Near-Infrared Fluorescent Probes: Synthesis and Spectroscopic Investigations of A Few Amphiphilic Squaraine Dyes

Kalliat T. Arun and Danaboyina Ramaiah*

Photosciences and Photonics Division, Regional Research Laboratory (CSIR), Trivandrum 695 019, India

Received: March 15, 2005; In Final Form: April 21, 2005

With the objective of developing near-infrared fluorescence probes for biological applications, a few squaraine dyes **3a**−**d**, containing amphiphilic substituents, were synthesized and their photophysical properties have been investigated in the presence and absence of the organized media. These dyes exhibited absorption in the range 630−650 nm, with significant absorption coefficients ($\epsilon = 1-3 \times 10^5$ M^{-1} cm^{-1}) in the aqueous medium. The fluorescence spectra of these dyes showed emission maximum from 660 to 675 nm, depending on the nature of substituents. The fluorescence quantum yields were in the range from 0.15 to 0.21 in ethanol, but 10 times lower values were observed ($\Phi_f = 0.01-0.02$) in the aqueous medium. In the presence of micelles such as cetyltrimethylammonium bromide, sodium dodecyl sulfate, and Triton X-100, these dyes showed negligible changes in their absorption properties, whereas a significant enhancement (5−10-folds) in their fluorescence yields was observed. Picosecond time-resolved studies indicated that these dyes show single-exponential decay in ethanol and ethanol−water mixtures; however, they exhibit biexponential decay with longer lifetimes in the presence of the micellar media. The results indicate that these novel amphiphilic squaraine dyes **3a**−**d**, which exhibit favorable photophysical properties, good solubility in the aqueous medium, and interact efficiently with micelles, can have potential biological applications as near-infrared fluorescence sensors.

1. Introduction

Squaraines form a class of dyes possessing sharp and intense absorption in the near to infrared region.[1] The intramolecular charge-transfer (CT) character of the S_0-S_1 electronic excitation combined with an extended conjugated π-electron network gives rise to the intense bands observed in the near-infrared region for the squaraine dyes.[2] The photochemical and photophysical aspects of these dyes have been studied extensively[1−3] because of their use as photogeneration pigments for photoreceptors in copiers and laser printers,[4] photoconductors in organic solar cells,[5] and IR absorbers in organic optical disks.[6] However, the biological applications of the squaraine dyes, especially their potential use as sensitizers in photodynamic therapy (PDT), have remained unexplored because of their insignificant triplet yields.[1,3] Recently, the biological applications of the appropriately substituted squaraine dyes as long wavelength fluorescent labels have been reported in the literature.[7]

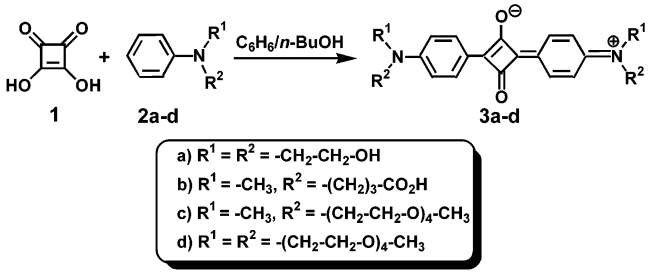
Our interest in this area originated from the idea of utilizing the squaraine dyes for PDT applications by appropriately modifying them with heavy atoms, which can increase their intersystem crossing efficiency. In this context, we have recently reported that substitution of the squaraine dyes with heavy atoms such as bromine and iodine results in their increased solubility in the aqueous medium and enhanced intersystem crossing efficiency, when compared to the parent squaraine dye.[8] These dyes exhibited absorption in the range from 600 to 620 nm and showed quantum yields of triplet excited states ($\Phi_T = 0.22-0.5$) and singlet oxygen ($\Phi(^1O_2) = 0.13-0.47$), depending on the nature of the halogen atoms. The cytotoxicity and mutagenicity studies using mammalian cell lines and bacterial strains indicated that these dyes exhibit significant cytotoxicity upon excitation with visible light and the mechanism of their biological activity could be attributed to the in vitro generation of singlet oxygen.[9] However, these heavy atom substituted dyes possess very low fluorescence quantum yields ($\Phi_f \leq 0.0003$) in aqueous medium, thereby limiting their use for the detection of tumors (diagnosis) by the fluorescence emission of the dyes that can localize selectively in tumor tissues.[10−13]

The objective of the present study has been to design fluorescent probes for the biological applications on the basis of the squaraine dyes, because of their advantageous photophysical properties and readily tunable substitution to introduce the required properties. In this context, we felt that the squaraine dyes **3a**−**d** (Scheme 1) would be promising candidates for biological sensing applications, since the substituents such as carboxyl, glycol, and alcohol groups present in them would render amphiphilicity to these dyes thereby increasing their solubility in aqueous medium and improving their fluorescence intensity and cellular uptake.[14] Here, we report the synthesis of a few novel squaraine dyes **3a**−**d** and the results of our investigations devoted to understanding their behavior in micellar media, which mimics the cell membrane structures.[15] The results indicate that the squaraine dyes **3a**−**d** (Scheme 1) under investigation exhibit reasonably good solubility in the aqueous medium and show significant fluorescence yields. Depending on the nature of the substituents present, these dyes were found to interact with the micellar media, effectively resulting in a 5−10-fold enhancement in their fluorescence yields. These results thus demonstrate that the appropriately substituted dyes based on squaraine moiety can have potential use as near-infrared fluorescent sensors in biological applications, where they would interact efficiently with cell membrane structures.

* To whom correspondence should be addressed. Tel: (+91) 471 2515362; fax: (+91) 471 2490186, 2491712; e-mail: d_ramaiah@rediffmail.com or rama@csrrltrd.ren.nic.in.

10.1021/jp051338o CCC: $30.25
Published on Web 06/04/2005

SCHEME 1: Synthesis of the Amphiphilic Squaraine Dyes 3a−d



2. Experimental Section

2.1. General Techniques. The equipment and the procedure for melting point determination and spectral recordings are described in our earlier publications.[16] Fluorescence quantum yields were measured by the relative method using optically matched dilute solutions. 4,4′-[Bis-(*N*,*N*-dimethylamino)phenyl]-squaraine dye ($\Phi_f = 0.70$) was used as the standard.[17] The quantum yields of fluorescence were calculated using eq 1

$$\Phi_u = \frac{A_s F_u n_u^2}{A_u F_s n_s^2}\Phi_s \qquad (1)$$

where A_s and A_u are the absorbance of standard and unknown, respectively. F_s and F_u are the areas of fluorescence peaks of the standard and unknown and n_s and n_u are the refractive indices of the standard and unknown solvents, respectively. Φ_s and Φ_u are the fluorescence quantum yields of the standard and unknown dye. A solution of Rhodamine 6G in water has been used as a quantum counter, which allows the correction in the spectral range up to 750 nm. Fluorescence lifetimes were measured on an IBH picosecond single photon counting system using a 635 nm IBH NanoLED source and Hamamatsu C4878-02 MCP detector. The fluorescence decay profiles were deconvoluted using IBH data station software V2.1, fitted with mono- or biexponential decay and minimizing the χ^2 values of the fit to 1 ± 0.1. The solvent orientation polarizability, Δf, was calculated as per eq 2[18]

$$\Delta f = \frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \qquad (2)$$

where ϵ and n are, respectively, the dielectric constant and refractive index of the solvents used. Measurements in the presence of additives were carried out by adding the freshly prepared dye solution in ethanol to the already prepared solution of the additive. The concentrations of the dyes were chosen in the micromolar range for all studies so that they exist as monomers as observed from their absorption spectra. Solvents used were purified before use. Doubly distilled water was used in all the studies. Petroleum ether used was the fraction with bp 60−80 °C. All experiments were carried out at room temperature (25 ± 1 °C), unless otherwise mentioned.

