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# Diphenylpolyene-cholesterol conjugates as fluorescent probes for microheterogeneous media

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ABSTRACT

Extrinsically conjugated fluorescent diphenylpolyene cholesterol derivatives are synthesized and spectroscopic investigations in homogeneous and aqueous micellar solutions are described. The emission of these cholesterol conjugates reveals characteristic intra-molecular charge transfer (ICT) behaviour in homogeneous solvents with a mono-exponential decay. Spectroscopic evidence in micellar aqueous solutions reveals a bi-exponential decay. This indicates the presence of two preferred locations of the cholesterol conjugated diphenylpolyenes sites of lower polarity and interfacial sites. The sensitivity of these fluorophores was utilized to determine the critical micelle concentrations.



## 1. Introduction

π-Conjugated materials based on dyes that fluoresce at various wavelengths and exhibit charge transfer are of great interest towards various optoelectronic [1–4] as well as diagnostic/analytical applications [5–9]. The unique modulation of the ground and excited state dynamics of diphenylpolyenes using variously substituted donor and acceptor groups have rendered them with potential applications in organic optoelectronic devices [3,10] and as photoresponsive materials [10,11]. Molecules having donor and acceptor groups on the aromatic ring can lead to strong intramolecular charge transfer (ICT) character responsible for environment sensitive emission behaviour [12]. For such systems, fluorescence quantum yield decreases with increase in solvent polarity accompanied by large bathochromic emission spectral shifts [13]. This unique behaviour can be utilized for fluorometric characterization of biological membranes [14]. Diphenylpolyenes substituted with suitable donor or acceptor groups have been examined as membrane probes in homogeneous and microheterogeneous environments [7,15,16]. A well-known example of an extensively studied non-covalent probe was diphenylhexatriene (DPH) which preferentially resides in the nonpolar regions of the cell membrane [17].

Biological membranes are composed of complex assemblies of lipids and proteins that allow many important cellular functions. Amphipathic cholesterol is an essential component of these biological membranes and has been used as a building block for creating photo responsive materials [18–20] and in bionanotechnology [21]. The rigid planar subunit of the rings and a flexible iso-octyl side chain 'tail' allows cholesterol to modulate various functions in the biological membrane organization. Many fluorescent cholesterol conjugates [22–27] containing fluorophores such as NBD [28,29], BODIPY [30,31], fluorescein [23] have been used to examine various functionalities of cholesterol in membranes. Functions such as cholesterol organization, trafficking, lipid interactions and modulation of activity of membrane proteins can be monitored by utilizing changes in fluorescence response with respect to their polar or non-polar environments.

In this paper, we report the synthesis and photophysical properties of two novel fluorescent diphenylpolyene-cholesterol analogues linked at the 3β-OH position. Due to the lack of 3-OH group these cholesterol probes lose the amphipathic property [14,32], but may show preference for partitioning into ordered micro domains and therefore can be a valuable tool for exploration of such dynamics. This structural design yields a fluorophore that can be monitored using non-invasive fluorescence methods. Presence of cholesterol also enables the fluorophores to be embedded in the biological hydrophobic environment rendering biosensing applications [33,34]. The fluorophores (**7**, **8**) and their cholesterol derivatives (**10**, **11**) (Scheme 1) that we utilized exhibit strong solvent dependent emission characteristics attributed to ICT behaviour [12]. We intended to tap into their emission properties and understand the feasibility of using these covalently linked cholesterol fluorophores for probing microenvironments.

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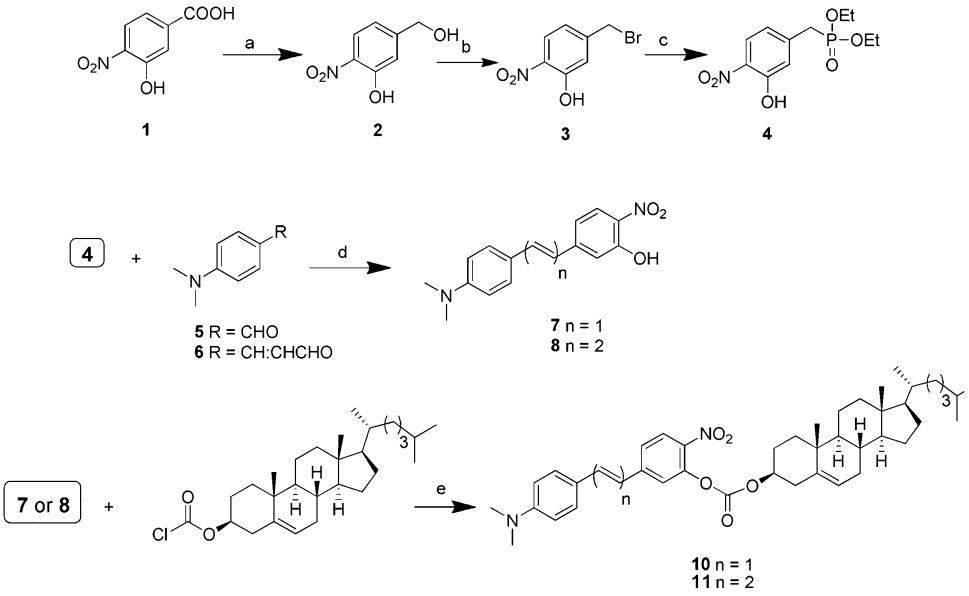


**Scheme 1.** Synthesis of cholesterol conjugated stilbene (**10**) and diphenylbutadiene (**11**). Reagents & conditions used: (a) $B(OMe)_3$, $BF_3{\cdot}Et_2O$, BH(Py), EDC, RT to 0 °C, 3 h; (b) $CBr_4$, DCM, $PPh_3$, 0 °C to RT, 3 h; (c) $P(OEt)_3$, DMF, 140 °C, 2 h; (d) NaH, THF, 0 °C to RT, 12 h and (e) pyridine, benzene, 80 °C, 24 h.

