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Fluorescence probe properties of intramolecular charge transfer diphenylbutadienes in micelles and vesicles

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Abstract

This paper reports absorption and fluorescence spectral studies of methyl 4-[(1*E*,3*E*)-4-phenylbuta-1,3-dienyl]benzoate (**1**), *N*,*N*-dimethyl-*N*-{4-[(1*E*,3*E*)-4-phenylbuta-1,3-dienyl]phenyl}amine (**2**), methyl 4-{(1*E*,3*E*)-4-[4-(dimethylamino)phenyl]buta-1,3-dienyl}benzoate (**3**) and 1-methyl-4-{(1*E*,3*E*)-4-[4-methoxyphenyl]buta-1,3-dienyl}benzoate (**4**) in homogeneous media of 1,4-dioxane and 1,4-dioxane–water binary mixtures, and in microheterogeneous media of cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and Triton-X-100 micelles, and dipalmotoyl phosphatidylcholine (DPPC) vesicles. The binding site of the diene probes in micelles and vesicles has been determined and it has been found that while in micelles dienes occupy the polar interfacial regions, in vesicles the probes are located deep inside the hydrophobic bilayer. The binding of dienes to the vesicles is stronger than their binding to the micelles as indicated by the binding constant values. The fluorescence emission of the probe dienes in micelles is from a conformationally relaxed intramolecular charge transfer excited state. However, in vesicles, since the excited state conformational motions are restricted due to the rigidity of the alkyl chain, the dienes fluoresce from their planar locally excited states.

Keywords: Diphenylbutadiene; Fluorescence; Charge transfer; Micelle; Vesicle

1. Introduction

Membrane mimetic systems provide interesting models to understand the structural and functional features of natural membranes [1–5]. The surfactant amphiphiles in solution can form a variety of membrane-mimetic assemblies such as micelles and vesicles whose molecular structure depends on the surfactant amphiphile and the experimental conditions used. Among the various physical methods used to study such molecular assemblies, photophysical methods based on fluorescence spectroscopy are widely used. In particular, the fluorescence probe studies have gained a great deal of attention due to the simplicity, wide scope and extreme sensitivity of the probe at very low solute concentration [6–10]. These methods can provide valuable information about the size, polarity, microviscosity, fluidity, diffusion, partitioning and reaction rates of the aggregated systems [11–16].

A variety of organic molecules containing donor–acceptor moieties exhibit solvatochromic emission properties arising due to conformationally relaxed, twisted intramolecular charge transfer emissive states [17–20]. 4-Dimethylamino benzonitrile, for example, exhibits dual fluorescence emission and it has extensively been studied for its solvent polarity dependent fluorescence [21–25]. It has been suggested that nonplanar, polar excited states are involved in the fluorescence of such donor–acceptor molecules. The twisted excited state can be formed as a result of rotation of the single bond connecting aromatic group to the *N*,*N*-dimethylamino substituent. It has also been proposed that the amino and benzonitrile moieties in 4-aminobenzonitriles are in fact not decoupled in the charge transfer state and the configurational change of the amino nitrogen from pyramidal towards planar is an important reaction coordinate in the intramolecular charge transfer reaction of these molecules. It is believed that the relaxed charge transfer state has an essentially planar structure with a less pyramidal amino nitrogen atom and different bond lengths than in the locally excited state, resulting in a larger dipole moment, and hence the solvent polarity dependent fluorescence [26–28].

Due to the high sensitivities of such excited states to solvation, polarity and molecular mobility changes render these compounds to be efficient fluorescence probes, which can be suitable reporters of their environments. Probes based

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on excimer and exciplex formation undergo a more complex and less well understood structural and conformation changes upon excitation [29]. Hence, fluorescence probes based on conformationally twisted excited state have been fruitfully employed in structural investigations of a variety of structures including aqueous micelles [30,31], vesicles [17,31], carbohydrates [32,33], cyclodextrins [30,34] and polymers [35–37].

Recent studies with the donor–acceptor substituted diphenylpolyenes have shown that these compounds are also capable of exhibiting solvatochromic fluorescence and in some cases, fluorescence emission can arise from conformationally relaxed intramolecular charge transfer excited states [38–41]. We have recently reported that nitro-substituted diphenylbutadienes can exhibit fluorescence emission from conformationally relaxed intramolecular charge transfer excited states and such fluorescence properties of the nitro-substituted diphenylbutadienes can be used to characterize the microenvironment of micelles [42–44]. However, the quantum yields of fluorescence (Φ_f) of the nitro-substituted dienes are found to be very low due to the internal rotation of the single bond connecting nitro substituent to the phenyl ring leading to the nonradiative transitions.

Herein we report that diphenylbutadienes (methyl 4-[(1*E*,3*E*)-4-phenylbuta-1,3-dienyl]benzoate (**1**), N,*N*-dimethyl-*N*-{4-[(1*E*,3*E*)-4-phenylbuta-1,3-dienyl]phenyl}-amine (**2**), methyl 4-{(1*E*,3*E*)-4-[4-(dimethylamino)phenyl] buta-1,3-dienyl}benzoate (**3**) and 1-methyl-4-{(1*E*,3*E*)-4-[methoxyphenyl]buta-1,3-dienyl}benzoate (**4**); Fig. 1) bearing *N*,*N*-dimethyl or methoxy as donor and carbomethoxy as acceptor on the phenyl ring are capable of solvatochromic fluorescence emission with greater efficiency (relatively higher Φ_f) as compared to the nitro-dienes, and that the fluorescence emissions of these dienes depends upon the type of donor–acceptor substituents and polarity of the medium. The strong medium-dependent fluorescence emission maximum and Φ_f of the dienes have been further employed to probe the micropolarity of micelles and vesicles.