2.2. Materials. Squaric acid, *N*-methylaniline, triethylene glycol, cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and Triton X-100 (TX-100) were purchased from Aldrich and were used without further purification. The starting materials, 4-bromobutyrate, (bp 81 °C at 10 mmHg),[19] *N*-methyl-*N*-(ethyl-4-butanoate)aniline, (bp 116−118 °C at 0.25 mmHg),[20] *N*-methyl-*N*-(carboxypropyl)aniline, (bp 147−150 °C at 0.2 mmHg),[20] *N*-methyl-*N*-(2-hydroxyethyl)-aniline, (bp 171 °C at 0.25 mmHg),[21] and the squaraine dye **3a** (mp 220−222 °C, lit. mp 219−221 °C)[22] were prepared by modifying the reported procedures.

2.3. Synthesis of 4,4′-[Bis-{*N*-methyl-*N*-(carboxypropyl)-amino}phenyl]-squaraine (3b). *N*-Methyl-*N*-(carboxypropyl)-aniline (319 mg, 1.74 mmol) and squaric acid (100 mg, 0.87 mmol) were refluxed in a mixture of *n*-butanol and benzene (1:3) by azeotropic distillation of water for 24 h. The solvent was distilled off under reduced pressure to obtain a residue which was chromatographed over silica gel. Elution of the column with a mixture (1:9) of methanol and chloroform gave 100 mg (13%) of the squaraine dye **3b**, mp 238−240 °C (d); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 30 °C, TMS): $\delta = 1.91$ (p, 4H, $-CH_2$), 2.40 (t, 4H, $J = 7.2$ Hz, $-CH_2$), 2.91(s, 6H, $-NCH_3$), 3.35 (t, 4H, $J = 7.3$ Hz, $-CH_2$), 6.99 (d, 4H, $J = 9.07$ Hz, Ar−H), 8.05 (d, 4H, $J = 8.97$ Hz, Ar−H); ^{13}C NMR (75 MHz, [D$_6$]DMSO, 30 °C, TMS): $\delta = 21.82, 31.41, 38.46, 52.03, 112.64, 116.73, 129.17, 149.13, 179.23$; IR (KBr): ν_{max} 3420, 2924, 1729, 1590, 1439, 1130 cm^{-1}; elemental analysis calcd (%) for $C_{26}H_{28}N_2O_6$: C, 67.23; H, 6.08; N, 6.03; found: C, 67.50; H, 5.81; N, 5.80.

2.4. Synthesis of 4,4′-[Bis-{*N*-methyl-*N*-(3,6,9,12-tetraoxatrideca)amino}-phenyl] Squaraine (3c). *Preparation of [1-(2-Methoxyethoxy)-2-(2-(4-methyl-2-sulfonyl)ethoxy)]ethane.* To a mixture of triethyleneglycol (5.0 g, 33 mmol) and sodium hydride (0.871 g, 36.3 mmol) in THF (40 mL), methyl iodide (4.69 g, 33 mmol) was added at once and stirred for 1 h. The solvent was removed under reduced pressure and the residue obtained was dissolved in water and extracted with dichloromethane. Evaporation of the solvent under reduced pressure gave a viscous liquid, to which pyridine (17 mL) was added and cooled to 0−5 °C. To this reaction mixture, *p*-toluenesulfonyl chloride (6.0 g, 31 mmol) was added in portions over a period of 1 h. Stirring was continued for 12 h maintaining the temperature at 0−5 °C, and then the mixture was treated with 30% HCl and was extracted with dichloromethane. Removal of the solvent under reduced pressure gave a viscous liquid, which was chromatographed over neutral alumina. Elution of the column with a mixture of ethyl acetate and petroleum ether (1:4) gave 5 g (50%) of [1-(2-methoxyethoxy)-2-(2-(4-methyl-2-sulfonyl)ethoxy)]ethane as viscous liquid ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C, TMS): $\delta = 2.44$ (s, 3H, Ar$-CH_3$), 3.37 (s, 3H, $-OCH_3$), 3.70−3.51(m, 10H, $-OCH_2$, $-NCH_2$), 4.16 (t, 2H, $J = 5.1$ Hz, $-OCH_2$), 7.35 (d, 2H, $J = 8.07$ Hz, Ar−H), 7.78 (d, 2H, $J = 7.97$ Hz, Ar−H); ^{13}C NMR (75 MHz, $CDCl_3$, 30 °C, TMS): $\delta = 21.55, 58.92, 68.60, 69.17, 70.45, 70.66, 71.82, 127.90, 129.75, 144.74$; IR (neat): ν_{max} 2924, 2877, 1362, 1181, 1098 cm^{-1}.

Preparation of N-Methyl-N-(3,6,9,12-tetraoxatrideca)aniline. To a stirred solution of *N*-methyl-*N*-(2-hydroxyethyl)aniline (2.0 g, 13 mmol) and KOH (1.04 g, 26 mmol) in THF (20 mL), [1-(2-methoxyethoxy)-2-(2-(4-methyl-2-sulfonyl)ethoxy)]-ethane (4.6 g, 143 mmol) in THF (10 mL) was added dropwise over a period of 1 h. The reaction mixture was refluxed for 24 h. Excess solvent was removed under reduced pressure and the residue was dissolved in water and was extracted with dichloromethane. Evaporation of the solvent under reduced pressure gave a viscous liquid, which was chromatographed over neutral alumina. Elution of the column with ethyl acetate and petroleum ether mixture (1:9) gave 2.4 g (60%) of *N*-methyl-*N*-(3,6,9,12-tetraoxatrideca)aniline as a viscous liquid. ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C, TMS): $\delta = 2.97$ (s, 3H, $-NCH_3$), 3.38(s, 3H, $-OCH_3$), 3.67−3.51 (m, 16H, $-OCH_2$), 6.73−6.66 (m, 3H, Ar−H), 7.21(t, 2H, $J = 7.3$ Hz, Ar−H); ^{13}C NMR (300 MHz, $CDCl_3$, 30 °C, TMS): $\delta = 38.81, 52.27, 58.94, 68.49, 70.54,$

70.61, 71.85, 112.04, 166.15, 129.08, 149.16; IR (neat): ν_{max} 2862, 1744, 1506, 1114 cm^{-1}.

*Preparation of the Squaraine Dye **3c***. A solution of *N*-methyl-*N*-(3,6,9,12-tetraoxatrideca)aniline (400 mg, 1.35 mmol) and squaric acid (77 mg, 0.67 mmol) in a mixture of *n*-butanol and benzene (1:3) was refluxed by azeotropic distillation of water for 18 h. The solvent was distilled off under reduced pressure, and the residue obtained was chromatographed over silica gel. Elution of the column with a mixture of methanol and chloroform (1:49) gave 110 mg (15%) of the squaraine dye **3c**, mp 100−102 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C, TMS): δ = 3.21(s, 6H, $-NCH_3$), 3.37 (s, 6H, $-OCH_3$), 3.72−3.52 (m, 32H, $-OCH_2$), 6.81 (d, 4H, J = 8.96, Ar−H), 8.39 (d, 4H, J = 8.95 Hz, Ar−H); ^{13}C NMR (75 MHz, $CDCl_3$, 30 °C, TMS): δ = 39.68, 52.29, 58.97, 68.53, 70.42, 70.52, 70.56, 70.81, 71.83, 112.43, 119.90, 133.17, 154.41, 183.32, 188.58; IR (neat): ν_{max} 2877, 1610, 1584, 1140, 1098 cm^{-1}; elemental analysis calcd (%) for $C_{36}H_{52}N_2O_{10}$: C, 64.27; H, 7.79; N, 4.16; found: C, 64.51; H, 7.69; N, 3.88.

