Chapter 2

Scope and summary of the work
Fig. 2.1: Topological description of an organized assembly.

Scheme 2.1

R = CnH2n+1 (n = 1, 3, 5, 6, 8, 10, 12, 14, 16, 18)
X = I, Br
Micelles have the potential to mimic membrane system and to interact with various neutral and charged molecules. The solubilization of an organic molecule in surfactant micelles depends on the nature of the hydrophobic group and charge in the substrate and the micelle. A micellar solution provides three topological regions (Fig. 2.1) i.e. inside (I), boundary (B) and outside (O) where solubilization may occur. Stilbazolium dyes have been widely used as fluorescent probes to identify the localization site of the dye in the micelle. The local concentration of the dye in any region is controlled by the mutual charge and hydrophobicity. These dyes have also been used as a potential sensor for the various microenvironments like micelle, microemulsion, membrane etc. Transportation of various solutes through cellular membranes has generated interest in studying the structure of carrier proteins linked with membrane. Several mechanisms have been proposed for the voltage sensitivity of fluorescent styryl dyes for visualization of voltage transient in membrane preparation such as, electrochromism, dye reorientation, formation of twisted intramolecular charged transfer (TICT) state.

In the present work embodied in the thesis, the interaction and solubilization of a series of styryl pyridinium dyes of varying chain length (2.1, C\textsubscript{1} to C\textsubscript{18}) with surfactant micelles of different charge i.e. Cationic (CTAB), nonionic (TX-100) and anionic (SDS) have been studied by absorption, fluorescence and time resolved spectroscopy.

Chapter-1 is a review of the work done on cyanine dyes during nineties especially in the fields of self-aggregation, nonlinear optical properties (NLO), adsorption, photoisomerization, association with organized assemblies, use as fluorescent sensor for different therapeutic treatment and as complexing agent.

The synthesis of styryl pyridinium dyes is represented in scheme-1. The carbon chain in ‘R’ is represented as C\textsubscript{n} and the dyes are referred to as C\textsubscript{1}, C\textsubscript{3}, etc. depending on the value of ‘n’ in C\textsubscript{n}. These dyes have been characterized by melting point, elemental analysis, NMR (\textsuperscript{1}H and \textsuperscript{13}C), Mass and UV-Vis spectroscopy. These compounds are trans in nature since the J value of the ethylenic protons (H\textsubscript{a} and H\textsubscript{b}) is 16.1Hz in all cases. From the X-ray crystallographic study of the C\textsubscript{1} dye, it has been observed that the dye exists in trans-isomer and is approximately planar. The dihedral angle between the phenyl ring plane and pyridyl ring plane is 10.5(3)°. The interesting feature of the
molecule is that the iodide ions are situated in the crystal lattice such that they are 0.216 Å closure to pyridine nitrogen than amino nitrogen. The position parameters, bond distance, bond angles and torsional angle have been obtained from the X-ray crystallographic study. The cis-isomer for C₁ and C₃ dyes has been synthesized and is found to be hygroscopic in nature. These isomers have also been characterized by NMR, Mass. C¹³ spectral data. The J value of the ethylenic protons is found to be 8.0 Hz. All the spectral data are consistent with the expected structure of the compounds. The purification of solvents and surfactants has been carried out by the literature method. Triple distilled water has been used to prepare the micellar solutions. Details of experimental procedures have been given in this chapter. The details are presented in Chapter-3.

Chapter-4 contains a detailed analysis of the species in the excited state in different solvents and micellar systems of CTAB, SDS and TX-100. The following observations have been made:

[a] Fluorescence spectra in organic solvents: The spectra of these dyes are very similar to each other in a single solvent except in water in which the solubility of some dyes with long alkyl chain is poor. The spectral peak position and Stokes shift show a variation of less than 5 nm in a single solvent indicating that the electronic transition of the styryl pyridinium chromophore is not perturbed significantly by the length of the alkyl chain attached to the pyridinium nitrogen. There is however a significant variation in the spectral peak position and Stokes shift for the dye in different solvents. The fluorescence decay is two exponentials in most of the solvents. In few solvents, however, the decay is a single exponential or a sum of three exponential. The fluorescence decays are fitted to two exponentials (butanol and chloroform) or a single exponential (methylene chloride). The lifetime results are similar for other dyes. These three lifetime ranges are: short lifetime (τ₁ < 0.3 ns), middle lifetime (τ₂ = 0.4 – 0.8 ns) or long lifetime (τ₃ > 1 ns). In the solvents of medium viscosity, the fluorescence lifetimes were found to consist of a short lifetime (τ₁) which is the major component except in chloroform and dichloromethane. The short lifetime component is associated with the trans-isomer of the dye, which is the major component. The species due to τ₂ and τ₃ are minor components in all the solvents under study except in chloroform and dichloromethane. The two lifetimes associated with cis- and quinoid forms of the dyes. Therefore the presence of cis and/or quinoid form is significant in these two solvents. The quinoid form of the dye is a mesomeric form of the trans isomer and can not be synthesized. The long lifetime τ₃ observed in few solvents and in surfactant solution is associated with the quinoid form.
Fig. 2.2: Potential energy diagram which explains the possible coexistence of the three isomers of the aminostyryl pyridinium dye in organic solvents and surfactant solutions. The quinoid form is the intermediate structure between the cis and trans-isomers.
[b] SDS: The interaction of these dyes with anionic SDS is expected to be coulombic one for the formation of dye-surfactant aggregate. The time-resolved fluorescence decay is measured for all the dyes in 2, 4 and 20 mM of SDS. The [dye] is maintained at 0.02 mM. The fluorescence decays are fitted to two or three exponentials. At low surfactant concentration ([SDS] = 2mM) all the three life times are observed but in [SDS] = 4 and 20 mM, the short lifetime is predominant.

c] CTAB: The change in absorption spectra of these dyes in CTAB solution depends on the alkyl chain length attached to the dye. The spectrum shifts to red end as the surfactant concentration increases to micellar regime. Time resolved fluorescence decay of these dyes is measured in 0.8, 4 and 10 mM CTAB solution. A significant deviation from the general trend is observed for C12 to C18 dyes at low [CTAB]. The long lifetime component is significantly higher in magnitude in these dyes. The short lifetime component, associated with the trans form of the dye, is the dominant component for the dyes (n<10), in SDS and CTAB. The τ2 and τ3 associated with cis and quinoid forms respectively are generally minor components in SDS and CTAB.

d] TX-100: The absorption spectra show no significant change for the dyes in this system as compared to SDS and CTAB. The fluorescence lifetime are measured at 0.6 and 11.88 mM of TX-100. The medium lifetime τ2 is significantly higher in TX-100 solution as compared to that in other surfactants. The oxyethylene head group in the surfactant monomer binds the dye of lower chain length. Like chloroform the solvation energy (as reflected in binding constant) is high in TX-100 (if TX-100 is taken as solvent).

The synthesized cis-isomer on excitation gives three lifetimes in all the solvents under study. So the presence of all the three species in the excited state from both cis and trans isomer have been proposed in this chapter. The correlation of v_max and Stokes shift with solvent polarity scale E(t)(30) and Δf have also been observed. A possible potential energy diagram for the dyes in the ground and excited state has been proposed (Fig. 2.2). The quinoid form shows a metastable or stable state depending upon the dye-solvent interaction. The quinoid form is the intermediate between the trans- and cis- species.

Chapter-5 deals with the role of alkyl chain in the localization of this styryl pyridinium dyes (2.1) in hydrophobic force field of cationic surfactant (CTAB) assembly.

Absorption spectra: All the dyes show λ_max at 450 nm in aqueous medium. The increase in absorption maxima in CTAB medium depends on the carbon chain length of the dyes. The C1-C5
dyes do not show any change in $\lambda_{max}$ and the optical-density values in presence of varying concentrations of CTAB surfactant (up to 10 nm), whereas a change is starting with $C_6$ dyes is noticed. The $\lambda_{max}$ values of the dyes in CTAB solutions increases with increasing chain length of the dye, the increase being sharp from $C_6$ to $C_8$ and then almost becomes constant. Both $C_{12}$ and $C_{14}$ dyes are found to form a turbid zone within 1-3.5 mM of CTAB. The $\lambda_{max}$ of $C_{12}$ dye does not change in the turbidity zone, whereas, that of $C_{14}$ dye decreases to 397 nm from 457 nm. The peak at 398 nm is nonexistant at 4.0 mM of CTAB, and only a peak at 478 nm is obtained. Both $C_{16}$ and $C_{18}$ dyes show a $\lambda_{max}$ value at 416 nm with shoulders at 400 and 478 nm in the absence of surfactant. Upon the addition of a surfactant the peak at 400 nm becomes prominent at the cost of the 416 nm peak, which completely disappears in favour of the 478 nm peak at 5 mM of CTAB. $C_{18}$ dye also exhibits a similar behaviour like that of the $C_{16}$ dye. The break points for CTAB in the presence of these dyes were found to be 3.5 mM. This value is much higher than the CMC value of CTAB (0.9 mM) as reported in literature.