## 2. Experimental

### 2.1. Materials and methods

The chemicals, surfactants (CTAB, Triton X-100 and SDS) and other reagents used for this study were obtained from Aldrich, Alfa Aesar, Acros or S.D. fine chemicals Ltd. Solvents were dried using reported procedures before their use in synthesis and optical spectroscopic studies. Double distilled Millipore water was used to prepare solutions of the desired concentration. A 20 μL tetrahydrofuran (THF) solution of the fluorophore ($1 \times 10^{-4}$ M) was added to the surfactant solution maintaining a uniform dye-concentration. $^1H$ and $^{13}C$ NMR characterization was done using Bruker Avance 500 (500 MHz) spectrometer and accurate mass analysis was performed using Waters-synapt G2S (ESI-QToF) mass spectrometer.

UV–vis absorption spectra were recorded using Analytik Jena Specord 210 plus and steady state fluorescence emission studies were performed using Horiba Jobin Yvon fluolorog-3 spectrofluorimeter using a slit-width of 1 nm. The fluorescence quantum yields were determined using a reference solution with a known quantum yield of fluorescence [35] with similar optical density. Picosecond pulsed diode laser-based time-correlated single photon counting (TCSPC) instrument from Horiba Jobin Yvon IBH (UK) set at a magic angle at 54.7° was used to determine the fluorescence lifetimes. The excitation sources used were 406 nm and 440 nm with the corresponding *fwhm* of 249, 248 ps having resolutions of 7 and 14 ps/channel, respectively. 406 nm excitation was used for molecule (**10**) and 440 nm excitation was used for molecules (**7**), (**8**) and (**11**). The excitation wavelength corresponds to their observed absorption maxima. The number of channels per decay was 5000 for both resolutions. The decays were fitted by using IBH DAS v6.2 software in mono and biexponential models by deconvolution by iterative reconvolution. The error associated with the determination of lifetime studies is 0.1–1.5%.

### 2.2. Synthesis of fluorescent cholesterol conjugates

The synthesis of cholesterol analogues of diphenylpolyenes (**10**) and (**11**) was achieved in five steps (Scheme 1). Carboxylic group of 3-hydroxy, 4-nitrobenzoic acid (**1**) was reduced to the corresponding alcohol using trimethylborate and borane-pyridine complex in quantitative yields [36]. The hydroxyl group was replaced with bromide using tetrabromomethane ($CBr_4$) yielding a yellow crystalline solid (**3**) with 83% yield [37]. Phosphonate (**4**) was obtained upon treatment of (**3**) with triethylphosphite in dimethylformamide (DMF). The phosphonate ester (**4**) was then subjected to a reaction with suitable aldehyde, 4-dimethylaminobenzaldehyde yielding (**7**) and 4-dimethylaminocinnamaldehyde yielding (**8**) with sodium hydride (NaH) as a base and THF as a solvent [37]. The final step of conjugation with cholesterol was done utilizing cholesteryl chloroformate to yield the carbonate linked fluorescent sterol derivatives (**10**) and (**11**).

### 2.3. General procedure for the synthesis of diphenylpolyene derivatives (7) and (8)

To a solution of phosphonate (**4**) (1 mmol) in dry THF (5 mL) at 0 °C, NaH (2.5 mmol) was added under $N_2$ atmosphere. After stirring for five minutes, aldehyde (1 mmol) in dry THF (5 mL) was added drop wise. Stirring was continued for further 30 min while maintaining the temperature at 0 °C. The reaction was later allowed to stir at room temperature for 12 h and the mixture was poured into ice-cold water. Extraction with dichloromethane (DCM) and concentrating under reduced pressure yields a dark red residue, which on elution by column chromatography (silica gel, 15% ethyl acetate/petroleum ether) afforded the desired product as dark red solid.

#### 2.3.1. (E)-5-(4-(dimethylamino)Styryl)-2-nitrophenol (7)

Yield: 30% (85 mg); dark red solid; $^1H$ NMR (500 MHz, $CDCl_3$) 3.03 (s, 6H), 6.71 (d, $J$ = 8.5 Hz, 2H), 6.83 (d, $J$ = 16.5 Hz, 1H), 7.09 (d, $J$ = 9.0 Hz, 1H), 7.13 (s, 1H), 7.19 (d, $J$ = 16.5 Hz, 1H), 7.44 (d, $J$ = 8.5 Hz, 2H), 8.04 (d, $J$ = 8.5 Hz, 1H), 10.79 (s, 1H). $^{13}C$ 125 MHz, $CDCl_3$) 40.3, 111.9, 115.9, 127.2, 128.6, 131.1, 141.1. HRMS [ESI] $[M-1]^-$ 283.1449.

#### 2.3.2. 5-((1E,3E)-4-(4-(dimethylamino)phenyl)buta-1, 3-dienyl)-2-nitrophenol (8)

Yield: 27% (83 mg); dark red solid; $^1H$ NMR (500 MHz, $CDCl_3$) 3.03 (s, 6H), 6.51 (d, $J$ = 15 Hz, 1H), 6.71 (d, $J$ = 8.5 Hz, 2H), 6.83 (d, $J$ = 16.5 Hz, 1H), 7.09 (d, $J$ = 9.0 Hz, 1H), 7.13 (s, 1H), 7.19 (d, $J$ = 16.5 Hz, 2H), 7.44 (d, $J$ = 8.5 Hz, 2H), 8.04 (d, $J$ = 8.5 Hz, 1H), 10.79 (s, 1H).

$^{13}$C (125 MHz, $CDCl_3$) 40.4, 112.3, 115.9, 116.0, 124.0, 125.6, 127.2, 128.4, 135.6, 137.9.HRMS [ESI] $[M+1]^+$ 311.1792.

### 2.4. Synthesis of cholesterol conjugated polyenes (**10**) and (**11**)

To a solution of stilbene or diene [(**7**), (**8**)] (0.3 mmol, 85 mg) in benzene (4 mL), pyridine was added (1.2 mmol, 145 μL) and stirred for 5 min. To this, cholesteryl chloroformate (0.3 mmol, 134 mg) in benzene (4 mL) was added drop wise. After complete addition of the cholesteryl chloroformate, the reaction was allowed to reflux for 24 h. Pyridine and benzene were removed under reduced pressure and was followed by extraction of the compound using DCM. Conjugates were recrystallized using DCM and methanol.