2.5. Preparation of 4,4′-[Bis-*N*,*N*-(3,6,9,12-tetraoxatridecaamino)phenyl]squaraine (3d). *Preparation of Bis-N,N-(3,6,9,12-tetraoxatrideca)aniline.* To a stirred solution of *N*-phenyldiethanolamine (600 mg, 3.31 mmol) and KOH (556 mg, 9.93 mmol) in THF (15 mL), [1-(2-methoxyethoxy)-2-(2-(4-methyl-2-sulfonyl)ethoxy)]ethane (2.22 g, 6.95 mmol) in THF was added dropwise over a period of 1 h. After the addition, the reaction mixture was refluxed for 24 h. Excess solvent was removed under reduced pressure and the residue obtained was dissolved in water and extracted with dichloromethane. The organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a viscous liquid, which was chromatographed over neutral alumina. Elution of the column with ethyl acetate−petroleum ether mixture (1:4) gave 900 mg (60%) of bis-*N*,*N*-(3,6,9,12-tetraoxatrideca)aniline as a viscous liquid. ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C, TMS): δ = 3.38(s, 6H, $-OCH_3$), 3.65−3.53 (m, 32H, $-OCH_2$), 6.71−6.63 (m, 3H, Ar−H), 7.19 (t, 2H, J = 7.2 Hz, Ar−H); ^{13}C NMR (75 MHz, $CDCl_3$, 30 °C, TMS): δ = 50.73, 58.91, 68.31, 70.38, 70.49, 70.53, 71.80, 111.54, 115.85, 129.15, 147.60; IR (neat): ν_{max} 2924, 2867, 1125 cm^{-1}.

*Preparation of the Squaraine Dye **3d***. A solution of bis-*N*,*N*-(3,6,9,12-tetraoxatrideca)aniline (350 mg, 0.74 mmol) and squaric acid (42 mg, 0.37 mmol) in a mixture of *n*-butanol and benzene (1:3) was refluxed by azeotropic distillation of water for 18 h. The solvent was distilled off under reduced pressure and the residue obtained was chromatographed over silica gel. Elution of the column with a mixture (1:99) of methanol and chloroform gave 40 mg (5%) of the squaraine dye **3d**, mp 78−80 °C; ^{1}H NMR (300 MHz, $CDCl_3$, 30 °C, TMS): δ = 3.37 (s, 12H, $-OCH_3$), 3.77−3.55 (m, 64H, $-OCH_2$), 6.84 (d, 4H, J = 8.96, Ar−H), 8.37 (d, 4H, J = 8.95 Hz, Ar−H); ^{13}C NMR (75 MHz, $CDCl_3$, 30 °C, TMS): δ = 50.73, 58.91, 68.31, 70.38, 70.49, 70.53, 71.80, 111.54, 115.85, 129.15, 147.60, 183.32, 188.58; IR (neat): ν_{max} 2918, 2867, 1610, 1584, 1114 cm^{-1}; elemental analysis calcd (%) for $C_{52}H_{84}N_2O_{18}$: C, 60.92; H, 8.26; N, 2.73; found: C, 61.20; H, 7.98; N, 2.49.

3. Results

3.1. Absorption and Fluorescence Emission Properties. The squaraine dyes **3a**−**d**, containing various substituents, were synthesized in reasonably good yields by the condensation reaction between squaric acid and the corresponding N-substituted aniline derivatives in benzene/*n*-butanol mixture with azeotropic distillation of water. These compounds were purified by column chromatography and characterized on the basis of analytical results and spectral evidence. Thus, for example, dyes **3a**−**d** showed strong absorption bands around 1610 and 1590 cm^{-1} in the IR spectra which corresponds to the C−O stretching frequency of cyclobutenediylium-1,3-diolate moiety. This is characteristic of resonance stabilized zwitterionic squaraine dyes. In the ^{1}H NMR spectra, the aromatic protons of the precursor aniline derivatives underwent a downfield shift of about 1 ppm in the dye because of the electron-withdrawing effect of the squarate anion. The ^{13}C NMR of these dyes showed two sp^2 carbons at δ 188.58 and 183.32 which are characteristic of the four carbon atoms of the central cyclobutene ring. In the case of dye **3c**, four sp^2 carbons of the aromatic aniline ring appeared at δ 154.41, 133.17, 119.90, and 112.43 while the other sp^3 carbons of the NCH_3 and OCH_3 groups appeared at δ 39.68 and 52.29, respectively. These dyes were quite stable under the experimental conditions and also in the presence of light (>600 nm).

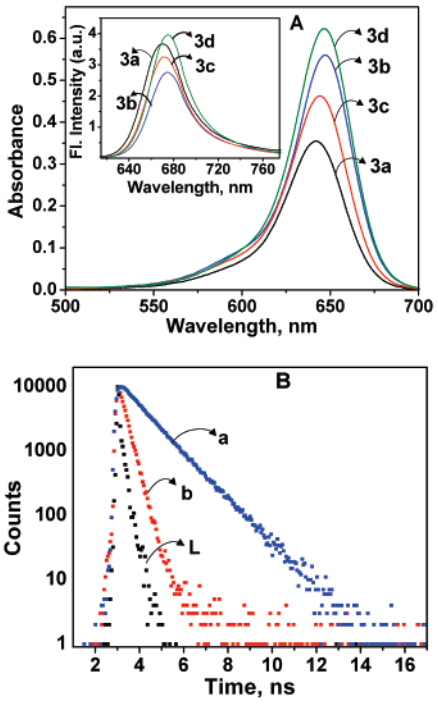


Figure 1. (A) Absorption spectra of **3a** (2.4 μM), **3b** (3.5 μM), **3c** (1.5 μM), and **3d** (1.8 μM) in 10% (vol/vol) ethanol−water mixture. Inset shows the fluorescence spectra of the dyes **3a**−**d** (optically matched solutions). Excitation wavelength, 600 nm. (B) Fluorescence decay profiles of **3c** in (a) ethanol, (b) 10% (vol/vol) ethanol−water mixture, and L is the lamp profile. Excitation and emission wavelengths, 635 and 670 nm, respectively.

Figure 1A shows the absorption and fluorescence emission spectra of the amphiphilic squaraine dyes **3a**−**d** in 10% (vol/vol) ethanol−water mixture, whereas Table 1 summarizes their photophysical properties in different solvent mixtures. These dyes exhibited absorption maxima in the range 640−646 nm in aqueous medium, whereas in ethanol, they exhibited a blue shift of about 1−8 nm. The fluorescence emission spectra of these dyes similarly showed a blue shift of about 9−14 nm, when aqueous medium was replaced with ethanol. For example, the squaraine dye **3d** showed the absorption and emission maxima at 646 and 676 nm in aqueous medium, while in ethanol it exhibited at 638 and 662 nm, respectively. All these dyes possess reasonably good fluorescence quantum yields in ethanol (Table 1). The symmetrical dye **3d** with long-chain glycol units (four units) exhibited a maximum fluorescence quantum yield

TABLE 1: Absorption (ab) and Fluorescence Emission (em) Properties of the Dyes 3a−d in Ethanol, 10% (vol/vol) Ethanol−Water Mixture, and in the Presence of Micelles Such as Cetyltrimethylammonium Bromide (CTAB), Sodium Dodecyl Sulfate (SDS), and Triton X-100 (TX-100)[a,b]

