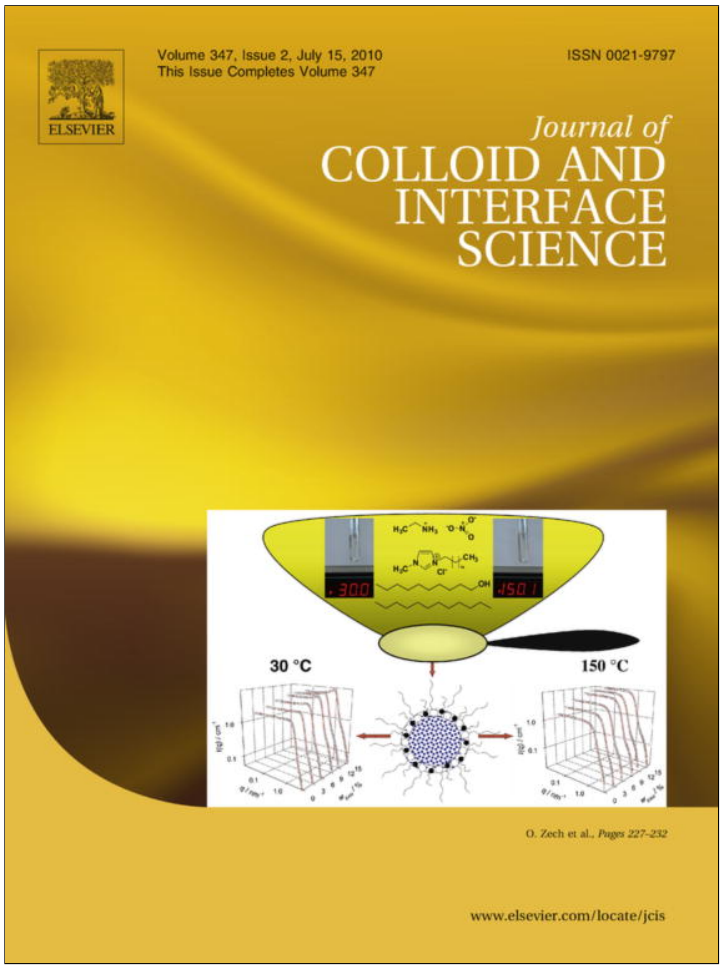

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Studies of Triton X-165–β-cyclodextrin interactions using both extrinsic and intrinsic fluorescence

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ABSTRACT

The interaction of β-cyclodextrin with the non-ionic micelle-forming surfactant Triton X-165 (TX-165) has been studied using steady state fluorescence and fluorescence anisotropy techniques. Both extrinsic and intrinsic fluorescence have been exploited for the purpose. Phenosafranin (PSF), a cationic phenazinium dye, has been used as the extrinsic probe while fluorescence of TX-165 has served as the intrinsic one. PSF shows discernible interactions with both TX-165 and β-CD. The experimental results reveal that the extent of interaction of PSF with TX-165 is greater than with β-CD. However, addition of β-CD to a micellar solution of TX-165 containing PSF leads to a disruption of the micelles whereby the fluorophore is released from the micellar environment to the bulk aqueous phase. It has been substantiated that an inclusion complex is formed between the non-ionic surfactant and the cyclodextrin. A 1:1 stoichiometry of the TX-165–β-CD inclusion complex has been proposed. Such a complexation between TX-165 and β-CD results in an inhibition in the micellization process of TX-165 leading to an enhancement in the apparent CMC value. The inferences are drawn from a series of experiments, *viz.*, binding studies, determination of micropolarity, heavy-ion quenching studies and steady state fluorescence anisotropy experiments monitoring both extrinsic and intrinsic fluorescences.

1. Introduction

The last two decades have witnessed growing importance of organized assemblies with regard to biological and photophysical processes. Reactants accommodated in molecular assemblies like micelles, cyclodextrins, micro-emulsions, vesicles and so forth often achieve a greater degree of organization compared to their geometries in the homogeneous solution [1]. Amphiphilic surfactant molecules capable of forming micelles, the dynamic nano-sized aggregates, are drawing serious attention of the chemists due to their importance as catalysts and solubility enhancers of a wide range of organic molecules [2,3]. Interactive features of innumerous fluorophores in micellar systems have been studied because of the fact that micelles, in a simple model, mimic the membranes of the biological systems [3,4]. Recent studies throw light on the interaction of bioactive fluorophores with the surfactants and pay attention towards the binding and partitioning of the former within the micellar environments. The photophysical/photochemical processes are often modified because of a change in the micropolarity and rigidity inside the micellar microenvironments compared to the situation in the bulk aqueous phase. Water–micelle interactions are still foggy especially in case of non-ionic surfactants [5–7]. Non-ionic micelles are characterized as organized assemblies consisting of the non polar hydrophobic core constituted from the hydrocarbon chains of the surfactant and micellar surface from the hydrophilic head groups. As the properties of the core and the head group regions are different, the fluorophore can reside in the core or in the palisade layer of the micelle or distribute itself within these two distinct regions depending on the polarity and charge characteristics of both the probe and the micelle. Triton X (TX) family, a trademark of Rohm and Haas [5], is the group of detergents all containing a *p-tert*-octylphenyl (OP) hydrophobic moiety with a polyethyleneoxide (PEO) chain constituting the hydrophilic part. TXs can be used as a self probe in fluorescence spectroscopy because they are naturally fluorescent [6,7]. The study of interactions between surfactant and sugars [8], in particular cyclodextrins [9], has an objective to improve and increase the field of application of the cyclodextrins (CDs). The potential application of CDs has been found in targeted drug delivery, as they are capable of solubilizing hydrophobic drugs [10–17]. This application requires an understanding of the effect of CDs on the cell membranes in as much as the utility of CDs in drug delivery would be little in the event of their disrupting