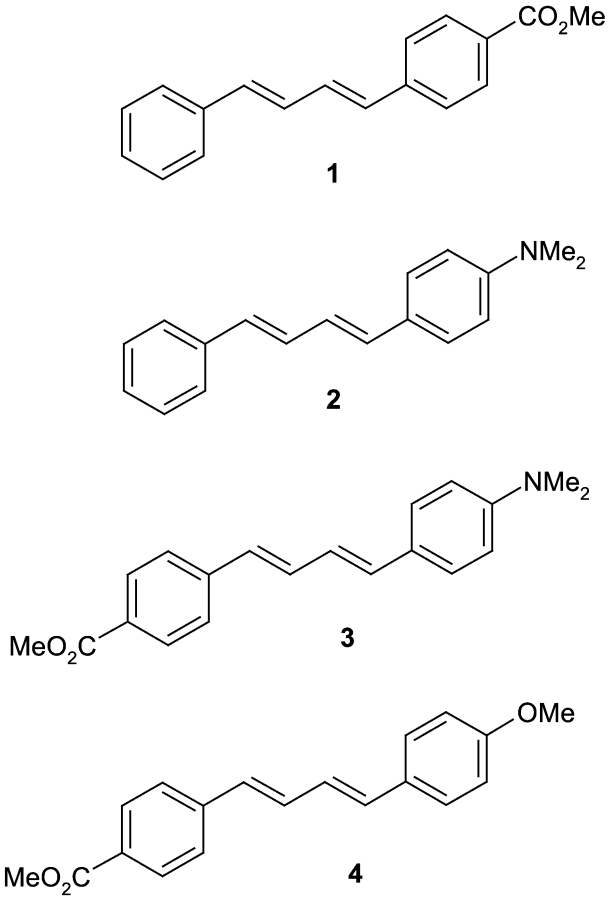

Fig. 1. Structure of the dienes **1**–**4**.

2. Experimental

2.1. Materials and general procedures

Cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulfate (SDS), Triton-X-100 and quinine sulfate were purchased from Sisco Research Laboratory, Mumbai, India and used as such without further purification. Dipalmotoyl phosphatidylcholine (DPPC) was from Sigma Chemical Co. USA. Chemicals used in synthesis were from Aldrich Chemical Co. USA. UV–Vis absorption measurements were done on Shimadzu U-160 spectrophotometer. ^{1}HNMR spectra in $CDCl_3$ as solvent and TMS as internal standard were taken on Varian 300 MHz FTNMR spectrometer at RSIC, IIT Bombay. IR spectra were recorded on Impact 400 FTIR instrument using KBr pellets. Elemental analysis was done on Theroquest CE instrument-1112 series CHNS autoanalyzer. Fluorescence measurements were performed on a Spex-fluorolog spectrofluorimeter having slit width of 1 and 1.5 mm for excitation and emission monochromators, respectively. Sonications were done in a Branson-1210 bath type sonicator or Branson B-12 probe sonicator (out put power 400 W, frequency 20 kHz) equipped with a microprobe. Centrifugations were done at 4 °C on Beckman L8-55 M, ultracentrifuge using a 45 Ti rotor. The pH values were measured by a pH meter (Toshniwal Instrumental Mfg., Ajmer, India). The HPLC analysis was done on Beckman HPLC instrument equipped with 110 A pump with 340 organizer and model 160 wavelength selective detector. The detection of the dienes was done near their absorption maximum. The quantum yield of fluorescence (Φ_f) was determined using quinine sulfate in 1 N sulfuric acid as standard [45]. Deionised and double distilled water was used for preparation of all the solutions. For all the absorption and emission studies, 1.0×10^{-5} M solutions of the dienes were used.

2.2. Synthesis

Dienes **1**–**4** were synthesized from appropriate phosphonates and aldehydes according to Emmons–Horner reac-

Table 1
UV–Vis absorption and fluorescence spectral data of dienes **1** and **2** in 1,4-dioxane–water binary mixtures

Diene	Percentage (%) of water in dioxane (ε)[a]	$\lambda_{ab\ max}$ (nm)	$\lambda_{f\ max}$ (nm)	$\lambda_{ex\ max}$ (nm)	Stokes' shift (cm^{-1})	Φ_f
1	0 (2)	348	406	345	4105	0.0300
	10 (5)	348	419	345	4869	0.0246
	20 (10)	348	421	346	4982	0.0237
	30 (16)	348	422	345	5038	0.0230
	40 (25)	348	423	345	5094	0.0227
	50 (32)	348	428	347	5371	0.0218
	60 (42)	348	430	346	5479	0.0212
	70 (50)	348	437	346	5852	0.1908
2	0 (2)	368	440	367	4446	0.0546
	10 (5)	371	474	369	5857	0.0534
	20 (10)	373	482	369	6062	0.0498
	30 (16)	372	486	369	6305	0.0497
	40 (25)	373	490	369	6401	0.0488
	50 (32)	373	494	369	6566	0.0417
	60 (42)	373	494	369	6566	0.0386
	70 (50)	373	496	369	6648	0.0365

[a] ε, Relative permittivity (dielectric constant) [49].

tion. In a typical experiment, a slight excess of triethylphosphite was refluxed with the respective benzyl bromides for 2 h to give the corresponding phosphonate. The phosphonate was slowly added to a stirring suspension of sodium hydride (prewashed with dry petroleum-ether) in THF at 0 °C under N_2 atmosphere. The aryl aldehyde taken in dry THF was added dropwise to the stirring reaction mixture. The reaction mixture was slowly brought to room temperature and the stirring was allowed to continue till most of the aldehyde disappeared as indicated by TLC on silica gel. After completion of reaction, the reaction mixture was quenched with brine and taken up in diethyl ether. The ether layer was washed with water and dried over anhydrous Na_2SO_4. Removal of the ether on a rotary evaporator yielded solid material, which was purified by column chromatography.

2.2.1. Diene 1

Yield: 86%; m.p.: 148–150 °C; HPLC: R_t = 7.0 min (Lichrosorb Si-60, 10 μ, 2% ethylacetate in hexane, 1.5 ml/min); UV–Vis (MeOH): λ_{max}, 346 nm, (ε, 74,400 l mol^{-1} cm^{-1}); IR ν_{max} (cm^{-1}): 1726 (C=O), 1601(C=C), 989 (C-H def for *trans* alkene); ^1H NMR ($CDCl_3$, 300 MHz) δ: 3.92 (3H, s, -COOMe), 6.69 (1H, d, J = 15.01 Hz, C_6H_5-C*H*=CH-), 6.74 (1H, d, J = 15.01 Hz, MeOOC-C_6H_4-C*H*=CH-), 6.97 (1H, dd, J = 14.83, 10.36 Hz, C_6H_5-CH=C*H*-CH=), 7.06 (1H, dd, J = 14.83, 10.35 Hz, MeOOC-C_6H_4-CH=C*H*-), 7.49 (2H, d of AB coupling, J = 8.24 Hz, MeOOC-C_6H_4-), 7.44–7.23 (5H, m, phenyl protons), 8.0 (2H, dd of aromatic quartet, J = 8.24, 1.48 Hz, MeOOC-C_6H_4); CHN: $C_{18}H_{16}O_2$ (264.33) calcd. C, 81.79; H, 6.10; found, C 81.86; H, 6.07.