	3a			**3b**			**3c**			**3d**		
	λ_{max} (nm)			λ_{max} (nm)			λ_{max} (nm)			λ_{max} (nm)		
additive	ab	em	Φ_f	ab	em	Φ_f	ab	em	Φ_f	ab	em	Φ_f
ethanol	639	663	0.15	636	662	0.18	637	660	0.19	638	662	0.21
10% EtOH−H_2O	644	671	0.021	647	673	0.015	645	674	0.018	646	675	0.026
CTAB[c]	642	667	0.11	644	667	0.14	642	663	0.12	644	667	0.096
SDS[d]	641	672	0.098	N[e]	N[e]	N[e]	640	663	0.15	643	668	0.16
TX-100[f]	647	671	0.15	649	672	0.12	645	669	0.12	646	671	0.14

[a] Average of more than two experiments. [b] Error involved in Φ_f values, ca. ±5%. [c] [CTAB] 129, 8, 21, and 110 mM for the dyes **3a**, **3b**, **3c**, and **3d**, respectively. [d] [SDS] 28, 21, and 22 mM for the dyes **3a**, **3c**, and **3d**, respectively. [e] Negligible changes were observed for the dye **3b** in the presence of SDS. [f] [TX-100] 109, 116, 118, and 135 mM for the dyes **3a**, **3b**, **3c**, and **3d**, respectively.

TABLE 2: Picosecond Time-Resolved Fluorescence Lifetimes of the Squaraine Dyes 3a−d in Ethanol, 10% (vol/vol) Ethanol−Water Mixture, and the Presence of Micelles[a,b]

	lifetimes, ps (relative amplitude)			
additive	**3a**	**3b**	**3c**	**3d**
ethanol	680 (100%)	560 (100%)	510 (100%)	910 (100%)
10% EtOH−H_2O	180 (100%)	29 (69%)	160 (100%)	320 (100%)
		211 (31%)		
CTAB[c]	350 (39%)	440 (4%)	560 (45%)	410 (25%)
	780 (61%)	860 (96%)	920 (55%)	1140 (75%)
SDS[d]	510 (57%)	Nd[e]	590 (100%)	690 (4%)
	610 (43%)	610 (43%)		1240 (96%)
TX-100[f]	630 (34%)	430 (31%)	130 (21%)	450 (37%)
	1320 (66%)	1140 (69%)	1120 (79%)	1450 (63%)

[a] Average of more than two experiments. [b] Error involved, ca. ±5%. [c] [CTAB] 20, 21, 42, and 41 mM for the dyes **3a**, **3b**, **3c**, and **3d**, respectively. [d] [SDS] 28, 21, and 22 mM for the dyes **3a**, **3b**, and **3c,** respectively. [e] Not determined. [f] [TX-100] 45, 30, 28, and 59 mM for the dyes **3a**, **3b**, **3c**, and **3d**, respectively.

of $\Phi_f = 0.21$, whereas $\Phi_f = 0.15$ was observed for the symmetrical dye **3a** with single glycol unit. However, all these dyes exhibited around 10-times lower quantum yields of fluorescence in aqueous medium when compared to those in ethanol.

To have a better understanding of the excited-state properties of these molecules, we have carried out picosecond time-resolved fluorescence analysis in different solvents. For example, Figure 1B shows the fluorescence decay profiles of the squaraine dye **3c** in ethanol and 10% (vol/vol) ethanol−water mixtures, whereas Table 2 summarizes the fluorescence lifetimes of the squaraine dyes **3a**−**d** under various conditions. The squaraine dyes **3a**−**d** showed a single-exponential decay in ethanol with lifetimes in the range 510−910 ps, while significantly reduced values were observed in 10% (vol/vol) ethanol−water mixture (Table 2). The dye **3b** with a carboxyl group, on the other hand, unusually showed biexponential decay in 10% (vol/vol) ethanol−water mixture when compared to ethanol. The major species of the dye **3b** exhibited a very short lifetime of 29 ps (69%), while the minor species has a longer lifetime of 211 ps (31%).

3.2. Effect of Micelles. The study of stability and photophysical properties of a photosensitizer under physiological conditions is important during its evaluation for various biological applications. Of these, the study of effect of the organized media such as micelles that are considered as model systems for biological environments would not only provide information on the sensitizer behavior but also useful for the designing of effective sensitizers.[10] To mimic the biological membranes, we have employed different surfactants that can form micellar structures at and above critical micellar concentrations (CMC) and have investigated their effect on the photophysical properties of the squaraine dyes **3a**−**d**. The surfactants that we have used include cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), and Triton X-100 (TX-100), which provide microheterogeneous environment by forming cationic, anionic, and neutral micellar structures, respectively.[15]

Figure 2A shows the effect of CTAB concentration on the absorption and fluorescence spectra of **3a** in 10% (vol/vol) ethanol/water mixtures. The increase in addition of cationic micelle (CTAB) resulted in significant decrease in absorbance of **3a**, with a bathochromic shift of about 2 nm. Similar observations were made with the dyes **3b**−**d** in the presence of CTAB (Table 1) (Figures S1−S3; Supporting Information). As shown in the inset of Figure 2A, the fluorescence emission spectra of **3a** showed a blue shift in the emission maximum from 670 to 666 nm along with a 5-fold enhancement in its fluorescent intensity. Similarly, the fluorescence spectra of the dyes **3b**−**3d** showed a blue shift of about 6−11 nm as the concentration of CTAB increased (Table 1, insets of Figures S1−S3; Supporting Information), with significant enhancement in their emission yields. As evident from Figure 2B, the fluorescence quantum yields of all these dyes increased with increase in concentration of CTAB. In the case of the dye **3b** with a carboxyl functional group in the side chain, the increase in fluorescence quantum yield reached saturation at about 8 mM of CTAB, while for the dye **3c**, about 20 mM of CTAB was required to reach the saturation (inset of Figure 2B). The squaraine dyes **3a** and **3d**, on the other hand, interacted with CTAB less efficiently when compared to **3b** and **3c**, since about 129 mM of CTAB was required to reach saturation in both these derivatives.

Figure 3A shows the change in the absorption spectra of the dye **3a** with increase in concentration of SDS (anionic micelles) in 10% (vol/vol) ethanol−water mixtures, while Figure 3B shows the changes in fluorescence spectra. Addition of the dye **3a** to a solution of SDS resulted in a small decrease in extinction coefficient at the absorption maxima with a hypsochromic shift

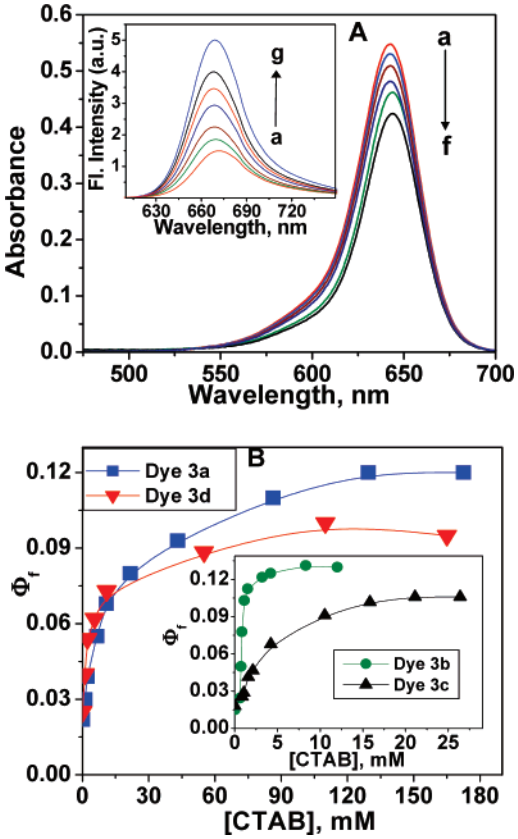


Figure 2. (A) Effect of CTAB on the absorption spectra of **3a** (0.5 μM) in 10% (vol/vol) ethanol−water mixture. [CTAB] (a) 0 and (f) 129 mM. Inset shows the effect of CTAB on the emission spectra of **3a**. [CTAB] (a) 0 and (g) 129 mM. Excitation wavelength, 600 nm. (B) Plots of fluorescence quantum yields of the dyes **3a** (1.0 μM) and **3d** (1.5 μM) and inset shows for the dyes **3b** (0.5 μM) and **3c** (1.6 μM) vs concentration of CTAB in 10% vol/vol ethanol−water mixture.