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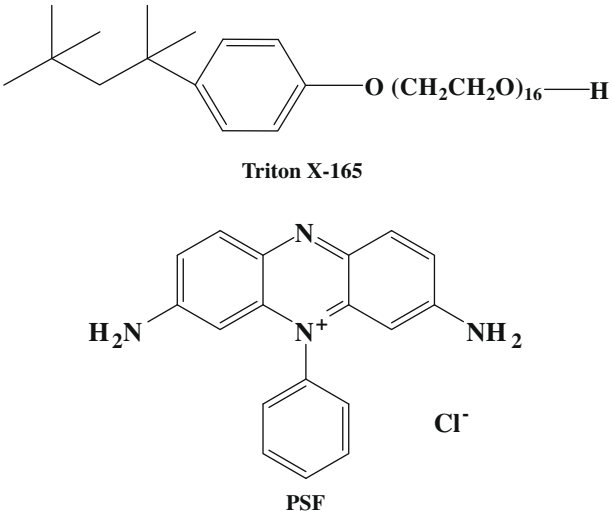
the membrane structure. Cyclodextrins are interesting microvessels capable of embedding appropriately sized molecules and the resulting supramolecules can serve as excellent miniature models for enzyme–substrate complexes. These doughnut-shaped cyclic oligosaccharides, mostly composed of 6, 7, and 8 D(+)-glucose units named as α-, β-, and γ-CD, respectively, have a hydrophilic outer surface and a hydrophobic inner surface. The cyclodextrin molecules have internal cavity accessible to the guest molecules of proper dimension through an opening of 4.5–5.3 Å, 6.0–7.0 Å and 7.5–8.5 Å for α-, β-, and γ-CD, respectively [1,8]. The depths of all the CDs are, however more or less the same (7.9 Å). Depending on the relative dimensions of the guest molecule, the CD cavity and the concentration of the CD, different host–guest stoichiometries are possible [8–10]. Cyclodextrin complexation can give beneficial modification of the guest molecules in terms of solubility enhancement, physical isolation of incompatible compounds, stabilization of labile guests and control on their volatility for the long-term protection of color, odor and flavor. Cyclodextrin nanocavity plays a key role in many biological processes to control the dynamics of a molecule confined in it [8].

Owing to their usefulness in the areas of synthetic, analytical, and pharmaceutical chemistry, a large number of studies have been undertaken to explore the nature of the surfactant–CD inclusion complexes [12]. There are reports of interaction of CDs with cationic and anionic surfactants by conductance, surface tension, NMR studies [18–22]. These studies interpret the formation of surfactant–CD complexes from various angles. Some of them report 1:1 while others favor 1:2 complexation. Association constants, diffusion coefficients, effect of additives, etc. have been determined in order to analyze the mode of complexation between the surfactants and CDs [18–22]. Also reports are available dealing with the interaction of the non-ionic surfactants with the CDs [23–26]. Although some studies deal with other non-ionic surfactants like alkyl-glucopyranosides exploiting isothermal titration calorimetry, cryo-TEM, X-ray, NMR, surface tension, MD simulations, etc. [25–29] majority of these studies are with Triton X-100. Reports on the interaction between TX-100 and β-CD systems are there, but the association types reported therein is not consistent to one another. It is generally reported that a 1:1 inclusion complex is formed between the surfactant and the CD [30–36]. A 1:2 inclusion complex is also suggested in a few reports [36,37]. Buschman et al. and He et al. have established the coexistence of 1:1 and 1:2 surfactant–cyclodextrin inclusion complexes [38,39]. However, a thorough study of surfactant–CD inclusion complexes is important mainly because these systems can be used to mimic the effect of cyclodextrin, an effective drug carrier, on the phospholipids, a major constituent of the cell membranes [40]. Such complexes are therefore able to alter chemical activity and act as molecular carriers. Recently, Lu et al. used the supramolecular assembly of CD and TX-100 as a precursor to synthesize mesoporous silica microtubules [18]. Thus the investigation of the interaction between different surfactants and CD is of both scientific and practical importance to the colloid and interface science. In spite of this apical significance, surfactant–CD complexation still remains unexplored to a good extent. To the best of our knowledge no thorough study has been performed on the formation of the inclusion complex between the CDs and any member of the Triton X series other than TX-100. In the present article we have carried out a systematic and detail study to decipher the interaction between TX-165 and β-CD. Triton X-165 contains 16 PEO groups in its hydrophilic head region (Scheme 1).

Phenosafranin (PSF), a cationic phenazinium dye, has been used as an extrinsic fluorophore for the present investigation by steady state and time resolved emission spectroscopy as well as steady state fluorescence anisotropy techniques. Extensive exploitation of phenosafranin (3,7-diamino-5-phenyl phenazinium chloride) (Scheme 1) for various photophysical and photobiological applications has been described elsewhere [41–46]. It is red in color with a planar tricyclic phenazinium moiety bearing a positive charge. PSF is a water-soluble dye with a characteristic broad absorption peak in the visible region [42].



Scheme 1. Structures of Triton X-165 and PSF.

In the presence of the surfactant and β-CD, the changes in the spectral characteristics of the probe allow us to investigate interaction of the surfactant with the added cyclodextrin. The intrinsic fluorescence of TX-165 has also been exploited to confirm the mode of binding interaction between the surfactant and the β-CD.

