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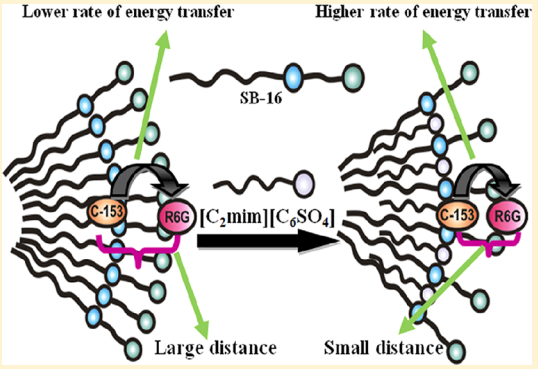
Study of Fluorescence Resonance Energy Transfer in Zwitterionic Micelle: Ionic-Liquid-Induced Changes in FRET Parameters

Vishal Govind Rao, Sarthak Mandal, Surajit Ghosh, Chiranjib Banerjee, and Nilmoni Sarkar*

Department of Chemistry, Indian Institute of Technology, Kharagpur 721302, WB, India

ABSTRACT: The fluorescence resonance energy transfer (FRET) using Coumarin-153 (C-153) as the donor and Rhodamine 6G (R6G) as the acceptor is studied in an aqueous solution of *N*-hexadecyl-*N*,*N*-dimethylammonio-1-propanesulfonate (SB-16) micelles by steady-state and picosecond time-resolved fluorescence spectroscopy. We have determined the rate of FRET (k_{FRET}) from the rise of the acceptor (R6G) emission. In the absence of donor (C-153), the acceptor (R6G) displays a single-exponential decay with average lifetime of 4.77 ns, whereas in presence of donor (C-153), the acceptor (R6G) exhibits a biexponential fluorescence transient having a distinct rise component of 0.94 ns and decay component of 5.16 ns. We have carried out a comparative study of changes in FRET parameters upon addition of three different ionic liquids (ILs), 1-ethyl-3-methylimidazolium ethylsulfate $[C_2mim][C_2SO_4]$, 1-ethyl-3-methylimidazolium *n*-butylsulfate $[C_2mim][C_4SO_4]$, and 1-ethyl-3-methylimidazolium *n*-hexylsulfate $[C_2mim][C_6SO_4]$, where each ionic liquid bears the same cationic part and the anionic parts differ in the alkyl chain length only. It has been observed that with gradual addition of the ILs $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, and $[C_2mim][C_6SO_4]$, the rise component gradually decreases and the rate of FRET (k_{FRET}) gradually increases. The k_{FRET} was found to be 1.06×10^9 s^{-1} in 28 mM aqueous SB-16 micelles. With the addition of 100 mM $[C_2mim][C_2SO_4]$, the k_{FRET} increases by a factor of 1.33 (1.41×10^9 s^{-1}), whereas with the addition of 100 mM $[C_2mim][C_6SO_4]$ it increases by a factor of 3.25 (3.45×10^9 s^{-1}). This rapid increase in k_{FRET} in the case of $[C_2mim][C_6SO_4]$ can be explained by our earlier observation (Rao, V. G.; Ghatak, C.; Ghosh, S.; Mandal, S.; Sarkar, N. J. Phys. Chem. B 2012, 116, 3690−3698), where we have shown that with the addition of $[C_2mim][C_6SO_4]$, C-153 moves toward the outer surface of the micelle. This movement of C-153 causes reduction in donor−acceptor distance and enhancement in FRET rate (k_{FRET}). This is well-supported by the reduced donor−acceptor distance (R_{DA}) observed with the addition of $[C_2mim][C_6SO_4]$. The R_{DA} was found to be 29.1 Å in 28 mM aqueous SB-16 micelles. With the addition of 100 mM $[C_2mim][C_6SO_4]$, the R_{DA} decreases to 24.8 Å. With further increase in the concentration of $[C_2mim][C_6SO_4]$, the R_{DA} decreases, but the time constant for the rise of acceptor emission decreases to such an extent that we are unable to observe it by our instrumental setup.