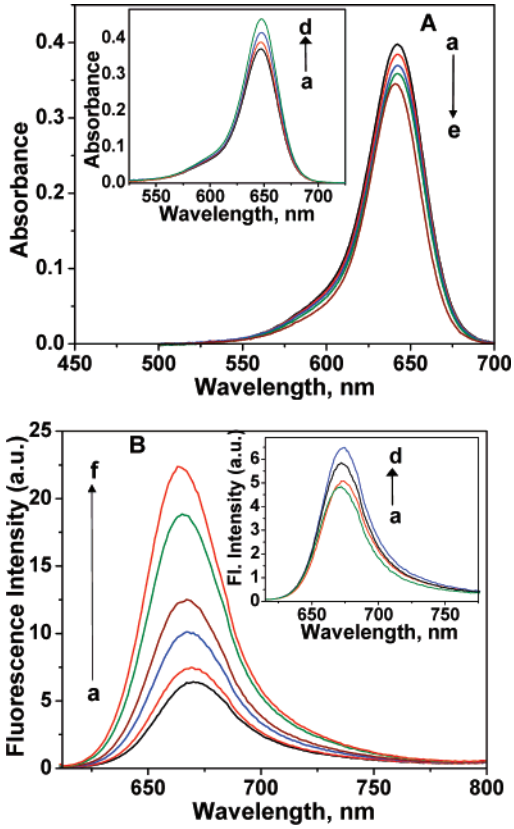


Figure 3. Effect of SDS on the absorption (A) and fluorescence (B) spectra of the dye **3a** (0.5 μM) in 10% (vol/vol) ethanol−water mixture. [SDS] (a) 0 and (e) 28 mM. Insets show the effect of SDS on the absorption (A) and fluorescence (B) spectra of the dye **3b** (2.3 μM). [SDS] (a) 0 and (d) 35 mM. Excitation wavelength, 600 nm.

of about 3 nm at the absorption maximum. The fluorescence intensity of the dye **3a** showed significant enhancement with the increase in concentration of SDS with a hypsochromic shift of about 1 nm at the highest concentration of SDS studied. The squaraine dyes **3c** and **3d** showed a similar trend in both absorption and emission properties (Figures S4 and S5; Supporting Information) wherein the dye **3c** exhibited a hypsochromic shift of 9 nm as the concentration of SDS increased, while the dye **3d** showed a 7-nm shift (Table 1). Insets of Figure 3A and 3B show the effect of SDS on the absorption and emission spectra of the dye **3b** in 10% (vol/vol) ethanol water mixtures. In contrast to the observations made with the dyes **3a**, **3c**, and **3d**, the addition of SDS to a solution of **3b** resulted in a nonnegligible increase in absorbance with a blue shift of about 2 nm. In contrast, by the addition of SDS, we observed negligible changes in the fluorescence spectrum of **3b**, indicating thereby its inability to interact with the anionic micellar structures. Figure 4A shows the change in fluorescence quantum yields of dyes **3a**−**d** with the increase in concentration of SDS. While the quantum yields of emission increased by 5-fold in the case of dye **3a**, the increase was about 8 and 6-folds for the dyes **3c** and **3d**. On the other hand, dye **3b** showed negligible increase in fluorescence quantum yields.

In addition to the cationic and anionic micelles, we have investigated the effect of the nonionic micelles such as TX-100. Figure 4B shows the effect of TX-100 on the absorption spectra of the dye **3a**, while the inset shows the effect on its emission spectra. As can be seen from the figure, the absorption coefficient of the dye **3a** decreases marginally with the increase in concentration of TX-100 with a bathochromic shift of about 3 nm. Similar observations were made with the dyes **3b**−**d** (Figures S6−S8; Supporting Information) (Table 1). The fluorescence quantum yields of dyes **3a**−**d** increased with increase in concentration of TX-100. (Figure S9; Supporting Information). Although all these dyes showed enhancement in fluorescence yields with the addition of TX-100 and reached saturation at about 140 mM of the neutral micelle, the maximum effect was observed for the dye **3a**.

To have a better understanding of the effect of the micellar media, we have analyzed picosecond time-resolved fluorescence lifetimes of the squaraine dyes **3a**−**d** in the presence of CTAB, SDS, and TX-100. Figure 5A shows the fluorescence decay profiles of the dye **3a** in 10% (vol/vol) ethanol−water mixtures and in the presence of these additives, whereas lifetimes of all dyes are summarized in Table 2. The dye **3a** exhibited a single-exponential decay in 10% (vol/vol) ethanol−water mixture, while biexponential decay was observed in the presence of various micelles. Thus, in the presence of CTAB, the dye **3a** showed lifetimes of 350 ps (39%) and 780 ps (61%), while in the presence of SDS, it showed lifetimes of 510 ps (57%) and 610 ps (43%). On the other hand, relatively longer lifetimes were observed for the dye **3a** in the presence of TX-100 (630 and 1320 ps). Similar results were obtained for the dyes **3b**−**d** in the presence of various micelles. However, in the presence of the anionic micelle SDS, the dye **3c** showed only a single-exponential decay (Table 2).

Figure 5B shows the concentration dependent time-resolved fluorescence decay profiles of the dye **3a** in the presence of neutral micelle TX-100. As the concentration of the micelle increases, the relative intensity of the long-lived species

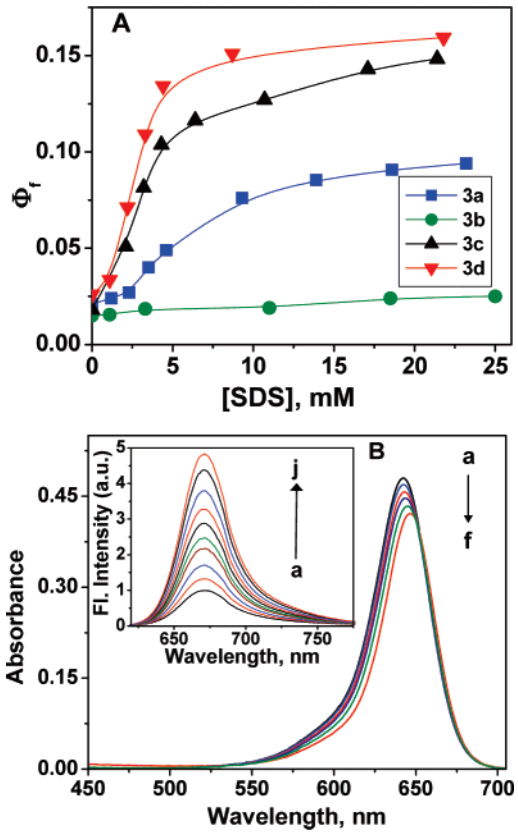


Figure 4. (A) Plot of fluorescence quantum yields of the dyes **3a**−**d** vs SDS concentration in 10% vol/vol ethanol−water solution. [**3a**] 2.67 μM, [**3b**] 2.3 μM, [**3c**] 1.43 μM, [**3d**] 1.3 μM. (B) Effect of TX-100 on the absorption and fluorescence (inset) spectra of **3a** (3.2 μM) in 10% (vol/vol) ethanol−water mixture. [TX-100] (a) 0 and (f) 109 mM. Excitation wavelength, 600 nm.