**Emission Spectra:** All the dyes show a maximum in the emission spectra at 581 nm in 2% (v/v) MeOH-H2O solvent system. The fluorescence maxima are not affected by a change in the [surfactant]; only a shift by about 3 nm takes place. However, the intensity increases by a great deal, and is dependent on the carbon chain (n) of the dye.

It is evident from the absorption data that the dyes can be classified to three categories, namely (i) $C_1$-$C_5$, (ii) $C_6$-$C_{14}$, (ii) $C_{16}$ and $C_{18}$. Though the absorption maxima, the molecular extinction coefficient and $\lambda_{em}$ values do not change for $C_1$ to $C_5$ dyes, the intensities of the emission of these dyes gradually increase upon addition of CTAB. The absorption at around 400 and 478 nm for both $C_{16}$ and $C_{18}$ dye are ascribed to dimeric and monomeric states respectively. With increasing temperature the [dimer] decreases along with a concomitant rise in the [monomer], the decrease/increase however, levels off at about 50 °C. The dimeric peak disappears completely at 5 mM CTAB, indicating complete absorption of the dye as a monomer into the micelle. Therefore, no change in $\lambda_{max}$ is noticed after 5 mM CTAB.

A micelle has both electrostatic and hydrophobic fields ($F_{H}$), the strength of which decrease with the distance from the surface of the micelle. Albeit the electrostatic repulsion of the cationic dyes due to the cationic surface of the CTAB micelle, their location around the micellar interface may be ascribed to the effect of hydrophobic field on the dyes having varied hydrophobicity. Thus, the $C_1$-$C_5$ dyes occupy a region of low hydrophobic field as a result small changes in the intensity of
Fig. 23. Schematic representation of localization of dye in a CTAB micellar force field gradient with respect to $E_T(30)$ scale. The asymptotic curve is a quantitative representation of the decrease of hydrophobic force ($F_h$) field with distance.
emission takes place. C_{16} and C_{18} dyes remain in dimer \leftrightarrow \text{monomer} in the absence of surfactant and form a mixed micelle with the surfactant. The region of occupation of the dyes in a CTAB micellar solution is expressed with the polarity scale $E_r(30)$ of the MeOH-H$_2$O system. The hydrophobic field ($F_H$) strength decreases exponentially with a function of the distance from the micellar droplet. The core of the micelle is of constant hydrophobic field corresponding to n-alkane, which then decreases with increasing distance/ $E_r(30)$ from the interface of the micelle (Fig. 2.3). Therefore, it is possible to delineate the location sites of the dyes from both the absorption and emission spectra.

The maximum wavelength of emission, $\lambda_{em}^{\text{max}}$, values of the dyes remain the same up to a certain concentration of CTAB (3.5 mM), after which a slight hypsochromic shift is noticed, which may be due to medium rheology. The increase in the emission intensity also becomes perceptible from C$_6$ dye.

A two-state binding model (scheme-2.2) is used to determine the ground-state association constant ($K_M$) for the association of the dye (D) and the micelle (M) to give the association species MD. In order to ascertain the 'open' or 'closed' environment of the dye (D), the binding constant values of the dyes to the micelle (M) have been determined by using eq. 2.2.

Scheme-2.2:

$$K_M$$

$$M + D \leftrightarrow \text{MD}$$

So,

$$K_M = \frac{[\text{MD}]}{[D][M]} \quad \ldots 2.1$$

$$I_i = \frac{I_w}{1 + K_M [M]} + \frac{I_m K_M [M]}{1 + K_M [M]} \quad \ldots 2.2$$

In this equation $I_i$, $I_w$ and $I_m$ are the intensities at any [surfactant], in water and in micellized surfactant respectively.

The binding constant ($K_M$, eq.-2.2) increases with increasing chain length up to C$_{16}$, and then decreases. The binding of C$_{16}$ with CTAB is maximum due to compatibility in the chain length.
Chapter-6 deals with the interaction of these dyes with nonionic surfactant (TX-100) solution and the role of alkyl chain during solubilization.