1. INTRODUCTION

Fluorescence resonance energy transfer (FRET) is a common photophysical process through which the nonradiative transfer of excitation energy from an electronically excited "donor" fluorophore molecule to a nearby "acceptor" molecule occurs. The efficiency of the FRET strongly depends on the distance between donor and acceptor. This distance-dependent behavior has provided physicists, chemists, and biologists with a highly effective tool for studying the structure and dynamics of large molecules in the condensed phases. It has been used to characterize a wide variety of macromolecular assemblies such as proteins,[1−10] biological membranes,[8,11,12] and also a bichromophoric molecular system.[13,14] FRET is also used to elucidate the structure of DNA[15] and in the characterization of organized assemblies such as micelles, reverse micelles, and vesicles.[16−25]

In recent years, the ultrafast FRET at short distances has gained huge attention.[2,4,26−34] However, at very short distances, when the electron clouds of the donor and acceptor overlap with each other, the point dipole approximation involved in Förster theory no longer remains valid.[26−28] Under these conditions, there is marked deviation from the inverse sixth power distance dependence of the rate predicted by the Förster theory. Systems where such deviations have been found to generally occur involve nanoparticles,[26,31] conjugated polymers,[4,35,36] and photosynthetic systems involving "dark" (forbidden) states.[2,28−30] According to the calculations of Scholes et al.[2] in photosynthetic systems and Wong et al.[4] in conjugated polymers, we can say that the rate of FRET shows a much weaker distance dependence ($\sim R_{DA}^{-2}$) when the distance between donor and acceptor is very small (~10 Å). In addition to this, Chance et al.,[37] Person et al.,[38] and Yun et al.[39] established that the rate of energy transfer from a dipole (fluorescent molecule) to a metallic surface shows R_{DA}^{-4} dependence.

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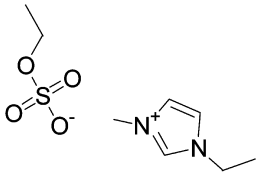
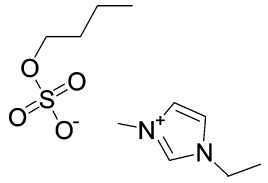
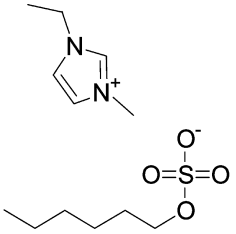
Scheme 1. Structures of the ILs $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, and $[C_2mim][C_6SO_4]$; the Fluorescence Probes Coumarin-153 and Rhodamine 6G (R6G); and the Zwitterionic Surfactant SB-16



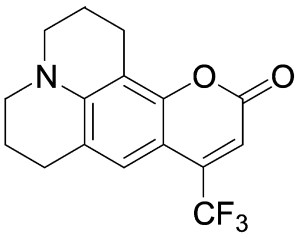
1-Ethyl-3-methylimidazolium ethylsulfate
$[C_2mim][C_2SO_4]$



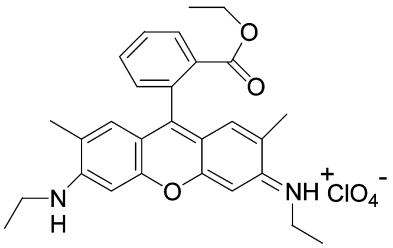
1-Ethyl-3-methylimidazolium n-butylsulfate
$[C_2mim][C_4SO_4]$



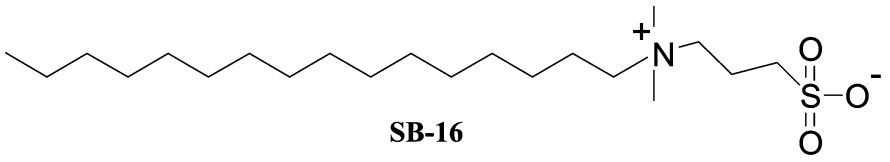
1-Ethyl-3-methylimidazolium n-hexylsulfate
$[C_2mim][C_6SO_4]$



Coumarin 153 (C-153)



Rhodamine 6G (R6G)