2. Materials and methods

PSF, TX-165 and β-CD were procured from Sigma–Aldrich (USA) and they have been used as received. Triply distilled water is used for making the experimental solutions. All the solvents used are of UV spectroscopic grade (Spectrochem, India). AR grade potassium iodide has been purchased from SRL (India). The concentration of PSF has been kept at ca. 1.0×10^{-5} mol dm^{-3}.

Absorption and steady state fluorescence measurements are performed using a Shimadzu UV-2450 spectrophotometer and a Horiba Jobin Yvon Fluoromax-4P spectrofluorimeter respectively. The steady state fluorescence anisotropy has also been measured using the same spectrofluorimeter. Steady state anisotropy, r, is defined by:

$$r = (I_{VV} - G \cdot I_{VH})/(I_{VV} + 2G \cdot I_{VH}) \quad (1)$$

where I_{VV} and I_{VH} are the intensities obtained with the excitation polarizer oriented vertically and the emission polarizer oriented vertically and horizontally, respectively. The G factor is defined as:

$$G = I_{HV}/I_{HH} \quad (2)$$

I terms refer to parameters similar to those mentioned above for the horizontal position of the excitation polarizer.

For the dynamic light scattering (DLS) measurements, we have used a Malvern Nano-ZS instrument employing a 4 mW He–Ne laser ($\lambda = 632.8$ nm) and equipped with a thermostated sample chamber. The sample is poured into a DTS0112 low volume disposal sizing cuvette of 1.5 ml (pathlength 1 cm). The operating procedure is programmed by the DTS software in such a way that there are averages of 25 runs, each run being averaged for 15 s and then a particular hydrodynamic diameter and size distribution is evaluated.

3. Results and discussion

Although fluorescent probes help in understanding and unveiling the interaction between various components, investigation of the surfactant–CD inclusion complex exploiting an extrinsic fluorescent probe is fraught with difficulties as there are three possible equilibria with respect to the mutual interactions which might operate simultaneously: surfactant–cyclodextrin, surfactant–fluorophore and cyclodextrin–fluorophore. The fluorescence intensity/lifetime might be affected by all these equilibria and so, it is not a trivial task to correlate the observed changes in the fluorometric parameters directly with the interaction between the CD and the surfactant. The results of such an experiment would depend strongly on the fluorophore used as well as the nature of the head group region of the micelle. This aspect highlights the importance of the suitability of the fluorescent probe to be used to study complicated systems and their interactions. With this perspective we intend first to shed some light on the individual equilibria and the extent of interaction of the probe with the surfactant and the cyclodextrin separately. We have then endeavored to study the interaction between TX-165 and β-CD using an extrinsic probe PSF. Independently, the intrinsic fluorescence of TX-165 has also been exploited to confirm the binding interaction between the surfactant and β-CD.

3.1. PSF–Triton X-165 interaction

PSF shows a broad unstructured absorption band at around 520 nm in aqueous solution. Addition of TX-165 leads to a red shift of ~10 nm indicating that the ground state of PSF experiences a lower polarity in the micellar environment (see *Supporting* material, S1).

Room temperature emission spectrum of an aqueous solution of PSF shows a single broad and unstructured charge transfer (CT) emission band with a maximum at around 587 nm [41,45]. With the addition of TX-165 at its lower concentration range the fluorescence intensity of PSF decreases gently. Such a variation in the fluorescence intensities in the presence of lower concentrations of surfactants is quite common and is attributed to the formation of pre-micellar aggregates [46,47]. Mishra et al. [17] rationalized that the decrease in the fluorescence occurs as because at low concentrations of the surfactant (in the pre-micellar region) several dye molecules crowd on each surfactant molecule and thereby, the dye molecules are brought in close proximity and such complexes are non fluorescent in nature due to rapid self-quenching process. This leads to a decrease in fluorescence intensity in the pre-micellar region. These non fluorescent adducts are disrupted at higher surfactant concentrations (at and above CMC), forming micellized monomers which are fluorescent and thereby fluorescence intensity increases above CMC [17]. Consistent with the literature [43], at higher surfactant concentrations, PSF shows a remarkable enhancement in the emission intensity with a reasonable blue shift of ~15 nm (Fig. 1).

Inset of Fig. 1 shows the variation of the fluorescence intensity as a function of the surfactant concentration and allows one to determine the critical micellar concentration (CMC) of the surfactant [48,49]. The blue shift of the fluorescence maximum indicates that the polarity of the micellar environment is less than the polarity of the bulk water. This is consistent with the previous studies of PSF in biological and bio-mimicking environments [41–45].

As mentioned above, depending on the polarity/charge characteristics of the probe and the micelle, a probe can reside either in the core or the palisade region of the non-ionic micelle or can distribute itself within these two distinct regions [50,51]. At the saturation level of the probe–micelle interaction, the final position of

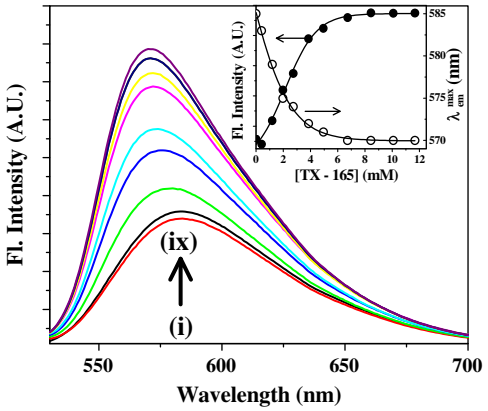


Fig. 1. Emission spectra of PSF solution with added TX-165 ($\lambda_{ex.}$ = 520 nm). Curves (i) → (ix) correspond to 0, 0.6, 1.8, 2.7, 3.8, 5.0, 6.7, 8.4 and 12.0 mM TX-165. Inset shows the variation of the fluorescence intensity and peak maximum with added surfactant.