#### 2.4.1. Stilbene-(**10**)

Yield: 55%; dark red solid; $^1$H NMR (500 MHz, $CDCl_3$) 0.71 (s, 3H), 1.87 (m, 38H), 2.55 (s, 2H), 3.07 (s, 6H), 5.45 (m, 1H), 5.46 (s, 1H), 6.90 (m, 3H), 7.22 (d, $J$ = 16.5 Hz, 1H), 7.38 (s, 1H), 7.47 (m, 3H), 8.15 (d, $J$ = 8.5 Hz, 1H). $^{13}$C (125 MHz, $CDCl_3$) 18.9, 19.4, 21.2, 22.7, 23.0, 24.0, 24.4, 27.8, 28.2, 28.4, 32.0, 32.1, 36.0, 36.3, 36.7, 37.0, 38.1, 39.7, 39.9, 40.4, 42.5, 50.2, 56.3, 56.9, 112.1, 121.7, 123.4, 126.7, 131.5, 133.5, 139.3, 142.8, 152.9. HRMS [ESI] $[M+1]^+$ 697.4642.

#### 2.4.2. Diene-(**11**)

Yield: 55%; dark red solid; $^1$H NMR (500 MHz, $CDCl_3$) 0.71 (s, 3H), 1.87 (m, 38H), 2.49 (s, 2H), 3.04 (s, 6H), 5.45 (d, $J$ = 4.5 Hz, 1H), 5.46 (s, 1H), 6.55 (d, $J$ = 15.5 Hz, 1H), 6.75 (m, 2H), 6.79 (m, 2H), 7.11 (dd, $J$ = 6.0/9.5 Hz, 1H), 7.26 (s, 1H), 7.39 (dd, $J$ = 5.0/7.0 Hz, 3H), 8.13 (d, $J$ = 8.5 Hz, 1H). $^{13}$C (125 MHz, $CDCl_3$) 12.0, 14.3, 18.9, 19.4, 21.2, 22.7, 22.8, 23.0, 24.0, 24.4, 27.7, 28.2, 28.4, 29.5, 29.8, 29.9, 32.0, 32.1, 35.9, 35.3, 35.7, 37.0, 38.0, 39.0, 39.7, 39.9, 42.5, 50.2, 56.3, 56.8, 121.4, 123.5, 123.8, 126.8, 128.4, 139.2, 145.3, 153.0. HRMS [ESI] $[M+1]^+$ 723.4721.

## 3. Results and discussion

Utilizing intensely solvatochromic probes to understand the micelle properties should lead to a molecule that can either reside in the interior or span the interface region of the media [12]. (**7**) and (**8**) are examples of such environmentally sensitive dyes with pronounced solvatochromic shifts in homogeneous solutions [12]. In this reported work, we have targeted synthesis of cholesterol analogues of diphenylpolyenes that preserves the ability to undergo charge transfer from donor to acceptor. To achieve this we have incorporated a phenolic hydroxyl group as a synthetic handle to conjugate receptors of choice which could lead to better photo responsive properties than a free dye [15,16]. The diphenylbutadiene and the cholesterol groups were covalently linked through a short "carbonate" linker. This structural design enables monitoring of solvent sensitivity through the solvatochromic fluorescence of these derivatives and helps to understand the properties of organized assemblies. Furthermore the short covalent spacer, linking fluorophore to the cholesterol appendage, could enable transfer of the probe to its natural resident sites leading to environment specific optical properties.

### 3.1. Absorption behaviour in homogeneous solvents

There is no discernible difference in absorption maxima ($\lambda_a$) of (**8**) when compared to (**7**) although the conjugation is extended by a double bond (Table 1). However, solvent polarity changes yields moderate $\lambda_a$ shifts in (**7**) and (**8**). In the case of (**7**), the observed shift is about 13 nm and in the case of diene (**8**) the shift is lesser (~8 nm) (Fig. 1C and D). The maximum absorption wavelength is observed in acetonitrile and the lowest in heptane. Planarity of the molecules in the ground state and solute–solvent H-bonding interaction accounts for the observed absorption spectral shifts. Conjugation of cholesterol with stilbene or diene moiety leads to intense absorption in the long wavelength region similar to the un-substituted derivatives. It is expected that bulky cholesterol substitution and subsequent loss of conjugation due to modification of the phenolic hydroxyl group may affect the absorption maxima. Likewise, butadiene conjugated (**11**) exhibits blue shifted absorption as compared to the free diene (**8**) (Fig. 1A) with a shift of 10–15 nm in non-polar solvents. On the other hand, cholesterol conjugated stilbene (**10**) has a large hypsochromic shift of up to 57 nm in the solvents studied (Fig. 1B) in comparison to the free stilbene (**7**). A combination of factors such as loss of H-bonding because of hydroxyl modification, bulkiness of the cholesterol moiety and aggregation or association because of the tendency due to the presence of cholesterol [22] contribute to the observed blue shift.

Thus among the molecules studied, it is found that (**8**) exhibits maximum $\lambda_a$ and (**10**) minimum $\lambda_a$. Cholesterol linked fluorophores (**10**) and (**11**) also show moderate shifts in absorption maxima as the solvent polarity is varied from heptane to methanol (Fig. 1C and D). Unlike (**7**) and (**8**), where additional double bond has no influence on $\lambda_a$, cholesterol conjugated diene (**11**) exhibit a strong bathochromic shift (~up to 47 nm) as compared to (**10**).