Table 2
UV–Vis absorption and fluorescence data of dienes **3** and **4** in 1,4-dioxane–water binary mixtures

Diene	Percentage (%) of water in dioxane (ε)	$\lambda_{ab\ max}$ (nm)	$\lambda_{f\ max}$ (nm)	$\lambda_{ex\ max}$ (nm)	Stokes' shift (cm^{-1})	Φ_f
3	0 (2)	395	508	394	5631	0.0846
	10 (5)	397	548	392	6940	0.1589
	20 (10)	397	573	394	7736	0.1923
	30 (16)	398	588	394	8118	0.2071
	40 (25)	399	597	392	8312	0.2301
	50 (32)	400	605	394	8471	0.2461
	60 (42)	401	608	396	8490	0.2545
	70 (50)	401	610	394	8544	0.2764
4	0 (2)	358	428, 464 (s)	356	4568	0.059
	10 (5)	358	469, 434 (s)	357	6689	0.046
	20 (10)	358	472, 434 (s)	356	6824	0.048
	30 (16)	359	482	356	7264	0.045
	40 (25)	359	485	359	7392	0.045
	50 (32)	358	495	357	7809	0.042
	60 (42)	358	498	356	7930	0.042
	70 (50)	358	501	356	8051	0.043

2.2.2. Diene 2

Yield: 60%; m.p.: 165–166 °C; HPLC: R_t=7.0 min (ODS-C_{18}, isopropanol, 0.5 ml/min); UV–Vis (MeOH): λ_{max}, 370 nm (ε, 47,975 l mol^{-1} cm^{-1}); IR ν_{max} (cm^{-1}): 1366 (-C-N str of -Ar-N), 945(-C-H def of *trans* alkene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.97 (6H, s, NMe_2), 6.57 (1H, d, J=15.38 Hz, NMe_2-C_6H_4-C*H*=CH-), 6.61 (1H, d, J=15.01 Hz, C_6H_5-C*H*=CH-), 6.69 (2H, dd of aromatic quartet, J=7.9, 1.83 Hz, NMe_2-C_6H_4-), 6.78 (1H, dd, J=15.01, 10.44 Hz, NMe_2-C_6H_4-CH=C*H*-), 6.94 (1H, dd, J=15.38, 10.26 Hz, C_6H_5-CH=C*H*-CH=), 7.19–7.42 (5H, m, phenyl protons), 7.34 (2H, dd of AB quartet, J=8.42, 1.83 Hz, NMe_2-C_6H_4-). CHN: $C_{18}H_{19}N$ (249) Calcd. C, 86.70; H, 7.68; N, 5.62; found C, 86.81; H, 7.79; N 5.66.

2.2.3. Diene 3

Yield: 62%; m.p.: 209–210 °C; R_t=6.5 min (ODS-C_{18}, 20% methanol in isopropanol, 0.5 ml/min); UV–Vis (MeOH): λ_{max}, 395 nm (ε, 26,644 l mol^{-1} cm^{-1}); IR ν_{max} (cm^{-1}): 1726 (-C=O str), 1606(-C=C- str), 1368 (-C-N str of -Ar-N), 958(-C-H def of *trans* alkene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.99 (6H, s, NMe_2), 3.91 (3H, s, -COOMe), 6.58 (1H, d, J=15.48, NMe_2-C_6H_4-C*H*=CH-), 6.67 (1H, d, J=15.56 Hz, MeOOC-C_6H_4-C*H*=CH-), 6.69 (2H, d, AB coupling J=8.79 Hz, NMe_2-C_6H_4-), 6.79 (1H, dd, J=15.65, 10.05 Hz, Me_2N-C_6H_4-CH=C*H*-CH=), 7.05 (1H, dd, J=15.36, 9.92 Hz, MeOOC-C_6H_4-CH=C*H*-), 7.35 (2H, d, AB coupling J=8.79 Hz Me_2N-C_6H_4-), 7.45 (2H, d of AB coupling, J=8.24 Hz, MeOOC-C_6H_4-), 7.98 (2H, dd of aromatic quartet, J=8.24, 1.83 Hz, MeOOC-C_6H_4-); CHN: $C_{20}H_{21}NO_2$ (307.40), Calcd. C, 78.15; H, 6.89; N, 4.56; found C, 78.19; H, 6.74; N, 4.63.

2.2.4. Diene 4

Yield: 65%; m.p.:152–155 °C; R_t=4.83 min (Lichrosorb Si-60, 10 μ, 8% ethylacetate in hexane, 1.5 ml/min); UV–Vis (MeOH): λ_{max}, 356 nm (ε, 38,790 M l^{-1} cm^{-1}); IR ν_{max}(cm^{-1}): 1726 (-C=O str), 1604 (-C=C-str), 960 (-C-H def of *trans* alkene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.83

Table 3
UV–Vis absorption and fluorescence spectral data of dienes **1**–**4** in microheterogeneous media of micelles and vesicles

Diene	Media	$\lambda_{ab\ max}$ (nm)	$\lambda_{f\ max}$ (nm)	$\lambda_{ex\ max}$ (nm)	Φ_f ($\pm$0.001)
1	CTAB	350	421	348	0.065
	SDS	350	421	346	0.092
	Triton-X-100	350	420	347	0.126
	DPPC	350	403	349	0.250
2	CTAB	374	490	369	0.082
	SDS	356	476	367	0.025
	Triton-X-100	376	474	370	0.116
	DPPC	376	468	375	0.280
3	CTAB	404	600	400	0.112
	SDS	395	596	392	0.089
	Triton-X-100	404	546	396	0.129
	DPPC	415	527	414	0.330
4	CTAB	361	484	358	0.078
	SDS	362	492	358	0.095
	Triton-X-100	355	470	360	0.128
	DPPC	362	438	361	0.246