the emission maximum of PSF is observed to be around 570 nm. The observed emission maximum of PSF in TX-165 is close to the fluorescence maximum of the probe in pure methanol solution [17]. Literature reports also suggest that the environment in the micellar interface often resemble to alcohol systems [52]. Thus the present observation indicates that the fluorophore resides in the micelle-water interfacial region and does not penetrate deep into the less polar micellar core which seems logical considering the cationic nature of the fluorophore.

3.1.1. Probe–micelle binding

Binding constant for the binding of the fluorophore with the non-ionic TX-165 micellar system has been determined to understand the degree of interaction between the dye and the micellar units. The binding constant calculation follows the method described by Benesi and Hildebrand using the fluorescence intensity data [53]. The corresponding equation is given below.

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{\max}} + \frac{1}{\Delta I_{\max} \times K_b} \times [L]^{-1} \tag{3}$$

where $\Delta I = (I - I_0)$ and $\Delta I_{\max} = (I_\infty - I_0)$. I_0, I and I_∞ stand for the integrated fluorescence intensities of PSF in the absence of surfactant, at an intermediate surfactant concentration and at the saturation level of PSF and TX-165 interaction; K_b being the binding constant and $[L]$ the micellar concentration. The micellar concentration $[L]$ is defined by Almgren as [54]

$$[L] = (S - \mathrm{CMC})/N \tag{4}$$

where S represents the surfactant concentration and N is the aggregation number of a micellar system (here $N = 82$) [5,48,49]. The binding constant has been determined to be 2.4×10^4 l mole^{-1} from the plot of $1/\Delta I$ against $[L]^{-1}$ (see *Supporting* material, S2).

The binding constant of PSF with TX-165 is found to be slightly less than those obtained for the same probe with the anionic S_nS micelles [43]. A higher value of the binding constant in case of anionic S_nS micelles as compared to that with the non-ionic TX-165 is justified from a favorable electrostatic interaction between the cationic probe and the anionic S_nS micelles [43].

3.2. PSF–β-CD interaction

In a different context, a detail study of the interaction of PSF with β-CD has been reported in one of our recent publications [55]. The essence of the study relevant to our present work is the value of the binding constant (K_b = 82.0 l mole^{-1}) for the formation of the PSF–β-CD inclusion complex. The low value of K_b implies a

partial encapsulation of the probe inside the CD cavity which is justified from the relative dimensions of PSF and β-CD cavity [55].

3.3. Triton X-165–β-CD interaction: probed by using external fluorophore PSF

After having an understanding relating to the interaction of PSF with TX-165 and β-CD individually, we endeavored to explore the surfactant–β-CD interaction through the fluorometric behavior of the external probe, PSF.

3.3.1. Steady state absorption and emission study

As discussed above, the absorption maximum of PSF is red shifted from 520 nm in water to 530 nm at the saturation level of PSF–TX-165 interaction. However, the absorption spectrum of PSF in aqueous medium does not change appreciably upon addition of β-CD. At the saturation level of interaction of PSF with TX-165, addition of β-CD results in a blue shift of the absorption maximum from 530 nm back to ~520 nm (figure not shown).

We have already noticed a remarkable increase in the fluorescence intensity of PSF associated with a hypsochromic shift from 587 nm in water to 572 nm at the saturation level of PSF–TX-165 interaction (*vide* Fig. 1). Addition of β-CD to an aqueous solution of PSF, however leads to a slight blue shift of ~5 nm with a nominal increase in its fluorescence intensity. Interestingly, at the saturation level of interaction of PSF with TX-165, addition of β-CD leads to a remarkable decrease in the fluorescence intensity with a large red shift from 572 nm (in 12 mM TX-165) to 585 nm (Fig. 2). The dramatic modification in the fluorescence properties of PSF upon addition of β-CD to a solution of TX-165 containing the probe reveals that there is a disruption of the PSF–TX-165 complex. Two possibilities may be ascribed to the role of the β-CD. It can act on either PSF or TX-165 depending on the specific characteristics of the latter two and their binding affinities with β-CD. We have tried to decipher the picture from different steady state studies to be described in the forthcoming sections. Micropolarity studies have been performed in this context and the results are found to be consistent with the other experiments. The detail of this study has been provided in the *Supporting* Material, S3. The fact that the final E_T (30) value approaches close to the E_T (30) value observed in water suggests that PSF is released in water.