### 3.2. Emission in homogeneous solvents

Both the cholesterol free and cholesterol linked molecules exhibit solvent dependent emission behaviour. As the solvent

**Table 1**
Absorption, emission data for the molecules (**7**), (**8**), (**10**) & (**11**) in homogeneous solvents.

| | Solvent | $\lambda_a$ (nm) | $\lambda_f$ (nm) | $\Phi_f$[a] | Stokes shift (nm) | | Solvent | $\lambda_a$ (nm) | $\lambda_f$ (nm) | $\Phi_f$[a] | Stokes shift (nm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Heptane | 442 | 523 | 0.09 | 81 | | Heptane | 443 | 523, 547 | 0.10 | 80 |
| | Cyclohexane | 447 | 529 | 0.11 | 82 | | Cyclohexane | 449 | 530 | 0.13 | 81 |
| | Toluene | 462 | 605 | 0.09 | 143 | | Toluene | 463 | 609 | 0.11 | 146 |
| 7 | Dioxane | 448 | 633 | 0.02 | 185 | 8 | Dioxane | 448 | 634 | 0.03 | 186 |
| | THF | 455 | | | | | THF | 458 | 686 | | |
| | $CH_3CN$ | 454 | – | | | | $CH_3CN$ | 451 | – | | |
| | $CH_3OH$ | 455 | – | | | | $CH_3OH$ | 446 | – | | |
| | Heptane | 392 | 503 | 0.29 | 111 | | Heptane | 433 | 502, 531 | 0.28 | 69 |
| | Cyclohexane | 396 | 503 | 0.29 | 107 | | Cyclohexane | 434 | 505, 535 | 0.29 | 71 |
| | Toluene | 405 | 554 | 0.18 | 149 | | Toluene | 453 | 595 | 0.17 | 142 |
| 10 | Dioxane | 400 | 573 | 0.09 | 173 | 11 | Dioxane | 447 | 620 | 0.08 | 173 |
| | THF | 412 | – | | | | THF | 454 | 677 | | |
| | $CH_3CN$ | 400 | – | | | | $CH_3CN$ | 454 | – | | |
| | $CH_3OH$ | 400 | – | | | | $CH_3OH$ | 448 | – | | |

[a] Quinine sulphate (0.545 in 1 N $H_2SO_4$)/rhodamine B (0.92 in ethanol)/fluorescein (0.79 in ethanol) were used as fluorescence standards [35] in determining the fluorescence quantum yield.

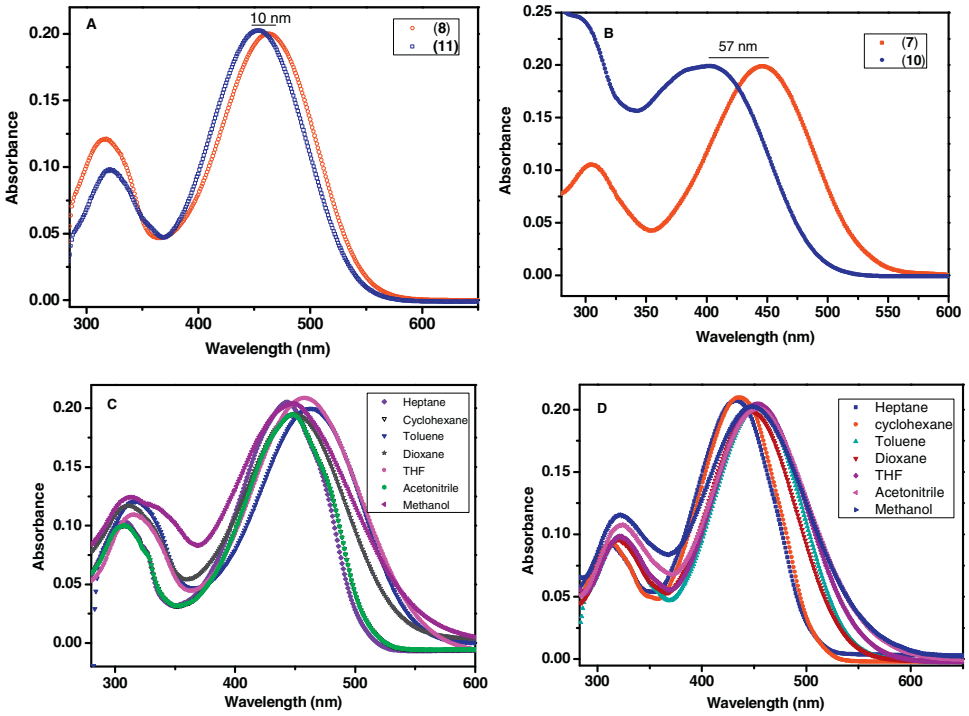


**Fig. 1.** Absorption spectra of the molecules investigated. (A) Absorption of (**8**) & (**11**) in toluene; (B) absorption of (**7**) & (**10**) in toluene; (C and D) absorption of (**8**) & (**11**) in solvents of varying polarity.

polarity is increased, a strong bathochromic shift in emission maxima ($\lambda_f$) is observed. Reorganization of polar solvent molecules around the fluorophore and emission arising from a non-planar, highly polar twisted intramolecular charge transfer (TICT) state contribute to these observed strong emission shifts [13]. Further large Stokes shift observed indicates the formation of the high dipole moment excited charge separated states. Decreased lifetimes with an increase in solvent polarity present other evidence of the presence of such charge transfer states. In contrast to the little or no absorption shifts, the additional double bond in (**8**) yields a stronger bathochromic shift (up to 110 nm) than the stilbene (**7**) (up to 70 nm) (Table 1). The spectral data are shown in Fig. 2A and B. This behaviour is akin to molecules, as reported in the literature [15], that do not contain the phenolic hydroxyl group. The quenched emission in polar solvents is strongly influenced by intermolecular H-bonding interactions as well as efficient nonradiative