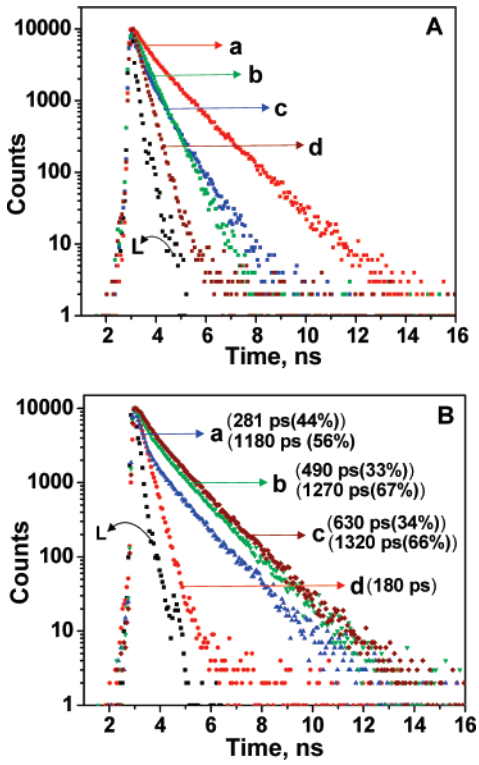


Figure 5. (A) Fluorescence decay profiles of **3a** in 10% (vol/vol) ethanol−water mixture in the presence of (a) TX-100, (b) SDS, (c) CTAB, and (d) alone. (B) Effect of TX-100 concentration on the fluorescence decay profiles of **3a**, under similar conditions. [TX-100] (a) 6, (b) 21, (c) 45, and (d) 0 mM. L is the instrument profile. Excitation and emission wavelengths, 635 and 670 nm, respectively.

increases with a concomitant decrease in the relative intensity of the short-lived component. Thus, at 6 mM of TX-100, it exhibited biexponential decay with a major species having a lifetime of about 1180 ps while the minor species has a lifetime of about 281 ps. At higher concentrations (45 mM), the relative intensity as well as the lifetime of the major species increased significantly with the concomitant decrease in the relative intensity of the minor species.

4. Discussion

The squaraine dyes **3a**−**d** under investigation have enhanced solubility in aqueous medium, when compared to the related derivatives.[1,3] The presence of side chains such as aliphatic alcohols and carboxyl and glycol units in **3a**−**d** renders amphiphilicity to these dyes thereby increasing their solubility in the aqueous medium. Further, these dyes showed nonnegligible solvatochromism and solvent dependent fluorescence quantum yields indicating that the squaraine chromophore in these molecules undergoes efficient interactions with the solvent molecules, which results in the change in the geometry of the squaraine chromophore because of solute−solvent complexation.[1] MNDO and CNDO semiempirical molecular orbital calculations have shown that the electron distribution in the squaraine dyes is highly polarized, with the anilino moiety being the electron donor (D) and the central cyclobutenone (C_4O_2) unit as the acceptor (A).[2] The ground state of the squaraine moiety therefore can be considered as an intramolecular D−A−D charge-transfer state where the solvent molecules can depolarize the D−A structure resulting in the red-shifted absorption as observed for the squaraine dyes **3a**−**d** in the aqueous medium.

Independent of various substituents present, the dyes **3a**−**d** exhibited around 10 times lower values of quantum yields in the aqueous medium when compared to ethanol. This could be attributed to the efficient interactions of these dyes with surrounding water molecules which results in the increased nonradiative decay processes. It has been proposed that the major nonradiative decay pathway for the excited states of the squaraine dyes is by the rotation of the C−C bond between the phenyl ring and the C_4O_2 unit.[1,23] The squaraine dyes **3a**−**d** with various substituents form complexes with water molecules in such a way that there is a greater distortion of the twist angle and the coplanarity of the D−A−D structure. This results in the increased nonradiative decay processes and, hence, quenching of the fluorescence was observed in the aqueous medium. The increase in fluorescence quenching with increase in solvent polarity has also been attributed to the twisted intramolecular charge transfer (TICT) processes in certain squaraine derivatives.[17,23a]

In tune with the observed fluorescence yields, the picosecond time-resolved fluorescence studies indicate that the lifetimes of these dyes are sensitive to the solvent mixture[24] as well as to the substitution pattern (Table 2). The dyes **3a**, **3c**, and **3d** showed monoexponential decay in ethanol with longer lifetimes, when compared to the lifetimes observed in the aqueous medium. On the other hand, the dye **3b** with the carboxyl functionality exhibited a single-exponential decay in ethanol, while it showed biexponential decay in the aqueous medium, indicating the involvement of two spectroscopically distinct species under these conditions.[25] On the basis of literature reports,[1] the biexponential decay of **3b** could be due to the

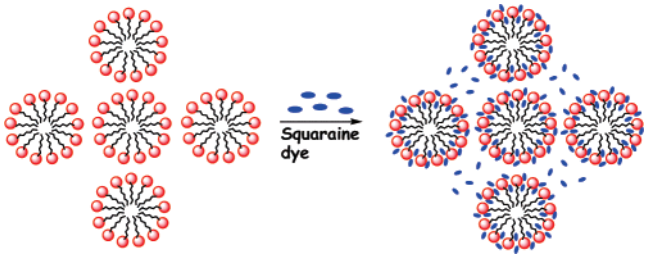


Figure 6. Schematic representation of microencapsulation of the squaraine dye by the micellar medium.

existence of either a solvent−solute complex or a rotational isomer of the monomeric form. The alternative assignment in terms of aggregates of **3b** could be ruled out, since such aggregates are expected to be nonfluorescent.[1,8b]

In the presence of micelles, all these dyes showed significant enhancement (5−10-fold) in fluorescent yields when compared to that in the aqueous medium. This could be attributed to the microencapsulation of the dye molecules into the micellar medium, which results in the change in the microenvironment experienced by the dye molecule. These microenvironment changes include the higher viscosity, lower dielectric constant and polarity, and poorer hydrogen bonding donor capabilities.[1b,24b] The squaraine dye **3b**, with the carboxyl group, exhibited an unusual selectivity for the micellar structures. It showed greater affinity for the cationic micelles CTAB, over the anionic micelles SDS, when compared to other dyes, as evidenced from their fluorescence properties. The negatively charged carboxyl group of **3b** undergoes electrostatic interactions with the positively charged headgroups present on the surface of the cationic micelles of CTAB. As expected, we observed negligible changes in the photophysical properties of the dye **3b** in the presence of SDS. When compared to CTAB and SDS, all these dyes required higher concentrations of TX-100, indicating thereby their poor affinity for the neutral micellar structures.