3.3.2. Iodide ion induced fluorescence quenching study

Fluorescence quenching study provides information about the accessibility of the entrapped fluorophore towards the quencher and thus an assessment about the location of the probe in different microheterogeneous environments can be made [45,56,57]. In the present work, quenching study using I^- quencher offers valuable information regarding the probable site of location of PSF within the micellar and β-CD environments [26]. It also helps to decipher the final location of PSF after addition of β-CD to the TX-165 micellar solution containing PSF. From this study we can also propose whether PSF is being released to the bulk water or is undergoing a preferential binding with β-CD. It is well known that iodide ion is preferentially available in polar region, i.e., in the water-bilayer interface and in the bulk aqueous phase. It is not supposed to be available in the micellar core due to the very low polarity in the said region. Hence, had the fluorophore been located into the micellar core, there would have been inappreciable fluorescence quenching due to the lack of availability of the quencher in this region. At the saturation level of interaction of PSF individually with TX-165, β-CD and TX-165–β-CD environments, fluorescence quenching studies have been performed and the quenching of the fluorescence of PSF with the addition of quencher (I^-) is found to follow the Stern–Volmer relation:

$$F_0/F = 1 + K_{SV}[Q] \quad (5)$$

where F_0 and F are the fluorescence intensities in the absence and the presence of the quencher respectively, [Q] is the molar concentration of the quencher, K_{SV} is the Stern–Volmer quenching constant. Fig. 3 depicts the Stern–Volmer plots as a function of bulk concentration of I^- in water and the three microheterogeneous environments. The slope of each plot gives the Stern–Volmer quenching constant (K_{sv}). The estimated K_{SV} values are collected in Table 1.

Fig. 3 and Table 1 reveal that the fluorescence quenching of PSF in micellar as well as β-CD environments is appreciably lower than that in water. This indicates that the fluorophore is buried both in the micelle and the CD, although to a different extent, as compared to that in water. The degree of exposure of the probe towards the ionic quencher is appreciably reduced in these organized assemblies making it inaccessible to the quencher and thereby resulting

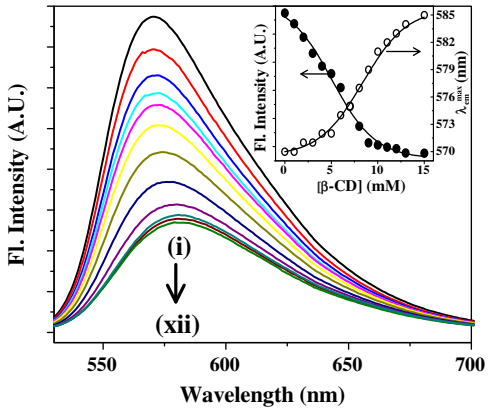


Fig. 2. Emission spectra of PSF in the presence of 12 mM TX-165 with added β-CD ($\lambda_{ex.}$ = 520 nm). Curves (i) → (xii) correspond to 0, 1, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 mM β-CD. Inset shows the variation of the fluorescence intensity and peak maximum with β-CD concentration.

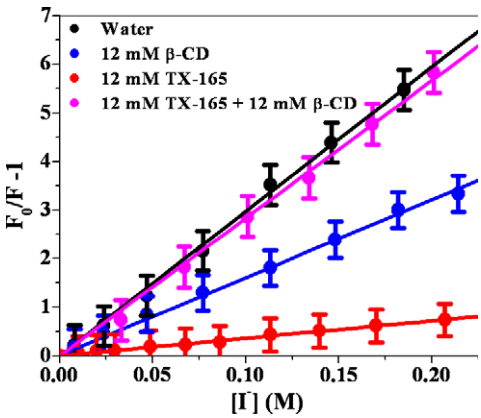


Fig. 3. Stern–Volmer plots for the fluorescence quenching of PSF by I^- ions in different environments. The environments are shown in the legends.

Table 1
K_{SV} values for the quenching of PSF in different environments.

Environments	K_{SV} (M^{-1})
Water	30.4 ± 1.5
TX-165 (12 mM)	9.6 ± 0.5
β-CD (12 mM)	16.1 ± 0.8
TX-165 (12 mM) + β-CD (12 mM)	28.2 ± 1.5

in a drop in the K_{SV} values. Since the concentration of both TX-165 and β-CD are in the millimolar range, whereas that of PSF is in the micromolar range, it is unlikely that there will be more than one PSF molecule per micellar unit or CD molecule. Upon gradual addition of β-CD to the probe bound TX-165, the K_{SV} value increases and in the presence of sufficient concentration of β-CD, approaches a value close to that obtained in water. Proximity of these two values confirms that addition of β-CD to a solution of PSF containing TX-165 disrupts the PSF–TX-165 complex resulting in the release of PSF into the bulk aqueous medium.

3.3.3. Steady state fluorescence anisotropy study

Increase in the rigidity of the environment surrounding a fluorophore results in an increase in the fluorescence anisotropy [56]. It reflects the degree of rigidity imposed on the probe in the microheterogenous medium. Fig. 4 shows the variation of the fluorescence anisotropy of PSF with increasing concentrations of TX-165 and β-CD separately.

As is obvious from the figure, the steady state fluorescence anisotropy increases in both the micellar and in β-CD environments from that in aqueous solution and finally reaches the corresponding saturation values. This indicates that the fluorophore is trapped in motionally restricted regions within β-CD as well as the micellar environments.

Interestingly, when β-CD is added to the probe bound TX-165, the fluorescence anisotropy value is found to decrease. The final value agrees well with that observed in the aqueous environment. This indicates a weakening of the interaction of PSF with TX-165 upon addition of β-CD; eventually leading to the release of PSF into water. This is consistent with the inference drawn from the I^- induced quenching study discussed above as well as the micropolarity measurements described in the *Supporting* material, S3.

3.3.4. Determination of apparent CMC of TX-165 in the presence of β-CD

We have determined the apparent CMCs of TX-165 in the presence of varying concentrations of β-CD. These CMCs are obtained from the fluorometric titration of PSF upon addition of TX-165 in the presence of different preset concentrations of β-CD (representative plots are shown in the *Supporting* material, S4). The CMC obtained (∼0.43 mM) in the absence of β-CD corresponds well with the literature values [48,49].