Absorption spectra: All the dyes except C_{16} and C_{13} show $\lambda_{\text{max}}$ values within 450-457 nm in 2% (v/v) MeOH-H_{2}O. The peak at 416nm with a shoulder at 400nm is ascribed to the dimer of the dyes. These dyes remain in the dimer form mostly because of the hydrophobic interaction of the long alkyl chains. The shoulder at 475 nm indicates a relatively low concentration of the monomer in the solvent system. On addition of TX-100 the peak due to the monomer only is obtained at 473 nm which indicates the interaction of the monomeric form of the dyes with the hydrophilic head group of the surfactant resulting in the shift of the dimer-monomer towards the monomer. Further addition of TX-100 results in a bathochromic shift in the absorption maximum of all the dyes. The $\lambda_{\text{max}}$ values for all the dyes however attain constant values after a certain concentration of the surfactant depending upon the chain length of the dye. These $\lambda_{\text{max}}$ values are fitted to a standard curve of $\lambda_{\text{max}}$ of dyes in various solvents and their $E_{T}(30)$ values. The $E_{T}(30)$ value is found to decrease linearly with increasing C_{n} up to about C_{10} and then remains constant. Thus the incorporation of the dyes in the micelle is in consistent with the current view that the longer the carbon chain greater is the extent of incorporation of the dyes. The break-point concentration of the surfactant due to the C_{1} dye agrees with the literature CMC value of TX-100 and the value decreases with increasing chain length of the dye.

Emission spectra: On addition of surfactant a bathochromic shift by about 3-9 nm is noticed initially followed by a hypsochromic shift by about 30-3 nm for C_{1} to C_{12} dyes. The C_{1} dye shows a maximum shift of 30 nm, which then decreases with increasing alkyl chain of the dye up to C_{12}. The C_{14} to C_{15} dyes do not exhibit such shifts. Only the C_{1} dye shows an interesting phenomenon that on addition of surfactant initially the emission band at 581 nm splits into two bands; one appearing at 590 and the other at 558 nm. Both the bands gradually converge on gradual addition of surfactant and a single peak at 560 nm obtained at a surfactant concentration of 2.95 mM. The $\lambda_{\text{em}}$ value of the C_{1} dye in dioxane (D=2.209) is 556 nm and therefore the C_{1} dye is perhaps distributed in aqueous and micellar phase, the micellar phase dye shows the emission at 558 nm and the aqueous phase dye shows at 590 nm. Further addition of surfactant increases the concentration of the micellar bound dye only with a resultant decrease in the concentration of the free dye. This behaviour is not exhibited by other dyes. All the dyes have very low emission intensity in 2%(v/v) MeOH-H_{2}O solvent system but on addition of the surfactant the intensity increases linearly and sharply up to a certain concentration of the surfactant. The concentration of the surfactant at which the break points
Fig. 2.4: Molecular model of Triton X-100.
is obtained corresponds to the literature value of the CMC of the surfactant for C\textsubscript{1} dye only. The decreasing concentration of the surfactant for the break point in case of dyes with increasing alkyl chain length suggests the decrease in CMC of TX-100. This decrease may be due to a co-operative effect where the alkyl groups of the dyes incorporate greater hydrophobicity to the system thus increasing the probability of micellization at earlier concentration. The intensity of the dye increases due to increasing rigidity of the dye in presence of surfactant. It appears that after the CMC of the surfactant, the micellar structure does not change and therefore the dyes do not experience any change in their environment. But before the CMC, increasing concentration of the surfactant changes the structural environment of the dye. So the sensitivity parameter has been used as a measure of the structural changes of the surfactant before CMC.

TX-100 contains a polyoxyethylene chain with a terminal hydroxy group (Fig.2.4) which has a crown ether like structure and the nonpolar part contains a benzene ring with branched carbon chain. The dye under consideration has a positively charged ionic group with carbon chain of increasing hydrophobicity attached to pyridyl nitrogen. Prior to the formation of the micelle, the monomeric surfactant may be aggregating to premicellar structures, which bind the dye and make it rigid as a result the intensity increases.

It is observed that the binding constant (K\textsubscript{M}, eq.2.2) increases with increasing carbon chain of the dyes. The dye molecules appear to have a strong affinity to a polyoxyethylene group and are incorporated into its exterior region. With micellization of the surfactant the binding becomes stronger as a result the value of K\textsubscript{M} increase by about 7 times. Increasing hydrophobicity of the dye due to increase in the chain length increases the sensitivity of interaction of the dye to the monomers or pre-micellar aggregates. The dyes may be getting adsorbed on the surface of the surfactant monomer or pre-micellar aggregate. Thus it is concluded that the hydrophobicity plays a dominant role in the interaction of the dyes with TX-100.