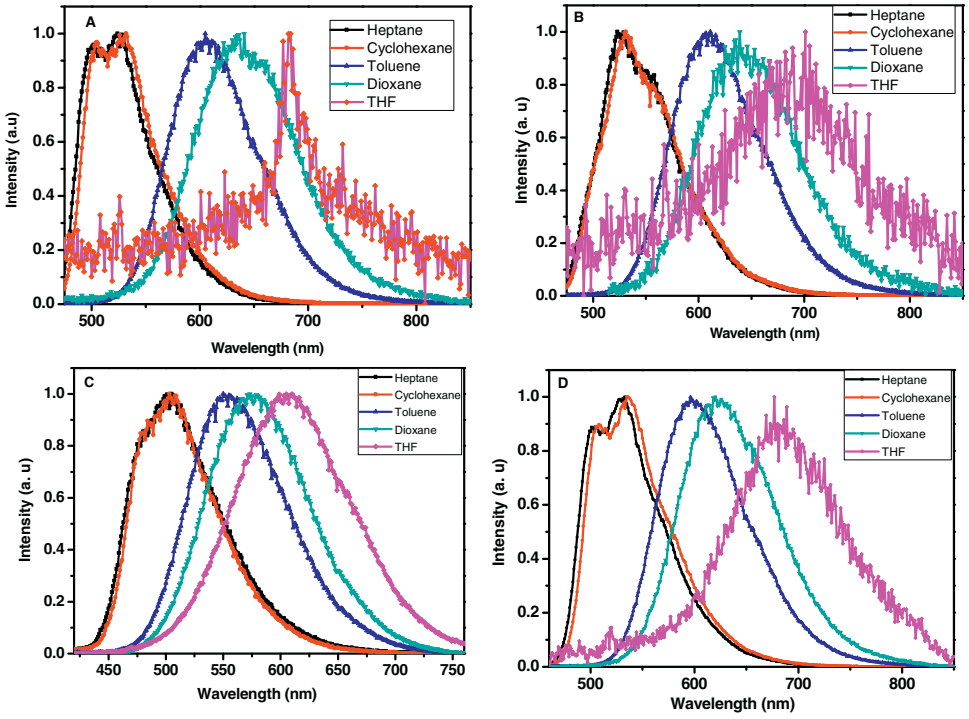


**Fig. 2.** Normalized emission spectra of the molecules investigated in solvents of varying polarity. A (**7**); B (**8**); C (**10**); D (**11**).

decay processes [15]. This observation is also confirmed by greater quantum yields of these compounds in non-polar solvents than in polar solvents.

Conjugating a cholesterol moiety that is largely unresponsive to light is expected to maintain the fluorescence properties of the cholesterol linked stilbenes or dienes as the core donor–acceptor structure is conserved. But in the case of cholesterol conjugated stilbene (**10**), hypsochromic shifts of emission are observed with a minimum shift of 20 nm in heptane and a maximum shift of 60 nm in dioxane as compared to free-cholesterol derivative (**7**). Along with the hypsochromic shift, an overall reduction in solvatochromic effect [70 nm (**10**) and ~110 nm (**7**)] is also seen (Fig. 2C). Bulky cholesterol closer to nitro moiety may slightly offset the donor–acceptor conjugation causing the reduction in the observed solvatochromism. Similar hypsochromic shifts are observed for (**11**) with a shifts up to 14–21 nm in nonpolar heptane or dioxane as compared to free dye (**8**). The solvatochromic shift of 163 nm from heptane to THF (Fig. 2D) is moderately lower than observed