The variation of the apparent CMC of TX-165, thus obtained, in the presence of different concentrations of β-CD using PSF as an extrinsic probe is shown in Fig. 5. A linear plot was obtained with a very good correlation coefficient ($R = 0.995$). The apparent CMC of TX-165 increases remarkably from 0.43 mM in water to 9.5 mM in the presence of 10 mM β-CD (Table 2).

The increase in the apparent CMC in the presence of β-CD implies that all the TX-165 molecules are not available for the micelle formation process. The obvious explanation to this is that TX-165 undergoes an inclusion complexation with β-CD thereby inhibiting the micellization process [5–7,39]. Hence micellization of TX-165 occurs at a higher surfactant concentration in the presence of β-CD. A similar change in the fluorescence of chlorine p_6 was observed by Mishra et al. upon addition of cationic surfactant CTAB and non-ionic TX-100 in the presence of varying concentrations of α- and β-CDs [17]. The apparent CMCs were reported to be much higher than the respective normal values. This was inferred in the light that the free surfactant monomers undergo interaction with the cyclodextrins forming surfactant–CD inclusion complexes.

The study although implies complexation between TX-165 and β-CD, fails to depict the stoichiometry of the complex. To decipher it we have performed the steady state fluorometric and fluorescence anisotropy studies exploiting the intrinsic fluorescence of TX-165 with the addition of β-CD.

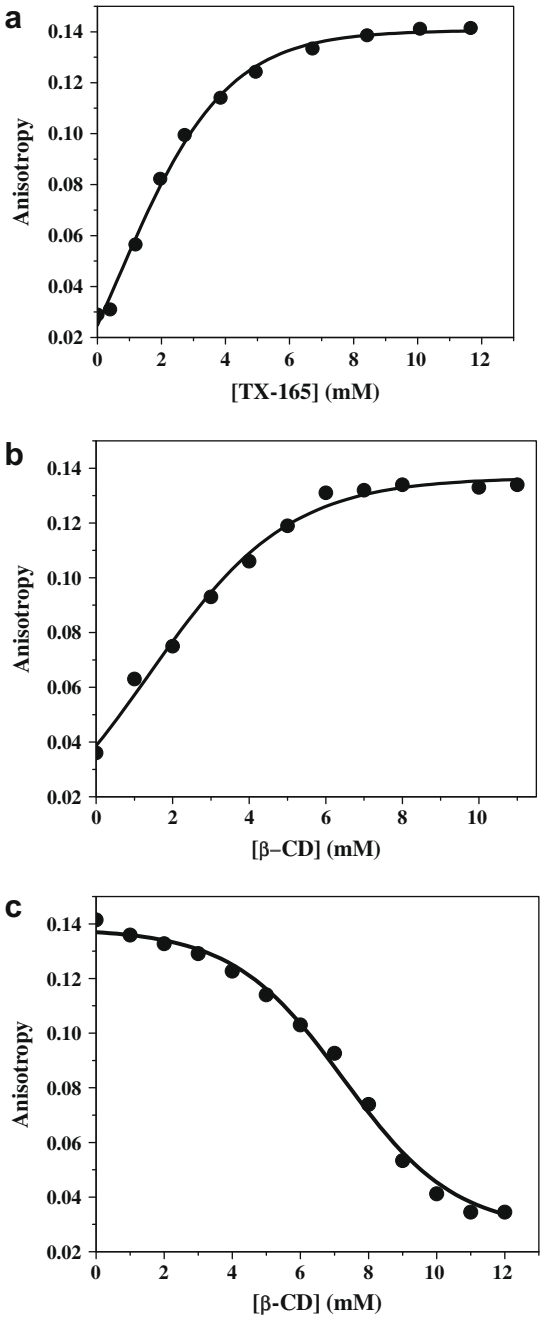


Fig. 4. Variation of fluorescence anisotropy of PSF as a function of increasing concentration of (a) TX-165, (b) β-CD and (c) β-CD in the presence of 12 mM TX-165.

3.4. TX-165–β-CD Interaction: probed by exploiting the intrinsic fluorescence of TX-165

Probing the intrinsic fluorescence of TX-165 is a smart technique that can be exploited to study the formation of the inclusion complex between TX-165 and β-CD. This is because it is free from the existence of multiple equilibria that develop when an external fluorophore is involved. The essence and originality of the present work lie in cleverly using both extrinsic and intrinsic fluorophore labels to describe the TX-165–β-CD interaction: a way-out which has hence before not been undertaken. He et al. investigated the interaction of TX-100 with β-CD using the intrinsic fluorescence of TX-100 only and thereby determined the stoichiometry of the surfactant–CD complex formed [39].

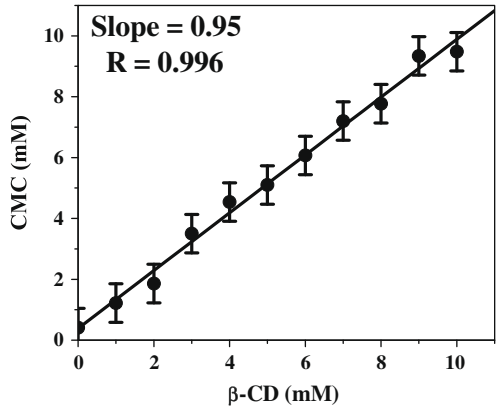


Fig. 5. Variation of apparent CMC of TX-165 in the presence of varying concentration of β-CD.

Table 2
Values of CMC of TX-165 at different concentrations of β-CD.