It has been observed that the squaraine dyes **3a**−**d** show longer lifetimes in the presence of micelles than in the aqueous medium. This is in conjunction with the observation that fluorescent quantum yields of these dyes are 5−10-fold higher in the presence of micelles when compared to that in the aqueous medium. However, as can be seen from the decay profiles (Figure 5) and Table 2, we observed biexponential decay for these dyes in the presence of micelles, indicating the existence of two spectroscopically distinct species (Table 2). As described earlier, these dyes get encapsulated in the hydrophobic pockets of the micellar media and therefore the long-lived component can be attributed to be arising from these encapsulated dye molecules. We assign the minor component with shorter lifetimes to be arising from the unbound dye molecules. However, this component showed a lifetime that is marginally higher than the lifetime of the dye molecules in 10% ethanol−water mixture. This could be attributed to the variation in the macroscopic environment experienced by the dye molecules in the micellar solution (outside the micellar structure), which is expected to be quite different from that of the pure solvent mixture. Similar effects of the macroscopic environment on the lifetimes of various dyes have been reported in the literature.[15,24a,26]

By taking into account the selectivity and sensitivity of the fluorescence quantum yields and lifetimes of the squaraine dyes **3a**−**d** in the presence of micelles, we propose that these dye molecules distribute within the micellar structure but close to the surface near the polar headgroups as shown in Figure 6. In this structure, the hydrophobicity rendered by the aromatic moieties can be overcome by the hydrophilicity of the side

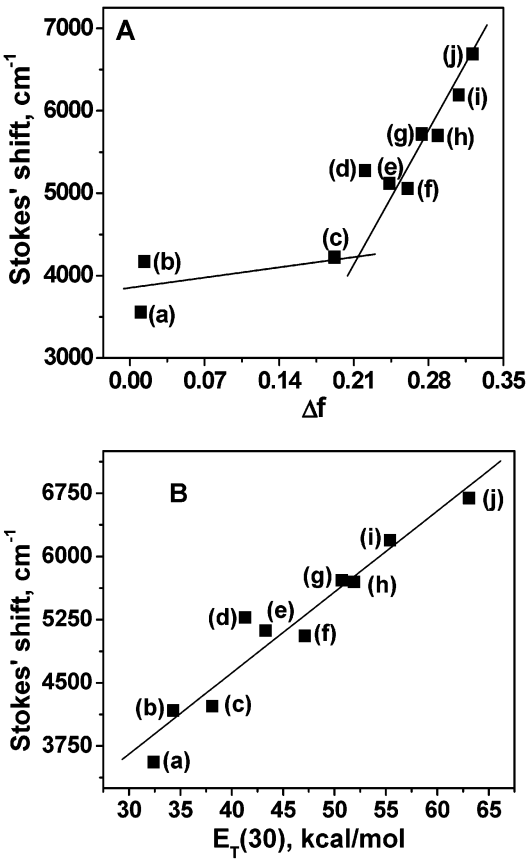


Figure 7. (A) Plot of Stoke's shift (cm^{-1}) vs solvent polarizability (Δf) for the squaraine dye **3c**. (B) Plot of Stokes' shift (cm^{-1}) vs E_T-(30) for the squaraine dye **3c**. (a) CCl_4, (b) toluene, (c) ethyl acetate, (d) 1,2-dichloroethane, (e) *t*-BuOH, (f) 2-BuOH, (g) *n*-propanol, (h) ethanol, (i) methanol, (j) water.

chains, which increase the affinity of these dyes toward the water molecules since water molecules penetrate up to a depth of six carbon chains inside the micelle.[15] Similarly, we believe that most of the nonencapsulated dye molecules remain distributed in the micellar solution as depicted in Figure 6. However, depending on the nature of substituents present, some of these unbound dye molecules can be residing close to the surface and can be undergoing electrostatic interactions with the headgroups of the micelles.

Further, to have a better understanding of the interactions of these dye molecules with micelles, we have estimated the polarity of the microenvironment using the Stokes' shift (cm^{-1}) and the orientation polarizability[18] and $E_T(30)$ empirical solvatochromic scale.[27] For the dye **3c**, the plot of Stokes' shift versus orientation polarizability indicated a significant deviation from the linearity for the solvents of high polarity (Figure 7A), revealing thereby that these dyes undergo effective interactions, particularly with the highly polar solvents. Furthermore, the polarity of the microenvironment in which the probe is allocated in micellar systems was estimated through the plot of the Stokes' shift versus $E_T(30)$ values of the various solvents (Figure 7B). The data gave a good linear correlation and the $E_T(30)$ values for the squaraine dye **3c** were 44.18, 48.38, and 49.91 and kcal/mol, respectively, for micelles CTAB, SDS, and TX-100. Although $E_T(30)$ cannot be considered as a complete solvatochromic scale to describe the polarity of the microenvironment, it gave a good description for these squaraine dyes because of their inherent charge-transfer characteristics. From the $E_T(30)$ values obtained, it is clear that in the presence of micelles, these

dye molecules experience a microenvironment which is less polar when compared to that of the aqueous medium, and hence we observed enhanced fluorescence yields and longer lifetimes for the dyes **3a**−**d**. The observation of similar $E_T(30)$ values for the micelles CTAB, SDS, and TX-100 indicates that these dyes are in an equivalent neighborhood in both ionic and nonionic micelles.

5. Conclusions

We have synthesized a few novel amphiphilic squaraine dyes soluble in the aqueous medium and have examined their photophysical properties devoted to understanding their behavior in micelles, which mimic biological environment. These dyes have absorption in the near-infrared region and exhibit reasonably good fluorescence quantum yields in the aqueous medium. The absorption spectra of these dyes showed negligible changes in the presence of micelles, however, exhibited considerable increase in their fluorescence quantum yields and lifetimes, indicating thereby that these molecules undergo effective microencapsulation. The efficiency of interactions of these dyes was much pronounced for the charged micelles when compared to the neutral micelles. The negligible photophysical changes observed for the dye with the carboxyl group in the anionic micelle SDS indicate that both the substituents on the dye molecules and charges present on the micelles play an important role in their effective interactions. The results of these studies demonstrate that these amphiphilic squaraine dyes, which exhibit favorable photophysical properties, can have potential use as fluorescence sensors for biological applications.

Acknowledgment. The authors thank the Council of Scientific and Industrial Research (COR-0003), Department of Science and Technology, DST-DAAD, Government of India for the financial support of this work. This work represents document no. RRLT-PRU-189 from the Photosciences and Photonics Division of the Regional Research Laboratory.

Supporting Information Available: Figures S1−S9 showing the effect of CTAB, SDS, and TX-100 on the absorption and fluorescence emission spectra of the squaraine dyes **3b**−**d** and plots of fluorescence quantum yields of the dyes 3a−d versus TX-100 concentration. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Law, K. Y. *J. Phys. Chem.* **1987**, *91*, 5184. (b) Law, K. Y. *J. Phys. Chem.* **1995**, *99*, 9818.

(2) Bigelow, R. W.; Freund, H.-J. *Chem. Phys*. **1986**, *107*, 159.

(3) (a) Kamat, P. V.; Das, S.; Thomas, K. G.; George, M. V. *J. Phys. Chem*. **1992**, *96*, 195. (b) Das, S.; Kamat, P. V.; la Barre, B. D.; Thomas, K. G.; Ajayaghosh, A.; George, M. V. *J. Phys. Chem*. **1992**, *96*, 10327. (c) Das, S.; Thomas, K. G.; George, M. V. Kamat, P. V. *J. Chem. Soc., Faraday Trans*. **1992**, *88*, 3419.

(4) (a) Law, K. Y.; Bailey, F. C. *J. Imaging Sci*. **1987**, *31*, 172. (b) Tam, A. C.; Balanson, R. D. *IBM J. Res. Dev.* **1982**, *26*, 186. (c) Wingard, R. E. *IEEE Ind. Appl*. **1982**, 1252. (d) Meiz, R. J.; Champ, R. B.; Chang, L. S.; Chiou, C.; Keller, C. S.; Liclican, L. C.; Neiman, R. B.; Shattuck, M. D.; Weiche, W. J. *Photogr. Sci. Eng.* **1977**, *21*, 73.