SB-16

However, there are cases in which the aforementioned deviation from inverse sixth power distance dependence of FRET rate is not observed even when the distance between the donor and acceptor is very small. Bhattacharyya et al. have shown that if the donor and acceptor are isolated by surfactant chains, then Förster theory remains valid at very short distances.[20] They have monitored ultrafast FRET in micelles,[19,21] mixed micelles,[24] microemulsions,[23] and cationic vesicles[20] and stated that the Förster theory provides a good estimate of the measured donor−acceptor distance due to the intervening surfactant chains. In this paper we investigate FRET in a zwitterionic micellar system, since such systems are one of the most widely studied micellar environments.[40−48] The unique properties of zwitterionic surfactants such as sulfobetaines and carboxybetaines can be attributed to the fact that even though they are formally neutral, their micelles incorporate anions owing to the higher charge density of the cationic ammonium inner surface compared to that of the anionic sulfonate or carboxylate surface.[40−44] Due to these unique properties they are quite useful in many separation processes. By the use of packed zwitterionic columns in liquid chromatography, one can separate inorganic anions or cations[49,50] and acidic or basic proteins[51] both independently and simultaneously in a single run using optimum conditions. Not only this, the chameleon-like nature of zwitterionic micelles can be utilized for performing electrophilic, nucleophilic, and base- and acid-catalyzed reactions.[52,53]

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Knowledge about the location of the different organic molecules (probe molecules of different polarity) inside the zwitterionic micelles would serve to increase their applicability. For this purpose we have chosen Coumarin-153 (C-153, hydrophobic in nature) as the donor and Rhodamine 6G (R6G, cationic) as the acceptor. We have also monitored the effect of addition of three different ionic liquids (ILs) on the FRET efficiency and distance between the donor and the acceptor. The use of ILs provides the added advantage of having control over the structure of these ions, since ILs can be designed according to our goal. Due to its unusual properties, an IL may demonstrate a unique role in altering the properties of aqueous surfactant solutions.[54−60] In our earlier work, we have shown a comparative study of changes in physicochemical properties of an aqueous solution of *N*-hexadecyl-*N*,*N*-dimethylammonio-1-propanesulfonate (SB-16) micelles upon addition of the three room temperature ionic liquids (RTILs), 1-ethyl-3-methylimidazolium ethylsulfate $[C_2mim][C_2SO_4]$, 1-ethyl-3-methylimidazolium *n*-butylsulfate $[C_2mim][C_4SO_4]$, and 1-ethyl-3-methylimidazolium *n*-hexylsulfate $[C_2mim][C_6SO_4]$.[60] We have also observed the effect of added RTILs on the nature of water molecules in the palisade layer of a zwitterionic micelle using solvation and rotational relaxation studies of C-153 dye.[61] In addition, the same study also revealed the movement of C-153 toward the outer surface of SB-16 micelles with the addition of ILs (particularly in case of $[C_2mim][C_6SO_4]$).[61] In this paper we have investigated FRET using C-153 as the donor and R6G as the acceptor because R6G, being cationic in nature, preferentially resides at the extreme outer surface of the micellar aggregate, whereas C-153, being hydrophobic in nature, preferentially resides in the inner nonpolar regions of the micellar aggregates. So, if the conclusion of the earlier paper (movement of C-153 toward the outer surface of SB-16 micelle) is correct, then we should observe a decrease in donor−acceptor distance with the addition of ILs, particularly $[C_2mim][C_6SO_4]$. Here, we have determined the time constant of FRET from the rise time of acceptor emission (vide infra).

2. EXPERIMENTAL SECTION

2.1. Materials and Sample Preparation. C-153 and R6G (laser grade, Exciton) were used as received. SB-16 was purchased from Sigma-Aldrich and used as received. $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, and $[C_2mim][C_6SO_4]$ were obtained from Bioniqs (>99% purity) and were also used as received. Doubly distilled deionized water (Milli-Q water) was used for sample preparation. The stock solution of C-153 was prepared in methanol. The final concentration of probe molecules C-153 and R6G in all the measurements was kept at 28 and 40 μM, respectively. Aqueous SB-16 solutions of the probe were prepared by taking appropriate aliquots of the probe from the stock and evaporating methanol using a stream of nitrogen gas. Aqueous SB-16 of desired concentration was added to achieve the required final probe concentration. The calculated amounts of ILs were added directly to the aqueous SB-16 solutions. All the experiments were performed at 298 K. The structures of $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, $[C_2mim][C_6SO_4]$, SB-16, C-153, and R6G are shown in Scheme 1.