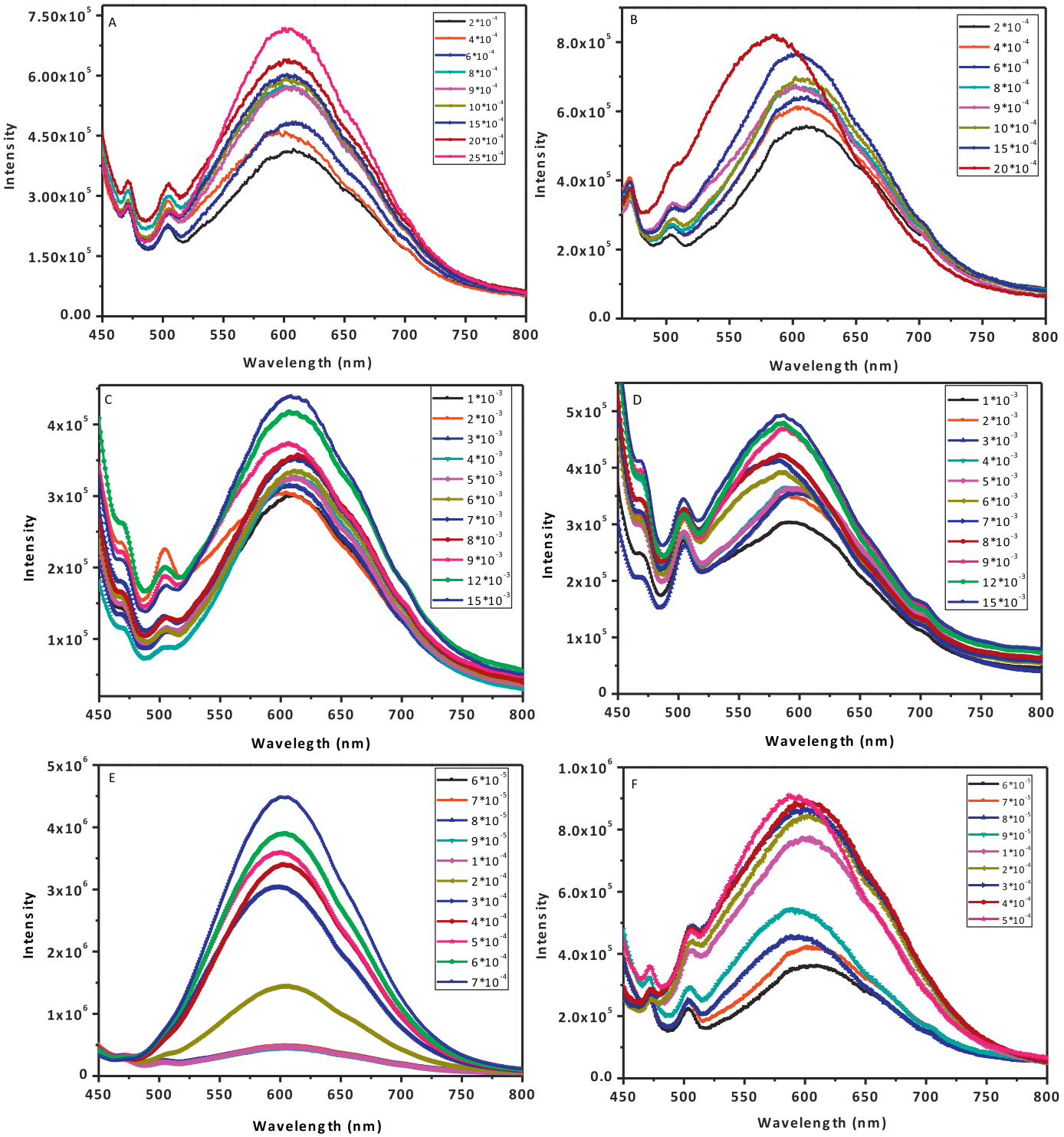


**Fig. 3.** Effect of change in surfactant concentration on fluorescence emission Spectra of (**10**) and (**11**). A, C and E are for (**10**) and B, D and F are for (**11**) in CTAB, SDS and Triton X-100 respectively.

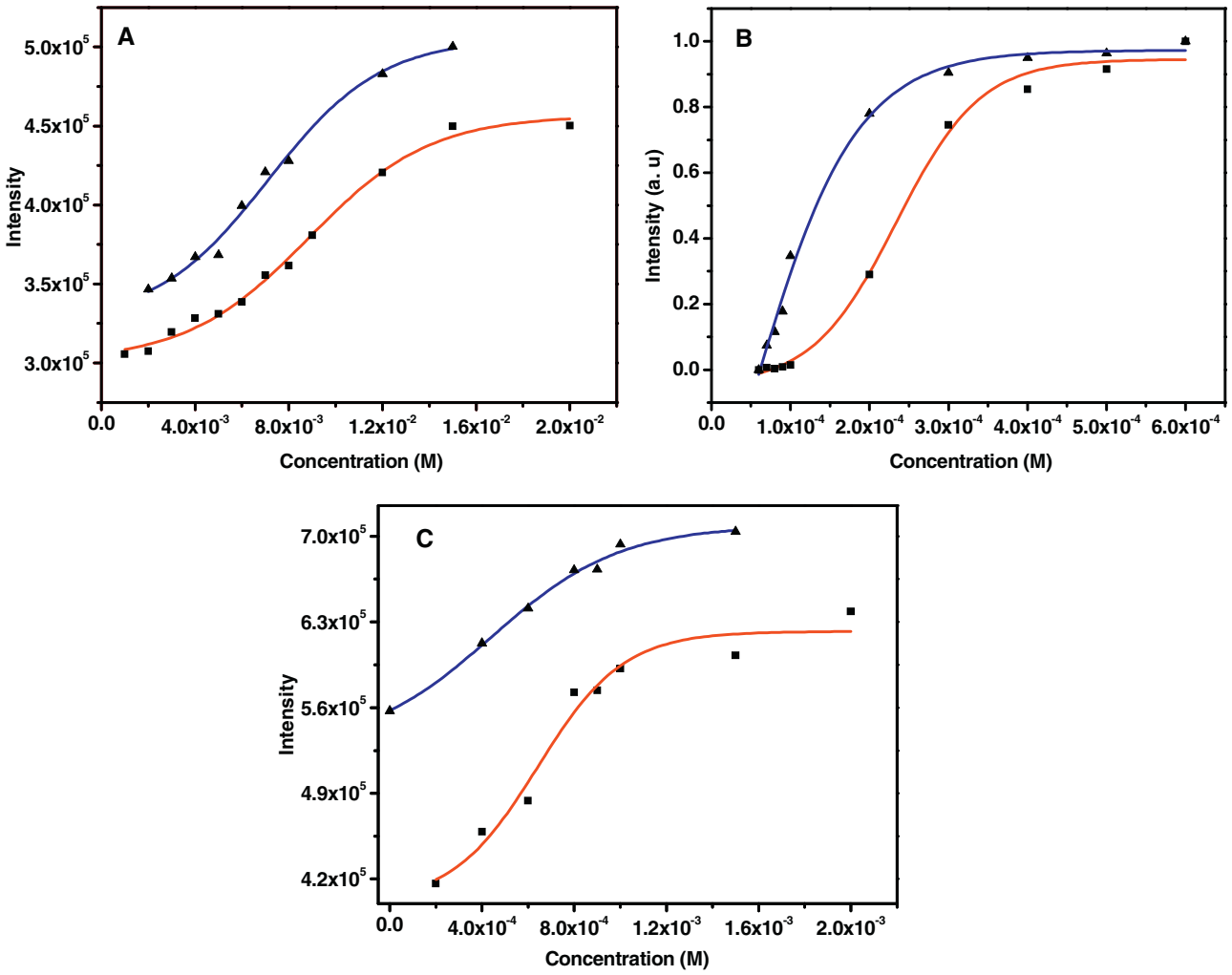


**Fig. 4.** Plots of emission intensity *versus* surfactant concentration for molecules (**10**-Red) and (**11**-Blue). A, B and C are for SDS, Triton X-100 and CTAB respectively. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)

in free diene (175 nm). Apart from a moderate reduction in the solvatochromic emission properties, conjugating a rigid cholesterol moiety contributes to 2–3 fold enhancement in the quantum yield of the molecules. The emission of both compounds, similar to the cholesterol-free dyes, was completely quenched in polar solvents. Table 1 summarizes the absorption and emission behaviour in the solvents studied.