[β-CD] (mM)	Apparent CMC of TX-165 (mM) (error ± 5%)
0	0.43
1	1.22
2	1.86
3	3.50
4	4.54
5	5.10
6	6.07
7	7.20
8	7.77
9	9.34
10	9.48

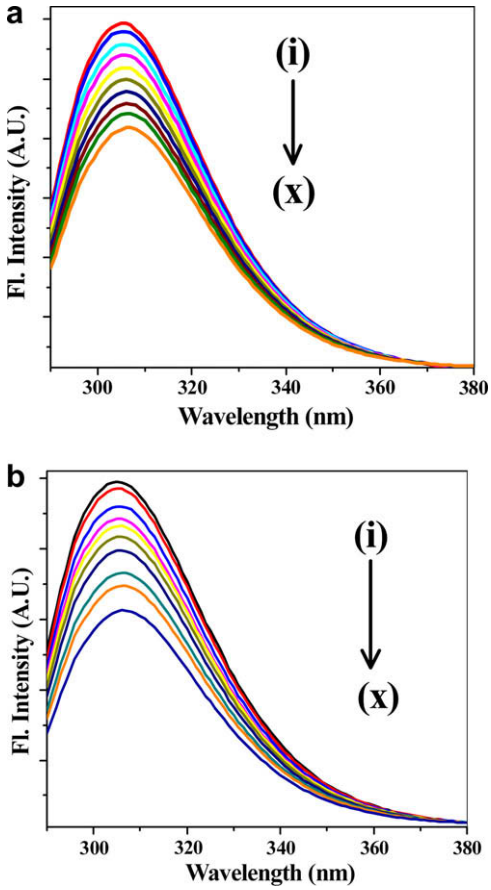


Fig. 6. Emission spectra of (a) 0.1 mM TX-165 and (b) 0.5 mM TX-165 with the addition of β-CD. Curves (i) → (x) corresponds to 0, 2, 3, 4, 5, 6, 7, 9, 10 and 12 mM β-CD. $\lambda_{ex.}$ = 280 nm.

Phenyl group present in TX-165 absorbs appreciably at 280 nm giving a somewhat broad fluorescence peaking at 306 nm. Addition of β-CD to a solution of TX-165 both in the pre-micellar and micellar concentration (0.1 mM and 0.5 mM) leads to a decrease in its fluorescence intensity of TX-165 (Fig. 6). This indicates that the environment around TX-165 is modified in the presence of cyclodextrin. The following discussion reveals that the formation of an inclusion complex between TX-165 and β-CD is responsible for the effect.

The dependence of the TX-165 fluorescence on β-CD concentration is analyzed using Benesi–Hildebrand equation at both pre-micellar and micellar conditions Eq. (3) [53]. Linearity of the plots (see *Supporting* material, S5) substantiates 1:1 complexation between TX-165 and β-CD. A similar stoichiometry as reported by Smith et al. for the TX-100–β-CD inclusion complex strengthen our findings [58].

Quenching study using I^- quencher is performed in order to examine the accessibility of the CD-bound TX-165 to the external ionic quencher. Fig. 7 presents the Stern–Volmer plots for the quenching of the fluorescence of TX-165 (0.1 mM and 0.5 mM) by I^- ion in the absence and the presence of 12 mM β-CD. The slopes give the Stern–Volmer quenching constants (K_{SV}). The K_{SV} values are estimated to be 12 ± 2 l mole^{-1} and 2 ± 0.5 l mole^{-1} in aqueous medium and in 12 mM β-CD respectively in both the cases. A substantial decrease in the K_{SV} value in the presence of β-CD as compared to that in aqueous solution reflects that the phenyl group is much less accessible to the ionic quencher and thereby corroborates the encapsulation of the phenyl group of TX-165 surfactant inside the β-CD cavity.

Self-aggregation of cyclodextrin is another matter that is necessary to be taken into account while making studies with β-CD. Cyclodextrins are known to self-aggregate through hydrogen bonding to form nanostructures. While α- and γ-CDs form only spherical aggregates in water medium, β-CD forms elongated nanostructure [59,60]. This difference is referred to the relatively poor water solubility of β-CD compared to the other two. However, β-CDs are known to self-aggregate only above ~16 mM in aqueous solutions [59]. In the present study all the experiments related to TX-165–β-CD inclusion complexation are performed within 0–12 mM β-CD to avoid the effect of self-aggregation. Formation of the TX-165–β-CD complex is supposed to prevent the self-aggregation process because of the presence of the long PEO chain of TX-165 (Scheme 2). Similar examples are available in the literature with other CDs and surfactants [61,62]. Lastly, to confirm the negligible role of self-aggregation of β-CD in the concentration range of our study, we have performed the DLS studies at both lower (5 mM) and higher concentrations (10 mM) of β-CD in the absence as well as in the presence of TX-165. Monomodal distributions are obtained in all the cases and the distribution patterns are same within experimental error limit (figure not given). This establishes that the self-aggregation of β-CD has insignificant role, if any at all, to play with the present study.