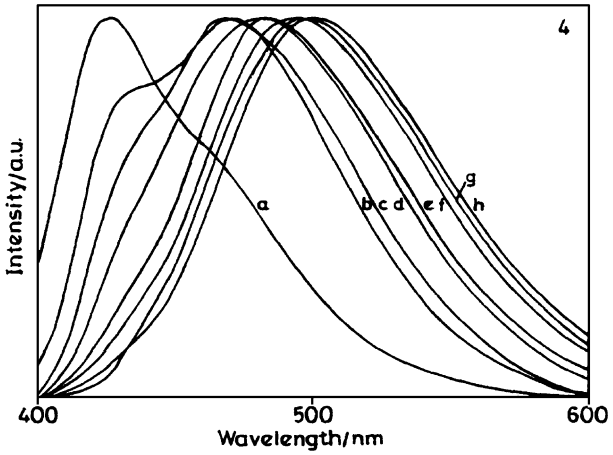

Fig. 2. Fluorescence spectra of diene **4** in 1,4-dioxane–water mixtures: percentage (%) of water in 1,4-dioxane is: (a) 0; (b) 10; (c) 20; (d) 30; (e) 40; (f) 50; (g) 60; and (h) 70.

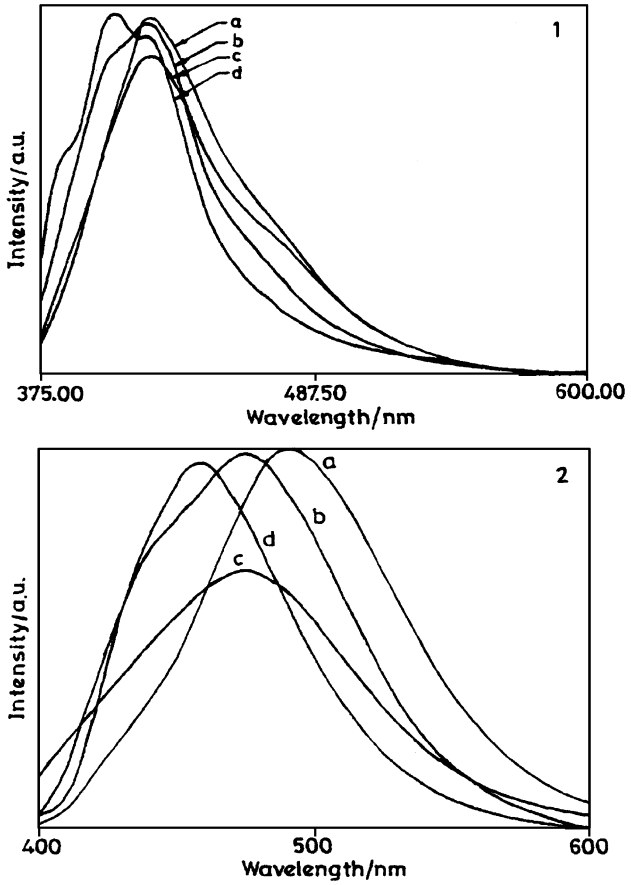

Fig. 3. Fluorescence emission spectra of the dienes **1** and **2** in CTAB (a), SDS (b), Triton-X-100 (c) and in DPPC (d). Spectra are normalized to fluorescence intensity.

(3H, s, OMe), 3.91 (3H, s, COOMe), 6.63 (1H, d, $J=16.21$ Hz, OMe-C_6H_4-C$\underline{H}$=), 6.76 (1H, d, $J=16.21$ Hz, MeOOC-C_6H_4-CH=), 6.85 (1H, dd, $J=15.38$, 10.32, OMe-C_6H_4-CH=C$\underline{H}$), 7.05 (1H, dd, $J=15.38$, 10.17 Hz, MeOOC-C_6H_4-CH=C$\underline{H}$-), 6.89 (2H, d, AB coupling, $J=8.79$ Hz, MeO-C_6H_4), 7.39 (2H, d, AB coupling, $J=8.79$ Hz, MeO-C_6H_4), 7.47 (2H, d, AB coupling, $J=8.42$ Hz, MeOOC-C_6H_4), 7.98 (2H, d, AB coupling, $J=8.42$ Hz, MeOOC-C_6H_4); $C_{19}H_{18}O_3$ (294.35) Calcd. C, 77.53; H, 6.16; found C, 77.57; H, 6.04.

2.3. Constitution of diene probes in micelles and vesicles and determination of binding constant

To prepare micelle solution of the dienes, 50 μl of 1.0×10^{-3} M diene in 1,4-dioxane was mixed with 5 ml of surfactant solution of varying concentrations in water. The mixture was briefly sonicated (bath type sonicator) to get a clear solution. The incorporation of dienes in DPPC vesicles was done by standard procedure [46]. In a typical procedure, a chloroform solution of the diene (30 μl, 1×10^{-3} M) was mixed with a chloroform solution of DPPC (3 ml, 1×10^{-3}M) in a 5 ml round bottom flask. Most of the organic solvent was removed from the mixture on a rotary vacuum evaporator at 4 °C. The mixture was further kept under vacuum for 8 h to ensure complete removal of organic solvent when a thin shiny film of the residual material is formed in the flask. To this, 3 ml of Tris–HCl buffer of pH 7.8 was added and the mixture was kept at 4 °C overnight, allowing the phospholipid to swell. The solution was sonicated (probe type sonicator) for 10 min to get small unilamellar vesicles. The solution was centrifuged at 5000 rpm for 20 min to remove the titanium particles from the probe, if any.