### 3.3. *Absorption and emission of (**10**) and (**11**) in surfactant media*

The ability of surfactants to form micelles and their microenvironmental similarity with biological macromolecules has significant applications in materials science as well as in biology [38,39]. The critical micelle concentration (CMC) is an important parameter to characterize micelle formation and various optical techniques

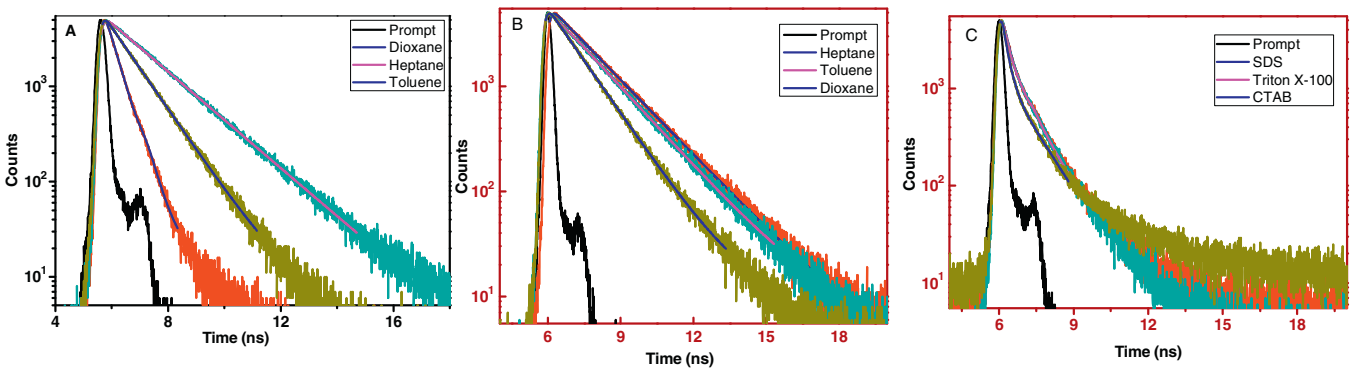


**Fig. 5.** Excited state decay profile of (**8**) and (**11**) in homogeneous and surfactant media. A (**8**) in homogeneous solvents, B (**11**) in homogeneous solvents and C (**11**) in surfactant media. Decay profile for (**8**) in surfactant media could not be observed (the samples were excited at 440 nm).

are used to measure the CMC of micelles [40]. Solubilization of probes at a number of different sites in these surfactant media leads to interesting medium and probe dependent solvatochromic properties. Such fluorophores have therefore been utilized as probes to detect the CMC of various surfactants or related systems [15,41–43] and as solvent polarity indicators [44]. The cholesterol conjugated fluorophores (**10**) and (**11**) were utilized to determine the CMC of surfactants in aqueous solutions. The structural changes induced by cholesterol addition, the type of microenvironment or temperature could affect the emission of these probes. It is therefore hypothesized that hydrophobic cholesterol appendage allows the fluorophores to interact with the hydrophobic chains of the surfactant. Subsequently we observed smooth changes to fluorescence intensity for these probes in the surfactant media.

The absorption of (**10**) in surfactant media (CTAB, SDS, Triton X-100) exhibits a moderate red-shift in comparison to non-polar solvents but is comparable to other solvents. Diene (**11**) has a blue-shifted absorption as compared to the organic solvents. Table 2 lists the absorption and emission maxima of these molecules in the micelle media utilized.

Similar to the absorption behaviour of these fluorophores, cholesterol probes have emission maxima comparable to those obtained in homogeneous solvents such as dioxane. The emission maxima show a slight red-shift and moderate blue shift in (**10**) and (**11**) respectively. The observations suggest that diene (**11**) has a greater propensity to occupy and interact with less polar domains in micellar media than (**10**). The emission of cholesterol free fluorophores (**7**) and (**8**) was completely quenched in the surfactant media owing to polar interactions. The quantum yields of the fluorophores in the micellar media are expected to increase because of their partitioning into the hydrophobic core as well due to the confinement provided by the micellar core. The observed emission quantum yields for (**10**) and (**11**) are comparable to that obtained in dioxane. This similarity indicates the possible localization of the fluorophores towards hydrophobic regions of the micelle.

As the concentration of surfactant is increased, the fluorescence intensity of (**10**) and (**11**) increases in all three media (SDS, Triton X-100, CTAB). The corresponding fluorescence intensity values are given in Table 1 in supplementary information. This concentration increase has minor effect on $\lambda_f$ of the molecules studied. The minor variations in emission maxima are not consistent across all the three micelles and local perturbations may be a contributing factor for such behaviour. Fig. 3A–F summarizes the emission intensity changes as a function of wavelength and surfactant concentrations for different micelle media. As can be seen clearly, in the case of neutral surfactant Triton X-100 (Fig. 3E and F), a distinct jump in fluorescence intensity was observed whereas in other surfactants the change is not demarcated. Nevertheless, this intensity change due to increase in surfactant concentration indicates partitioning of the probe to preferential domains of the micelle. This also explains the observed blue shifted emission of diene (**11**) in comparison to dioxane. As soon as the surfactants reach a CMC, measurable emission intensity changes were observed because of probes preferential localization.

### 3.4. Determination of critical micelle concentration

As part of our investigation to determine the CMC and to prove the efficacy of our compounds as fluorescence probes, we chose non-ionic (Triton X-100), anionic (SDS) and cationic (CTAB) surfactants as a representative selection. The CMC data for these surfactants were well documented in the literature [42,45,46] and offer an easy comparison to probe the fluorophore's suitability for such measurements. The plot of emission intensity changes *versus* the change in concentration of surfactant has been utilized as a tool to determine the CMC. As described earlier, a sharp increase

**Table 2**
Absorption, emission behaviour of (**7**), (**8**), (**10**) and (**11**) in microheterogenous media and calculated CMC of SDS, CTAB and Triton-X 100 using (**10**) and (**11**). Absorption and emission data of fluorophores were obtained at the CMC of the given surfactant.

| | (**10**) | | | | CMC | (**11**) | | | | CMC | (**7**) | | (**8**) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\lambda_a$ (nm) | $\lambda_f$ (nm) | Stokes shift (nm) | $\Phi_f$[a] | | $\lambda_a$ (nm) | $\lambda_f$ (nm) | Stokes shift (nm) | $\Phi_f$ | | $\lambda_a$ (nm) | $\lambda_f$[b] | $\lambda_a$ (nm) | $\lambda_f$[b] |
| SDS | 406 | 584 | 178 | 0.12 | 8.5 ± 0.08 | 426 | 611 | 185 | 0.10 | 7.7 ± 0.07 | 421 | - | 435 | - |
| CTAB | 401 | 599 | 198 | 0.11 | 0.82 ± 0.04 | 436 | 603 | 167 | 0.09 | 0.20 ± 0.02 | 422 | - | 435 | - |
| Triton X-100 | 405 | 589 | 184 | 0.09 | 0.247 ± 0.06 | 437 | 602 | 165 | 0.09 | 0.77 ± 0.03 | 421 | - | 436 | - |

[a] Rhodamine B (0.92 in ethanol) were used as fluorescence standards [35] in determining the fluorescence quantum yield.
[b] No fluorescence was detected.