Chapter-7 deals with the interaction dyes with SDS surfactant aggregates and chain folding of the dyes during solubilization.

Absorption spectra: The $\lambda_{\text{max}}$ values of C\textsubscript{1}-C\textsubscript{8} dyes increase on addition of SDS with a break point at 4.2 mM of SDS, which is taken as the CMC of SDS. This value of CMC however, is lower than the reported value (8.1mM) of SDS, may be because of electrostatic interaction with the dyes. The absorption bands of C\textsubscript{10} to C\textsubscript{18} dyes are broad and are not symmetric. In order to quantify the change, the spectra have been fitted as a sum of Gaussian peaks with the help of a Gaussian
Fig. 2.5. Structural hierarchy of the dyes (C1-C16) in presence of SDS.

Cationic dye molecules with varying chain length:

Surfactant molecules (SDS):
curve-fitting programme. On the basis of absorption data the dyes can be placed in three categories: (i) C_1-C_8 dyes absorb at about 450-455 nm in the absence of surfactant and show a bathochromic shift to about 485 nm at [SDS] > CMC. The C_8 dye shows broad and asymmetric spectrum when [SDS] < CMC, which on deconvolution give two gaussian peaks at 493 and 436 nm, (ii) C_{10}-C_{14} dyes show two gaussian peaks at 461-471 nm and 392-420 nm in the absence of SDS. On addition of surfactant the peaks undergo bathochromic shift, (iii) the nature of spectra of C_{16} and C_{18} dyes in the absence of surfactant are same, which are explained by three gaussian peaks at 472, 418 and 403 nm. On addition of SDS, both 472 and 418 nm peaks undergo bathochromic shift and the 403 nm peak disappears.

On the basis of the solubility behaviour in 2% MeOH-H_2O (v/v) solvent system and SDS surfactant solution in 2% MeOH-H_2O (v/v) the dyes can be classified into four categories: (i) C_1 dye is soluble in water and at all concentration of SDS. (ii) C_3 and C_5 dyes are soluble in water and form turbidity (Fig.2.5C) within a certain range of SDS concentration. On further addition of SDS complete solubilization occurs. (iii) C_6-C_{12} dyes are insoluble in water, but are soluble in 2% MeOH-H_2O system. The solution in MeOH-H_2O exhibits a turbidity zone within a certain concentration range of SDS and then goes into complete solution on further addition of SDS. (iv) C_{14}-C_{18} dyes are insoluble in water and solution of these dyes in 2% MeOH-H_2O do not form any turbidity with SDS. This situation is comparable to the formation of an ionic crystal. As the solubility of the dye decreases with increasing chain length, the number of surfactant molecules required to form the turbidity zone decreases sharply.

**Emission spectra:** The break points at 4.2 mM, which is same as that obtained from absorption spectra. The appearance of a single symmetric peak in the emission spectra of all the dyes clearly indicates the existence of a singular species in the excited state. On the basis of the \( \lambda_{em} \) values, the dyes can be categorized as: (i) C_1 to C_6 dyes show a constant \( \lambda_{em} \) value at 581 nm at all concentration of SDS; (ii) C_8 to C_{14} dyes show a hypsochromic shift from 581 nm to about 560 nm followed by a bathochromic shift to about 578 nm, with increasing [SDS]; (iii) C_{16} and C_{18} dyes under similar condition show a hypsochromic shift from 590-580 nm to 564-570 nm throughout the concentration range of SDS. The emission intensity of the dye is much larger for C_1 to C_{10} whereas it decreases from C_{12} to C_{18} dye.

The C_1 to C_8 dyes exist as monomers with \( \lambda_{max} \) values around 450-455 nm at the experimental concentration in 2% (v/v) methanol-Water system (Fig. 2.5A). The bathochromic shift
associated with $C_1$ to $C_8$ dyes on addition of surfactants and enhancement of emission intensity indicate the passage of the fluorophore from polar aqueous medium to a relatively non-polar site in micellar environment. The $C_1$ dye ($\lambda_{\text{max}} = 478 \text{ nm}$) has an environment corresponding to an $E_T(30)$ value of 57.5 (Fig. 2.5E) whereas that of $C_3-C_8$ dyes ($\lambda_{\text{max}} = 484 \text{ nm}$) have a less polar environment $E_T(30) = 52.4$) (Fig. 2.5F). The emission intensity values of $C_{12}$ to $C_{18}$ dyes at 20 mM of SDS are far less than that of $C_1$ to $C_{10}$ dyes indicating that the microenvironment around the fluorophores of $C_{12}$-$C_{18}$ dyes are less polar than water. Since the extrapolated value of absorption maxima of the dyes in hexane/decane is 441 nm, it is proposed that a fraction of the dye occupies a hexane like environment and the other fraction occupies an environment of $E_T(30)$ value of 47-49.7.