To calculate binding constants of the dienes in micelles and vesicles, equal volumes of dienes were incorporated in varying concentration of micelle/vesicle solutions by using same procedure as described above. From the values of the change in fluorescence intensity at emission maximum with change in concentration of the micelle/vesicle, binding constants were calculated using literature procedure [47,48]. Binding constants (K) were determined by a plot

Table 4
Dielectric constant (ε) of the solubilization sites of dienes **1**–**4** in micelles and vesicles

Diene	Media	ε
1	CTAB	10
	SDS	10
	Triton-X-100	10
	DPPC	2
2	CTAB	25
	SDS	5
	Triton-X-100	5
	DPPC	5
3	CTAB	32
	SDS	25
	Triton-X-100	5
	DPPC	5
4	CTAB	25
	SDS	32
	Triton-X-100	5
	DPPC	5

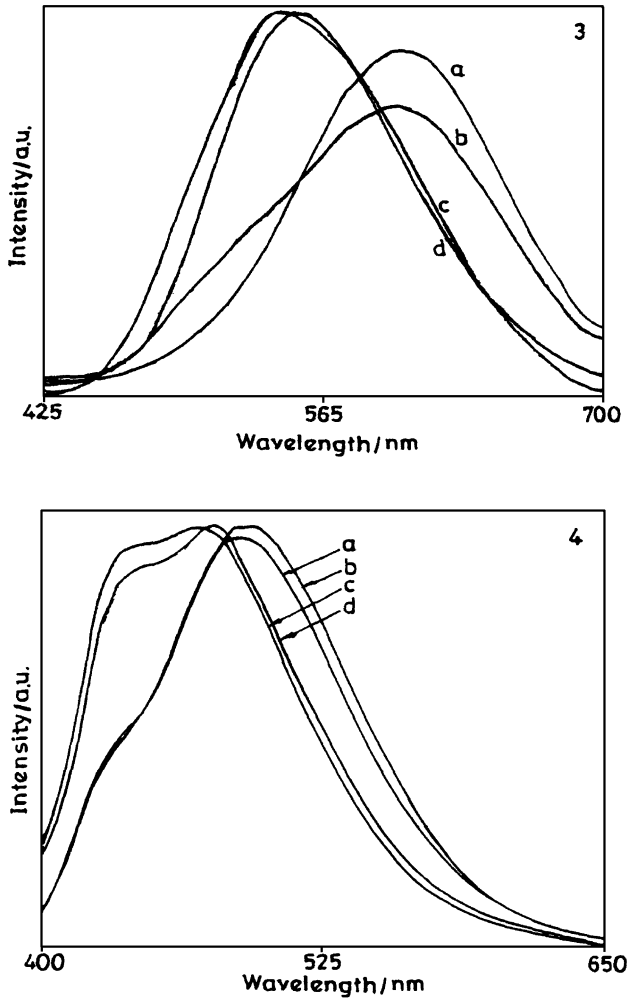

Fig. 4. Fluorescence emission spectra of dienes **3** and **4** in CTAB (a), SDS (b), Triton-X-100 (c) and in DPPC (d). Spectra are normalized to fluorescence intensity.

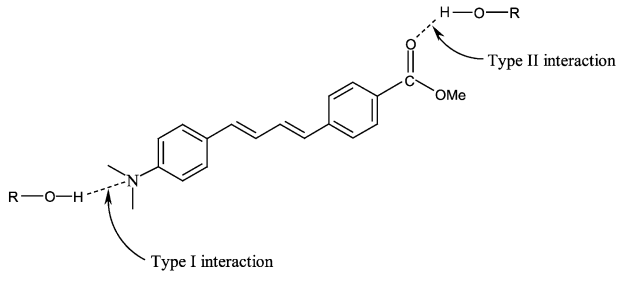

Fig. 5. Possible hydrogen bonding interactions in diene **3** in micelles.

of $(F_i^{\text{micelle or DPPC}}/F_i^{H_2O} - 1)^{-1}$ vs. C_1^{-1}; where $F_i^{\text{micelle or DPPC}}$ is the fluorescence intensity of dienes in micelles or in DPPC vesicles; $F_i^{H_2O}$ is the fluorescence intensity of dienes in aqueous solutions, and C_1 is the molar concentration of surfactant (for micelles) or DPPC (for vesicles).