(5) (a) Loufty, R. O.; Hsiao, C. K.; Kazmaier, P. M. *Photogr. Sci. Eng*. **1983**, *27*, 5. (b) Morel, D. L.; Ghosh, A. K.; Feng, T.; Stogryn, E. L.; Purwin, P. E.; Shaw, R. F.; Fishman, C. *Appl. Phys. Lett*. **1978**, *32*, 495. (c) Merritt, V. Y.; Hovel, H. J. *Appl. Phys. Lett*. **1976**, *29*, 414.

(6) (a) Gravesteijn, D. J.; Steenbergen, C.; van der Veen, J. *Proc. SPIE* **1983**, *420*, 327. (b) Jipson, V. P.; Jones, C. R. *IBM Technol. Bull.* **1981**, *24*, 298.

(7) (a) Terpetsching, E. H.; Szmacinski, H.; Ozninkas, A.; Lakowicz, J. R. *Anal. Biochem.* **1994**, *217*, 197. (b) Oswald, B.; Patsenker, L.; Duschl, J.; Szmacinski, H.; Wolfbeis, O. S.; Terpetsching, E. *Bioconjugate Chem.* **1999**, *10*, 925. (c) Nakazumi, H.; Colyer, C. L.; Kaihara, K.; Yagi, S.; Hyodo, Y. *Chem. Lett.* **2003**, *32*, 804. (d) Welder, F.; Paul, B.; Nakazumi, H.; Yagi, S.; Colyer, C. L. *J. Chromatogr., B* **2003**, *91*, 793. (e) Ros-Lis, J. V.; Garcia, B.; Jimenez, D.; Martinez-Manez, R.; Sancenon, F.; Soto, J.; Gonzalvo, F.; Valldecarbres, M. *J. Am. Chem. Soc.* **2004**, *126*, 4064.

(8) (a) Ramaiah, D.; Joy, A.; Chandrasekhar, N.; Eldho, N. V.; Das, S.; George, M. V. *Photochem. Photobiol.* **1997**, *65*, 783. (b) Arun, K. T.; Epe, B.; Ramaiah, D. *J. Phys. Chem. B* **2002**, *106*, 11622. (c) Ramaiah, D.; Arun. K. T.; Das, S.; Epe, B. U. S. Patent 6,770,787, 2004.

(9) (a) Ramaiah, D.; Eckert, I.; Arun, K. T.; Weidenfeller, L.; Epe, B. *Photochem. Photobiol*. **2002**, *76*, 672. (b) Ramaiah, D.; Eckert, I.; Arun, K. T.; Weidenfeller, L.; Epe, B. *Photochem. Photobiol*. **2004**, *79*, 99.

(10) Moser, J. G. *Photodynamic Tumor Therapy: 2nd and 3rd generation photosensitizers;* Harwood Academic Publishers: Amsterdam, 1998.

(11) Wagnieres, G. A.; Star, W. M.; Wilson, B. C. *Photochem. Photobiol*. **1998**, *68*, 603.

(12) (a) Aalders, M. C. G.; van der Vange, N.; Stewart, F. A.; Klein, M. G.; van der Vijver, M. J.; Sterenborg, H. J. C. M. *J. Photochem. Photobiol., B: Biol*. **1999**, *50*, 88. (b) Campbell, D. L.; Gudgin-Dickson, E. F.; Forket, P. G.; Pottier, R. H.; Kennedy, J. C. *Photochem. Photobiol*. **1996**, *64*, 676. (c) Leunig, A.; Rick, K.; Stepp, H.; Gutmann, R.; Alwin, G.; Baumgartner, R.; Feyh, J. *Am. J. Surg*. **1996**, *172*, 674.

(13) Baumgartner, R.; Kriegmair, M.; Stepp, H.; Lumper, W.; Heil, P.; Riesenberg, R.; Stocker, S.; Hofstetter, A. *Proc. Soc. Photo-Opt. Instrum. Eng*. **1991**, *1881*, 20.

(14) (a) Richert, C.; Wessels, J. M.; Muller, M.; Kisters, M.; Benninghaus, T.; Goetz, A. E. *J. Med. Chem*. **1994**, *37*, 2792. (b) Szeimies, R.-M.; Karrer, S.; Abels, C.; Steinbach, P.; Fichweiler, S.; Messmann, H.; Baumler, W.; Landthaler, M. *J. Photochem. Photobiol., B: Biol.* **1996**, *34*, 67.

(15) Kalyanasundaram, K. *Photochemistry in Microheterogeneous Systems*; Academic Press: New York; 1987.

(16) (a) Sajimon, M. C.; Ramaiah, D.; Thomas, K. G.; George, M. V. *J. Org. Chem*. **2001**, *66*, 3182. (b) Joseph, J.; Eldho, N. V.; Ramaiah, D. *Chem. Eur. J*. **2003**, *9*, 5926.

(17) Gude, C. C.; Rettig, W.; Lapouyade, R. *J. Phys. Chem. A* **1997**, *101*, 9673.

(18) (a) Lakowicz, J. R. In *Principles of Fluorescence Spectroscopy*; Plenum Press: New York, 1983. (b) Rosenberg, H. M.; Eimutus, E. *Spectrochim. Acta*. **1966**, *22*, 1751.

(19) Vogel, A. I. In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Furniss, B. S., Hannaford, A. J., Smith, P. W. G., Tatchell, A. R., Eds.; ELBS and Longman Group Ltd.: London, 1989.

(20) (a) Chen, H.; Herkstroeter, W. G.; Perlstein, J.; Law, K. Y.; Whitten, D. G. *J. Phys. Chem.* **1994**, *98*, 5138. (b) Desai, R. D. *J. Indian Inst. Sci.* **1924**, *7*, 235.

(21) Thomas, K. G.; Biju, V.; George, M. V.; Guldi, D. M.; Kamat, P. V. *J. Phys. Chem.* **1998**, *102*, 5341.

(22) (a) Sprenger, H.-E.; Ziegenbein, W. *Angew. Chem., Int. Ed. Engl*. **1968**, *7*, 530.(b) Ajayaghosh, A.; Arunkumar, E.; Daub, J. *Angew. Chem., Int. Ed*. **2002**, *41*, 1766. (c) Ros-Lis, J. V.; Martinez-Manez, R.; Soto, J. *Chem. Commun*. **2002**, 2248.

(23) (a) Gude, C.; Rettig, W. *J. Phys. Chem. A* **2000**, *104*, 8050. (b) Rettig, W.; Gleiter, R. *J. Phys. Chem*. **1985**, *89*, 4676. (c) Rettig, W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 971.

(24) (a) Chen, H.; Law, K. Y.; Perlstein, J.; Whitten, D. G. *J. Am. Chem. Soc.* **1995**, *117*, 7257. (b) Law, K. Y. *J. Photochem. Photobiol., A: Chem.* **1994**, *84*, 123.

(25) Chen, H.; Farahat, M. S.; Law, K. Y.; Whitten, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 2584.

(26) (a) Datta, A.; Mandal, D.; Kumar, P. S.; Das, S.; Bhattacharya, K. *J. Mol. Liq*. **1998**, *77*, 121. (b) Sarkar, N.; Datta, A.; Das, S.; Bhattacharya, K. *J. Phys. Chem*. **1996**, *100*, 15483. (c) Tatikolov, A. S.; Costa, S. M. B. *J. Photochem. Photobiol., A: Chem*. **2001**, *140*, 147. (d) Laia, C. A. T.; Costa, S. M. B. *Phys. Chem. Chem. Phys*. **1999**, *1*, 4409. (e) Laia, C. A. T.; Costa, S. M. B. *J. Chem. Soc., Faraday Trans*. **1998**, *94*, 2367. (f) Laia, C. A. T.; Costa, S. M. B. *Langmuir*, **2002**, *18*, 1494.

(27) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319.