Analysis of the molecular dimensions of the Triton X surfactants and the CD cavity sizes aid in the understanding of the binding behavior. For TX-100, a 1:1 stoichiometry is normally accepted [27–34], though some workers showed the coexistence of both 1:1 and 1:2 stoichiometry [38,39]. The study of Smith et al. through fluorescence and NMR experiments showed that the terminal methyl groups of TX-100 are not involved in the interaction inside the β-CD cavity [58]. We adopt a similar model for the present case of TX-165. A substantial decrease in the K_{SV} value in the

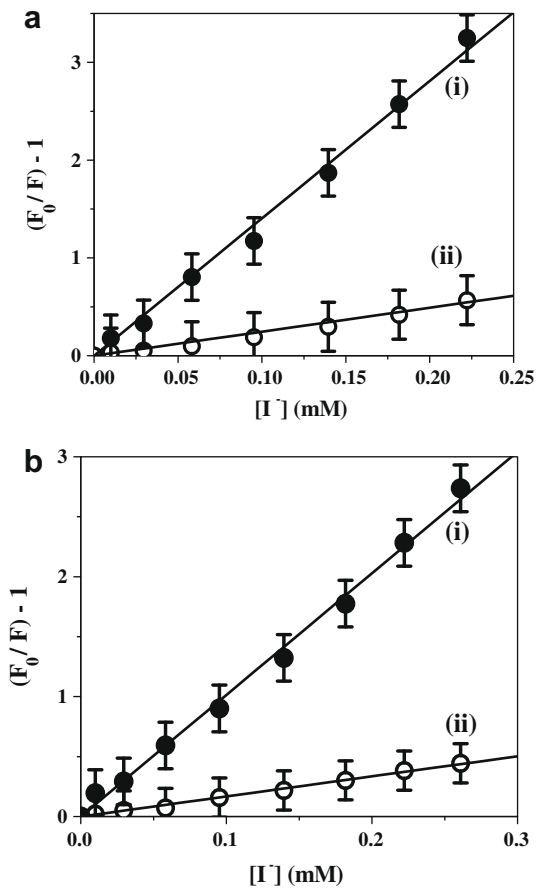
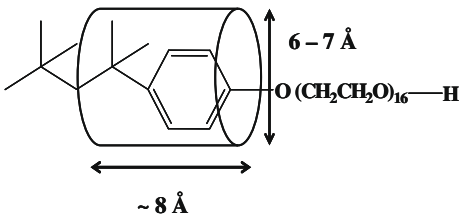


Fig. 7. Stern–Volmer plots for the fluorescence quenching of (a) 0.1 mM and (b) 0.5 mM TX-165 by I^- ions in aqueous solution (i) and in 12 mM β-CD (ii).



Scheme 2. Proposed structure of the inclusion complex between TX-165 and β-CD.

presence of β-CD as compared to that in aqueous solution reflects that the phenyl group is encapsulated inside the β-CD cavity. As obtained from the optimized dimension of TX-165 molecule at the semi-empirical AM1 level using Hyperchem 6.0 software package, the cross sectional diameter of TX-165 with fully extended PEO chain is found to be 6.27 Å which matches well with the diameter of β-CD (6.5 Å) [1,26]. Since the cavity depth of β-CD is too small (7.9 Å) to accommodate the extended PEO chain lengthwise (34.6 Å), the possibility of encapsulation of the head group region of TX-165 containing the PEO chain is ruled out. However, there is a report by Du et al. on TX-100–β-CD complexation claiming that the flexible PEO chain (10.4 Å long) stays in a curled fashion inside the CD cavity [26]. They inferred from NMR and molecular optimization studies that the volume of the CD cavity is already small enough compared to the added volume of the n-octyl group of the TX-100 along with the phenyl group and therefore 1:2 complexation is impossible [26]. Pineiro et al. investigated the host-guest stoichiometry for the surfactant octyl-β-D-glucopyranoside and β-CD and found that the predominant species is 1:1 and to a very minuscule extent 2:1, disregarding the existence of the 1:2. TX-165 having a longer head group chain length compared to TX-100 makes the formation of >1:1 stoichiometry negligible, if not impossible. Linearity of the Benesi–Hildebrand plots monitoring the intrinsic fluorescence of TX-165 with the addition of β-CD substantiates the formation of 1:1 complexation between TX-165 and β-CD. In all, the experimental observations and analysis of the molecular dimensions, in the present case, propose the formation of 1:1 inclusion complex between TX-165 and β-CD. Calculations suggest that the dimension of the part of TX-165 molecule excluding the terminal methyl groups and the PEO chain turns out to be ~7 Å, which can be accommodated comfortably within the β-CD cavity. The PEO head group of TX-165 gains extra stabilization through the hydrogen bonding interactions with the alcoholic –OH groups available at a higher concentration in the rim region of the CD. Thus, the inclusion complex of TX-165 may have a schematic structure as depicted in Scheme 2.

4. Summary

The interaction between TX-165, a non-ionic micelle-forming surfactant, and β-CD has been studied following a series of steady state fluorescence and fluorescence anisotropy studies. The work is interesting from the viewpoint that both extrinsic and intrinsic fluorescences have been exploited for the purpose. It is found that addition of β-CD to a solution of PSF bound TX-165 leads to the preferential complexation of TX-165 with the added β-CD resulting in the release of the fluorophore into the bulk aqueous phase. Formation of the inclusion complex between TX-165 and β-CD is found to inhibit the micellization process leading to an increase in the apparent CMC of TX-165 in the presence of β-CD. 1:1 stoichiometry for the TX-165–β-CD inclusion complex has been confirmed exploiting the intrinsic fluorescence of TX-165. It is well known that the study of the surfactant–CD inclusion phenomenon is of paramount significance, particularly in the field of drug delivery and transport, since surfactants often mimic the cell membranes. The present work gets importance since this is an exhaustive study on the formation of the inclusion complex between β-CD and a member of the Triton X series other than TX-100. The study is expected to trigger more exploration in the field of drug delivery and transport.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2010.03.048.

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