**Table 3**
Fluorescence lifetime data in homogeneous solvents and in surfactant media. (**10**) was excited at 406 nm and other molecules were excited at 440 nm. Lifetime data are in nano seconds. For biexponential decay the bracket mentioned values are pre-exponentials.

| Solvent | (**7**)[a] | $\chi^2$ | (**10**) | $\chi^2$ | (**8**)[a] | $\chi^2$ | (**11**) | $\chi^2$ |
|---|---|---|---|---|---|---|---|---|
| Heptane | 1.64 | 1.133 | 2.00 | 1.177 | 1.65 | 1.039 | 1.83 | 1.009 |
| Toluene | 1.31 | 1.063 | 1.73 | 1.022 | 1.32 | 1.159 | 1.73 | 1.086 |
| Dioxane | 0.62 | 1.099 | 1.59 | 1.143 | 0.41 | 1.068 | 1.09 | 1.064 |
| SDS | – | – | 2.06(0.86) 0.33(0.14) | 1.061 | – | – | 1.01(0.49) 0.25(0.51) | 1.062 |
| Triton X-100 | – | – | 1.02(0.71) 0.20(0.29) | 1.054 | – | – | 0.83(0.62) 0.17(0.38) | 1.135 |
| CTAB | – | – | 1.96(0.70) 0.20(0.30) | 1.132 | – | – | 1.10(0.494 0.21(0.506) | 1.041 |

[a] Complete fluorescence quenching seen for (**7**) and (**8**).

in emission intensity for both the cholesterol linked fluorophores was observed as soon as the surfactant adopts the micelle conformation. Fig. 4A–C depicts plots of emission intensity changes of fluorophore with the surfactant concentration. The inflection point of the curve has been taken as the CMC for all the surfactants. Table 2 summarizes the obtained values of CMC and these results are in harmony with results obtained with similar probes [12,13,15]. Substitution of cholesterol moiety preferentially pushes the fluorophore to more non-polar locations and this attribute can be useful in probing complex biological membranes. As a control experiment, when cholesterol free dye (**7**) was used to determine the CMC, the fluorescence was heavily quenched and no changes either to the intensity or wavelength were observed indicating that presence of cholesterol moiety is advantageous in probing these hydrophobic environments.

### 3.5. Fluorescence lifetimes (**7**)–(**11**) in homogeneous and microheterogeneous media

The fluorescence decay profile of substituted diphenylbutadiene derivatives in homogeneous solvents (heptane, toluene and dioxane) and micellar media when excited at 406 or 440 nm are shown in Fig. 5 (Fig. S1 for (**7**) and (**10**)). The obtained lifetime values are listed in Table 3. In organic solvents, all the molecules (**7**), (**8**), (**10**) & (**11**) decays single-exponentially with a lifetime of ~0.41–2.00 ns depending on the polarity of solvent used. The observed lifetimes show a decreasing trend with an increase in solvent polarity. Reliable fluorescence lifetimes in highly polar solvents (acetonitrile/methanol/$H_2O$) could not be obtained due to very low fluorescence quantum yields and rapid non-radiative decay. On cholesterol substitution (**10**, **11**) the observed lifetime increases as compared to cholesterol free dyes (**7**, **8**) in the given solvents.

Interestingly, in the case of micellar media, a bi-exponential decay was observed for cholesterol linked fluorophores (Fig. 5C) indicating the ability of the molecules to reside in two different microenvironments leading to more than one emitting species. Alternately one part of a solute could have an inclination towards polar properties associated with the interface while another part has a disposition towards non-polar interior. The shorter lifetimes, 0.33 ns (SDS), 0.20 ns (Triton X 100), 0.20 ns (CTAB) for (**10**) and 0.25 ns, 0.17 ns and 0.21 ns for (**11**) observed could be correlated to the solute occupying the polar interface (Table 3). The relatively low quantum yields obtained for these fluorophores in polar solvents as well as in surfactant media also support the interaction of the fluorophore with polar surroundings. The decay profile of non-cholesterol conjugated molecules (**7**, **8**) in surfactant media data could not be resolved to generate a best fit as a result of heavy quenching of the free fluorophore in these media. Based on the structure of our molecule, it is likely that the cholesterol end favours the non-polar domain and dimethyl amino moiety favours polar domain.

## 4. Conclusions

The investigation describes the synthesis and fluorescence spectroscopic investigations of cholesterol free [(**7**), (**8**)] and cholesterol conjugated donor–acceptor diphenylpolyenes [(**10**), (**11**)]. The diphenylpolyene moiety containing dimethylamino group as a donor and a nitro group as an acceptor is extrinsically linked to the 3-hydroxyl position *via* a carbonate linker. The spectroscopic investigations in homogeneous solvents reveal preservation of charge-transfer emission for these molecules despite cholesterol modification. The fluorescence emission behaviour of these molecules in surfactant media indicates assistance of cholesterol

in preferentially placing the fluorophore to less polar locales of the micelle yielding a bi-exponential decay profile. Thus cholesterol conjugated fluorophores can be excellent biological microenvironment distinguishing reporters [22,47]. This study, although limited to cholesterol conjugation, can pave the way for similar donor–acceptor based fluorophore conjugated biological receptors to probe complex biological media.

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