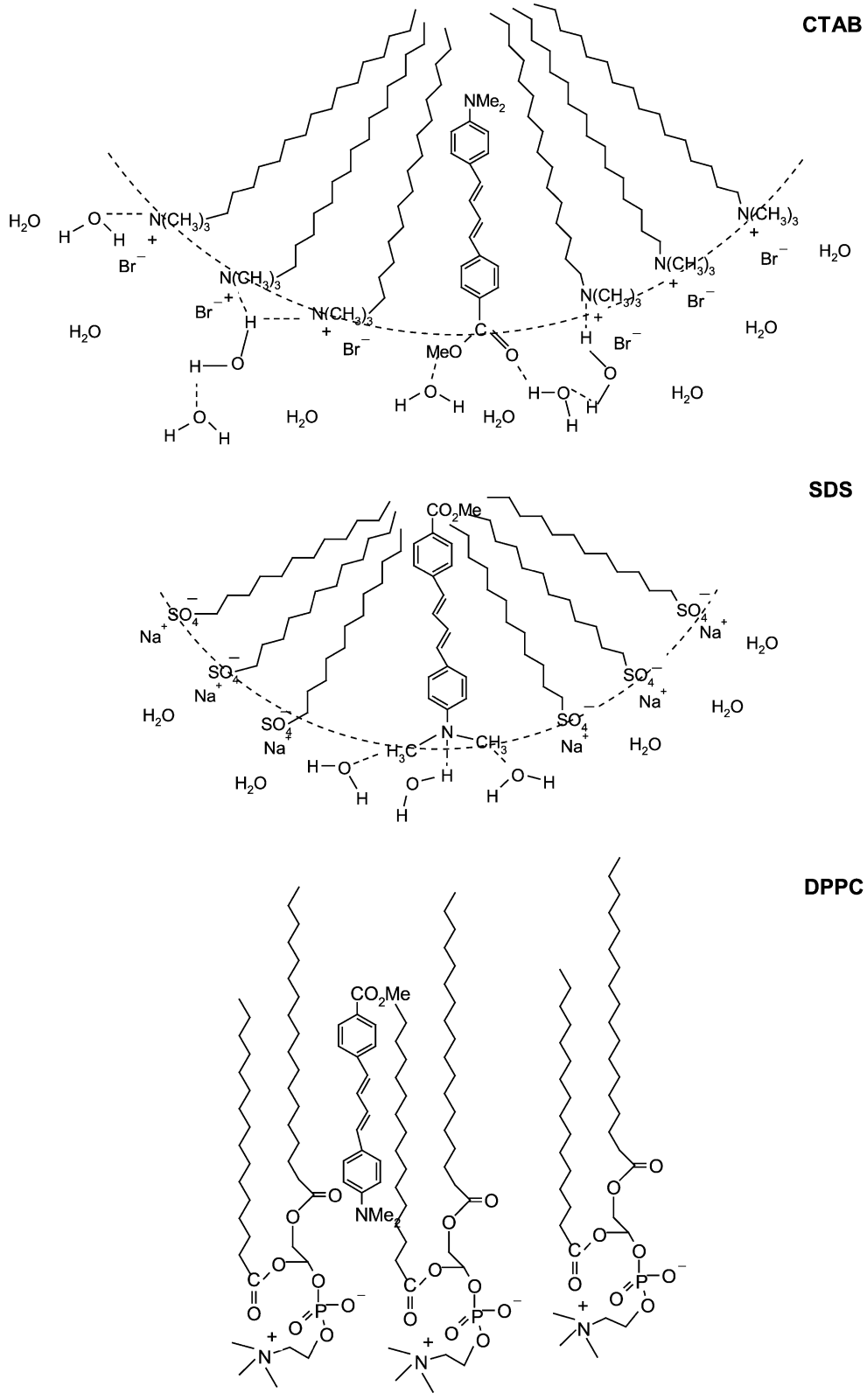

Fig. 6. Localization of diene **3** in CTAB, SDS and in DPPC vesicles.

3. Results and discussion

UV–Vis absorption and fluorescence spectral studies of dienes **1**–**4** were done in 1,4-dioxane and 1,4-dioxane–water binary mixtures and the data are given in Tables 1 and 2. No significant changes are observed in the absorption maximum of the dienes with increase in percent of water in 1,4-dioxane. However, the fluorescence maximum of the dienes is considerably influenced by the water content in 1,4-dioxane. Thus, diene **1** shows a moderate red shift of 31 nm in its fluorescence maximum when the solvent is changed from 1,4-dioxane to 70% water in 1,4-dioxane mixture. Dienes **2**–**4** exhibit more pronounced red shift of 56, 102 and 73 nm, respectively, in their fluorescence when the solvent is changed from 1,4-dioxane to 70% water in 1,4-dioxane mixture. In addition, diene **4** in 1,4-dioxane, and in 10% and 20% water–1,4-dioxane binary mixtures showed dual fluorescence emission. The fluorescence intensity of longer wavelength band increases with increase in percent of water in 1,4-dioxane. In fact, the shorter wavelength emission totally disappears above 20% water content in 1,4-dioxane (Fig. 2). The Φ_f of dienes **1**, **2** and **4** decreased with increase in percent of water in 1,4-dioxane whereas diene **3** showed increase in Φ_f in similar conditions.

The large red shift and greater sensitivity of the fluorescence to solvent properties in the case of dienes **2**–**4** can be due to the participation of a polar, conformationally relaxed intramolecular charge transfer excited state. The excited state conformational relaxation in these dienes is possible by bond rotation at a number of sites including the carbon–carbon bonds between the aromatic centers as well as the C–N bond between the dialkylamino group and the aromatic ring. As the conformational relaxation of a single bond connecting aryl or arylidene group to the chain double bond requires larger reaction volume, it can be suggested that such conformational changes occur in the single bond connecting ***N***,*N*-dimethylamino or carbomethoxy group to the aromatic ring (i.e., Ar–NMe_2 or Ar–CO_2Me). It is also possible that conformational relaxation across more than one single bond occurs in the excited state of these dienes, particularly in diene **3** wherein the C–C single bond between the carbomethoxy and the aromatic ring as well as the C–N bond between the dialkylamino group and the aromatic ring can undergo excited state conformational changes simultaneously.

A highly solvatochromic and efficient fluorescence emission in these dienes prompted us to examine the fluorescence probe efficacy of these dienes as reporters of the microenvironment of organized assemblies. The UV–Vis absorption and fluorescence spectral data of dienes **1**–**4** in CTAB, SDS, Triton-X-100 micelles and in DPPC vesicles are summarized in Table 3 and the corresponding fluorescence emission spectra are presented in Figs. 3 and 4. As can be seen, there is considerable difference in absorption maximum and fluorescence maximum of dienes **2**–**4** in anionic, cationic and neutral micelles. However, diene **1** does not show difference in its absorption and fluorescence in different micelles. *N*,*N*-Dimethylamino substituted dienes **2** and **3** show absorption at relatively shorter wavelengths in SDS and it is comparable to those obtained in 1,4-dioxane. In contrast, CTAB solutions of the same probes give absorption spectra shifted towards longer wavelengths and comparable to those obtained in more polar media like 1,4-dioxane–water mixtures. Diene **4** in CTAB and SDS shows similar absorption wavelength. However, in Triton-X-100 there is blue shift in the absorption maxima. In DPPC vesicles all the dienes (**1**–**4**) showed red-shifted absorption maximum comparable to that in polar solvents.

In contrast to the moderately sensitive nature of absorption maximum of the dienes with micelle charge, the fluorescence spectra of **2**–**4** are significantly affected by the charges on the micelles. All the dienes in charged micelles (CTAB and SDS) show red-shifted fluorescence emission, which is comparable to that of the emissions of the dienes in polar homogeneous media. Similar to that of absorption maxima, the fluorescence emission maximum for **2**–**4** is red-shifted in CTAB as compared to that in SDS. However, in Triton-X-100 all the dienes show blue-shifted fluorescence as compared to in CTAB and SDS. In DPPC vesicles, all the dienes show blue shift in their fluorescence as compared to in micelles and it is comparable to that observed in nonpolar homogeneous solvents. The Φ_f of the dienes in micelles are comparable to that found in polar solvents. In DPPC vesicles, dienes **1**–**4** showed Φ_f that is much higher than in the micelles and organic solvents.