The hexane like environment in the surfactant assembly is provided by the `dry core' of the micelle. The incorporation of the cationic dye into the `dry core' would be against the attractive force of the micellar surface and further the long alkyl chain would experience steric effect of the disordered `dry core'. Therefore the long chain of the dye may be folded such that the chromophoric group enjoys a hydrocarbon like environment. Further addition of surfactant does not produce any perceptible change in the spectrum. The two/three Gaussian peaks for $C_{10}$ to $C_{18}$ dyes are, therefore, ascribed to the presence of monomer $\leftrightarrow$ dimer (Fig. 2.5B) in the absence of surfactant. With addition of surfactant a competition between homomolecular and heteromolecular interaction sets in, as a result, the dissociation of the dimer occurs resulting in a broadening of the spectrum, which separates into two gaussian peaks. These two species continue to exist till 20 mM of SDS.

The binding properties of the cationic dyes are of intrinsic interest since their binding to SDS has both an electrostatic and a hydrophobic component. The cationic dyes have hydrophilic head group of 12.1 Å length and hydrophobic chain length varying from 1-21 Å as the carbon chain changes from $C_1$ to $C_{18}$. In the present case each dye has a delocalized positive charge and alkyl chain of increasing hydrophobicity. The $K_M$ values (eq.2.2) instead of increasing with the carbon number decrease steeply for $C_{10}$ to $C_{14}$ dyes and are negligible for $C_{16}$ and $C_{18}$ dyes as compared to other dyes. Both the Fromherz and Menger model can explain our experimental results where the distribution of dye not only occurs in the spherical ionic surface but also in the hydrophobic pocket on the surface of the micelle. The binding of $C_1$ to $C_8$ dyes at the interface is both electrostatic and hydrophobic in nature (Figs. 2.5E and 2.5F). The $C_{10}$ to $C_{18}$ dyes appear to exist in two different environments in the ground state when [SDS] < CMC, which is reflected in the absorption spectra. These two environments are due to the folded or sandwiched form of one of the species with the
surfactant (Fig. 2.5D) and presence of the other in a more polar environment of SDS. On further addition of SDS micellization occurs and one fraction of the dye most probably remain in a folded form to a large extent and occupy the hydrophobic patch of the micellar surface (Fig. 2.5H), and another in the polar region of the micellar surface (Fig. 2.5G). Since the folded form is expected to have a hydrophobic surface, the 'open structure' of the micelle adsorbs it at the hydrophobic patch of the surface rather then move deep into the micellar interior. The driving force behind such an arrangement is due to the ionic nature of the dye. A negligible binding constant indicates the decrease of both coulombic and hydrophobic force of attraction for C16 and C18 dyes. This study, therefore is a unique example of surfactant-assisted folding occurring in dyes containing long alkyl chains. However, the values of the association constant do not unambiguously state the localization sites of the dyes within the micelle.

The following conclusions are arrived at from the present work.

1. All the dyes (C1 to C18) exist in trans form. The C10 to C18 dyes exist in monomer ↔ dimer in water. The amount of dimeric species increases with the length of the carbon chain.

2. All the dyes on excitation in various solvents (except in water, dioxane and in dichloromethane) give two species as is indicated by two lifetimes, the shorter one being for the trans isomer. The two species are the trans isomer and the quinoid form of the dyes. The third species in water and in dioxane is the cis isomer.

3. The surfactants act as fluorescent switch as the intensity of emission increase on addition of the surfactant. The behaviour of the dyes in CTAB and TX-100 is same whereas the behaviour in SDS is different.

4. All the surfactants bind the dyes as monomer, when [surfactant] > CMC. Though the binding constant, $K_M$, values generally increase with increasing carbon chain, yet the behaviour in different surfactants differs. In CTAB the $K_M$ value increase up to C16 and then show a decreasing trend whereas in SDS the decrease is initiated at C16. In TX-100, the increase is slow initially followed by a rapid increase.

5. The localization of the dyes in CTAB has been delineated by a force field gradient depending on the hydrophobicity of the dyes. It is proposed that in SDS the dyes undergo surfactant stimulated coiling like the milipede under the influence of an external stimulation. The TX-100 mostly behaves like CTAB for the localization sites of the dyes.