The solubilization sites of these probes in the micelles and in DPPC vesicles were assigned on the basis of the spectral shifts in the absorption and fluorescence maxima and Φ_f in comparison to that found for the dienes in homogeneous media of 1,4-dioxane–water binary mixture. The polarity of the probe-binding site was calculated by comparing the fluorescence maxima of the dienes in different micelles and in 1,4-dioxane–water mixtures. The fluo-

Table 5
Binding constant (K, M^{-1}) of dienes **1**–**4** in micelles and vesicles

Diene	Media	K (M^{-1})
1	CTAB	1.25×10^4
	SDS	2.7×10^2
	Triton-X-100	5×10^4
	DPPC	2×10^5
2	CTAB	1.1×10^3
	SDS	2.7×10^2
	Triton-X-100	2.5×10^3
	DPPC	3.3×10^5
3	CTAB	2.5×10^4
	SDS	3.3×10^3
	Triton-X-100	3.3×10^4
	DPPC	1.25×10^6
4	CTAB	2.5×10^3
	SDS	4.1×10^2
	Triton-X-100	3.3×10^3
	DPPC	1.25×10^5

rescence maximum of **1** in all the three micelles is close to that observed in 1,4-dioxane–20% water mixture. Perusing the known dielectric constants of 1,4-dioxane–water mixtures, dielectric constant of the micelle medium localizing dienes **1**–**4** is determined (Table 4).

The micelle charge-dependent shifts in the absorption and fluorescence of dienes **2**–**4** in different micelles, Φ_f and dielectric constant of the probe solubilization sites indicate that these dienes experience polar environment in the micelles of CTAB and SDS. This suggests that dienes are solubilized in interfacial site where they can interact with the surfactant head groups but can be solvated partially by surrounded water molecules. The red-shifted absorption and fluorescence emission for dienes **2**–**4** in CTAB compared to that in SDS can be due to the hydrogen bonding effect of the probes in micelle environments. These dienes can interact with hydrogen bonding solvents in two types as illustrated in Fig. 5. In type I interaction, the hydrogen bonding can occur between the lone pair of electrons on the *N*,*N*-dimethylamino group with that of the OH group of the solvent molecule. Because of this, the lone pair cannot participate in the conjugation; hence, decrease in the absorption maxima and also in charge transfer nature of the *N*,*N*-dimethylamino group can result in blue-shifted fluorescence emission. However, excited state hydrogen bonding can also lead to the stabilization of the excited state, which can reduce the energy difference between the ground state and excited state, because of which blue shift in emission wavelength is not prominent compared to the blue shift in absorption wavelength. In type II interaction, hydrogen bond interaction can be possible between -CO_2Me group and solvent molecules. In this case, the hydrogen bond can stabilize the ground state, but conjugation in the molecule remains same. Hence, absorption maximum in type II interactions can be red-shifted compared to that of type I interaction. Dienes **2**–**4** in CTAB can be located in inter-

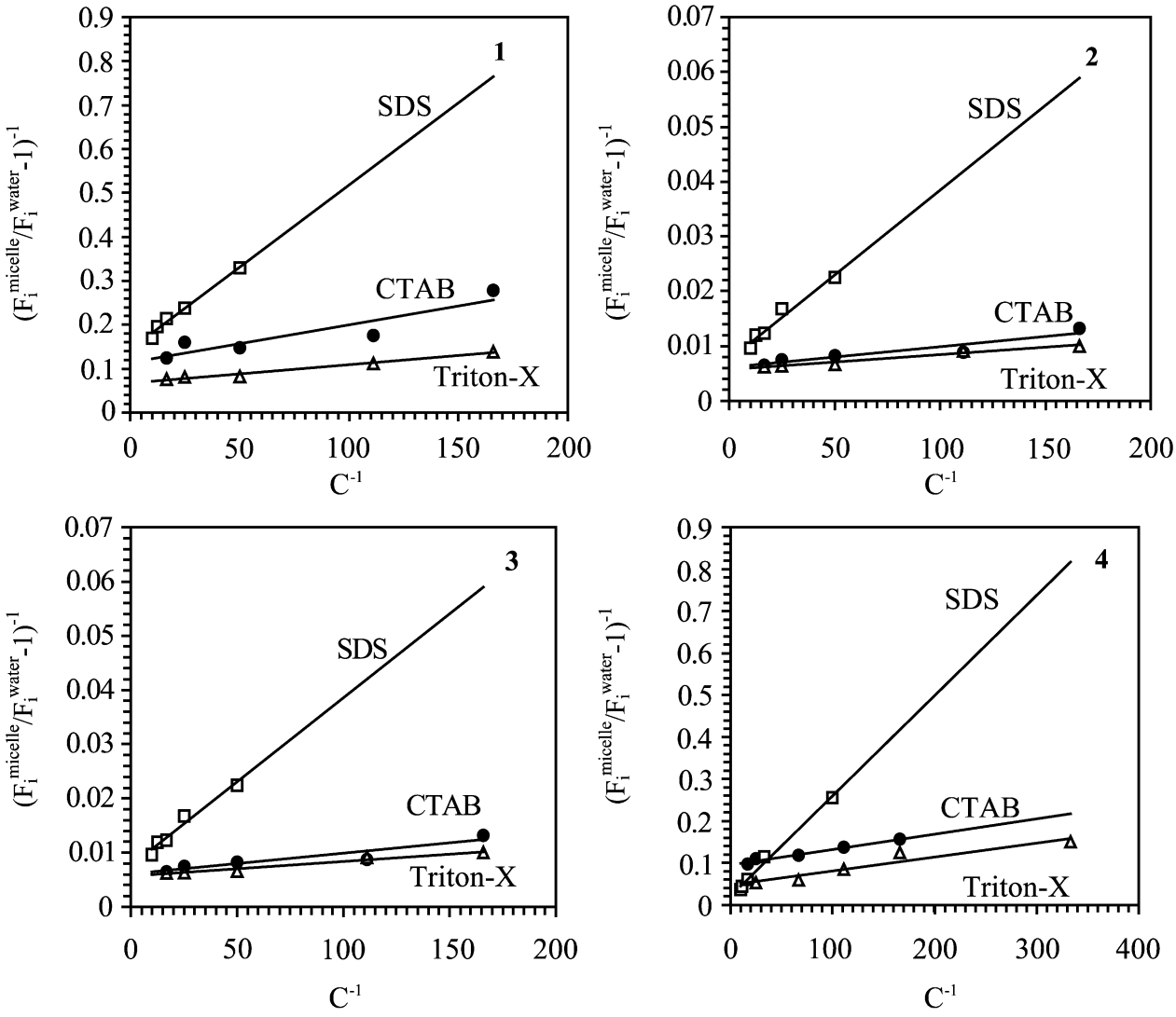

Fig. 7. Plots of $((F_i^{micelle}/F_i^{H_2O}-1)^{-1}$ vs. C_1^{-1} for **1**–**4** for calculating binding constants in CTAB, SDS and Triton-X-100 micelles.

facial regions with *N*,*N*-dimethylamino group (for **2** and **3**) or methoxy group (for **4**) inside hydrophobic alkyl chains and with CO_2Me group of the **3** and **4** towards polar environment. In SDS, these dienes can be aligned with *N*,*N*-dimethylamino group or methoxy group towards polar aqueous region and hence due to type I hydrogen bonding interaction in **2** and **3**, there is a blue shift in absorption and emission maxima compared to that of the CTAB. Diene **4** with methoxy substituent cannot undergo type I interaction; hence, there is no effect in absorption and emission spectra for this diene. Fig. 6 shows the possible localization sites of diene **3** in CTAB, SDS and DPPC vesicles. Different localization of the dienes in CTAB and SDS micelles can be due to the specific interaction of the polar head groups or counter ions of the micelles with the polar substituents of the dienes. In Triton-X-100, as can be seen from the calculated dielectric constant values of the solubilization sites, all the dienes are located in a hydrophobic environment. The red-shifted emission of the dienes in CTAB and SDS micelles can be due to the involvement of a polar, conformationally relaxed intramolecular charge transfer states, whose stability is expected to be influenced by the changing micropolarity of the micelle domains.

A blue-shifted fluorescence emission for dienes **1**–**4** in DPPC vesicles indicates that these dienes can reside in a distinctly different environment in DPPC bilayers compared to that of the micelles. As shown in Fig. 6, in vesicles, the dienes can be solubilized in sites of lower polarity as compared to that of micelles. This is also evident from the calculated dielectric constant values (Table 4). High Φ_f in DPPC vesicles indicates that dienes can be located inside the alkyl chains. Because of the rigidity of the alkyl chains, this arrangement will reduce all other molecular motions and nonradiative transitions resulting in increase in Φ_f. The fluorescence emission of these dienes in DPPC vesicles is due to locally excited planar state. This can be clearly seen from the fluorescence emission of diene **4** in DPPC vesicles. The constrained environment of the lipid bilayer can inhibit conformational motions of the single bonds in the diene, thereby diminishing intramolecular charge transfer and hence the polarity of the excited state, which fluoresces at relatively lower wavelength.

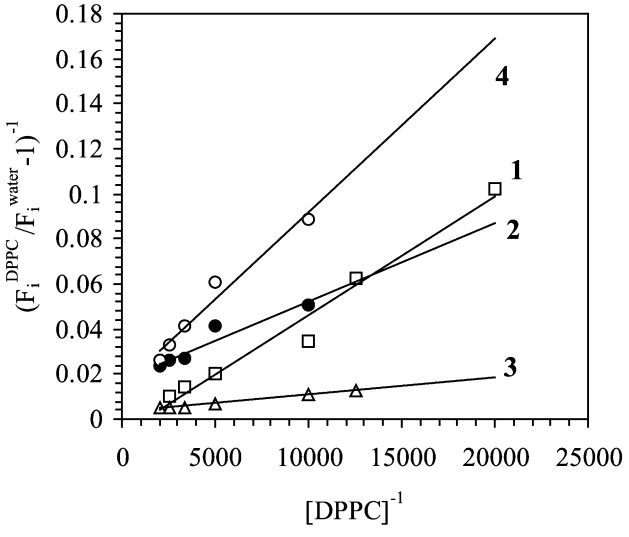

Fig. 8. Plots of $(F_i^{DPPC}/F_i^{H_2O}-1)^{-1}$ vs. C_1^{-1} for **1**–**4** for calculating binding constants in DPPC vesicles.

With a fixed concentration of dienes in micelles and vesicles, the fluorescence intensities of all the dienes increased with increase in the concentration of the surfactants. Binding of these dienes with micelles and DPPC vesicles is further analyzed by determining the binding constant (*K*) values, and the binding constant values so obtained are given in Table 5. Plots of C^{-1} vs. $[(F_i^{micelle\ or\ DPPC}/F_i^{H_2O})-1]^{-1}$ are shown in Figs. 7 and 8. The *K* values indicate that the dienes bind strongly to vesicles as compared to the micelles. This further indicates that these dienes solubilize in the rigid bilayer of the vesicles.

4. Conclusions

In conclusion, UV–Vis and fluorescence spectral studies of dienes **1**–**4** in micelles suggests that these dienes occupy polar environment of the micelles. Calculated dielectric constant values are less than that of the bulk water and more than that of the hydrophobic alkyl chains, which shows that the dienes occupy interfacial regions of the micelles. Charge-dependent shifts in absorption and fluorescence emission further supports the location of the dienes in interfacial regions, which can interact differently with the different head groups or counter ions. Polar environment of the micelle solubilization site of the dienes results in fluorescence emission from the twisted intramolecular charge transfer excited states, similar to that of the polar solvents. Difference in the dielectric constant values of the micelle water interface calculated with the different dienes incorporated in micelles indicates variations in solubilization sites of dienes in micelles depending on the substituents. Red shift in absorption and fluorescence emission in CTAB for the dienes **2**–**4** compared to that of SDS indicates that in addition to polarity, hydrogen bonding also plays a role at the binding site. Studies in DPPC vesicles show that dienes solubilize differently in vesicles as compared to that of the micelles. Blue-shifted fluorescence emission for all the dienes in vesicles indicates that these dienes occupied a more hydrophobic region, which can be inside the bilayers of the vesicles, resulting in fluorescence emission from planar locally excited states. High Φ_f in DPPC vesicles further indicates the dienes are located in the rigid bilayer, which can reduce all the molecular motions and nonradiative transitions. Higher binding constant values obtained in case of vesicles compared to that of micelles show that the dienes are strongly solubilized in the vesicle media compared to that in micelle domains.

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