2.2. Instrumentation. The absorption and fluorescence spectra were collected using a Shimadzu (model number, UV-2450) spectrophotometer and a Hitachi (model number, F-7000) spectrofluorimeter, respectively. For steady-state experiments, all the samples were excited at 408 nm. For time-resolved fluorescence measurements, we have used a time-correlated single photon counting (TCSPC) instrument from IBH. The instrument response function of our setup is ~0.09 ns. The detailed time-resolved fluorescence setup is described in our earlier publication.[62,63] Briefly, the samples were excited at 408 nm using a picosecond laser diode (IBH, Nanoled), and the signals were collected at the magic angle (54.7°) using a Hamamatsu microchannel plate photomultiplier tube (3809U). The data analysis was performed using IBH DAS version 6 decay analysis software. The temperature was kept constant (298 K) by circulating water through the cell holder using a JEIO TECH thermostat (RW-0525GS).

Following the Förster theory[64] we have calculated the rate of FRET (k_{FRET}) by using eq 1

$$k_{FRET} = \frac{1}{\tau_{rise}^{A}} = \frac{1}{\tau_0^{D}}\left(\frac{R_0}{R_{DA}}\right)^6 \quad (1)$$

where, τ_0^D is the lifetime of the donor in the absence of the acceptor, τ_{rise}^A is the rise time of the acceptor emission in the presence of the donor, R_{DA} is distance between donor and acceptor, and R_0 is the Förster distance at which the k_{FRET} is equal to the decay rate of the donor $(1/\tau_0^D)$ in the absence of the acceptor. For the calculation of the Förster distance we used eq 2

$$R_0 = 0.211[\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6} \quad (2)$$

where Q_D is the quantum yield of the donor in the absence of the acceptor, n is the refractive index of the medium (following earlier studies we have taken n = 1.4, which is commonly used for macromolecule in water systems), κ^2 is the orientation factor (vide infra), and $J(\lambda)$ is the overlap integral, which expresses the degree of spectral overlap between the donor emission and acceptor absorption. The $J(\lambda)$ can be defined using the normalized fluorescence intensity (F_D) of the donor in the absence of the acceptor and the extinction coefficient of the acceptor (ε_A) as

$$J(\lambda) = \frac{\int_0^\infty F_D(\lambda)\, \varepsilon_A(\lambda)\lambda^4\, d\lambda}{\int_0^\infty F_D(\lambda)\, d\lambda} \quad (3)$$

Depending upon the relative orientation of the donor and the acceptor, the orientation factor κ^2 can vary from 0 to 4. Bagchi and co-workers showed that the rate of FRET crucially depends on the orientation factor.[65−67] When κ^2 = 0, it indicates that the FRET is forbidden. Here, the occurrence of ultrafast FRET indicates a large value of κ^2. κ^2 is generally assumed to be equal to 2/3, which is the value for random orientation of the donor and the acceptor. Following our earlier study,[25] where we have shown that for the C-153 and R6G pair in the lipid bilayer the κ^2 lies between 0.30 to 1.92, we have used κ^2 = 2/3 here (since the donor and the acceptor molecules are free to move).

3. RESULTS AND DISCUSSION

3.1. Steady-State Studies. The absorption and emission spectra of acceptor (R6G) and donor (C-153) in neat water and in 28 mM aqueous SB-16 solutions in the presence of different amounts of ILs were recorded at 298 K. In neat water, the absorption maxima of R6G and the emission maxima of C-153 was found to be 527 and 550 nm, respectively. In the case of 28 mM aqueous SB-16 solution the absorption maxima of R6G and the emission maxima of C-153 were found to be 538

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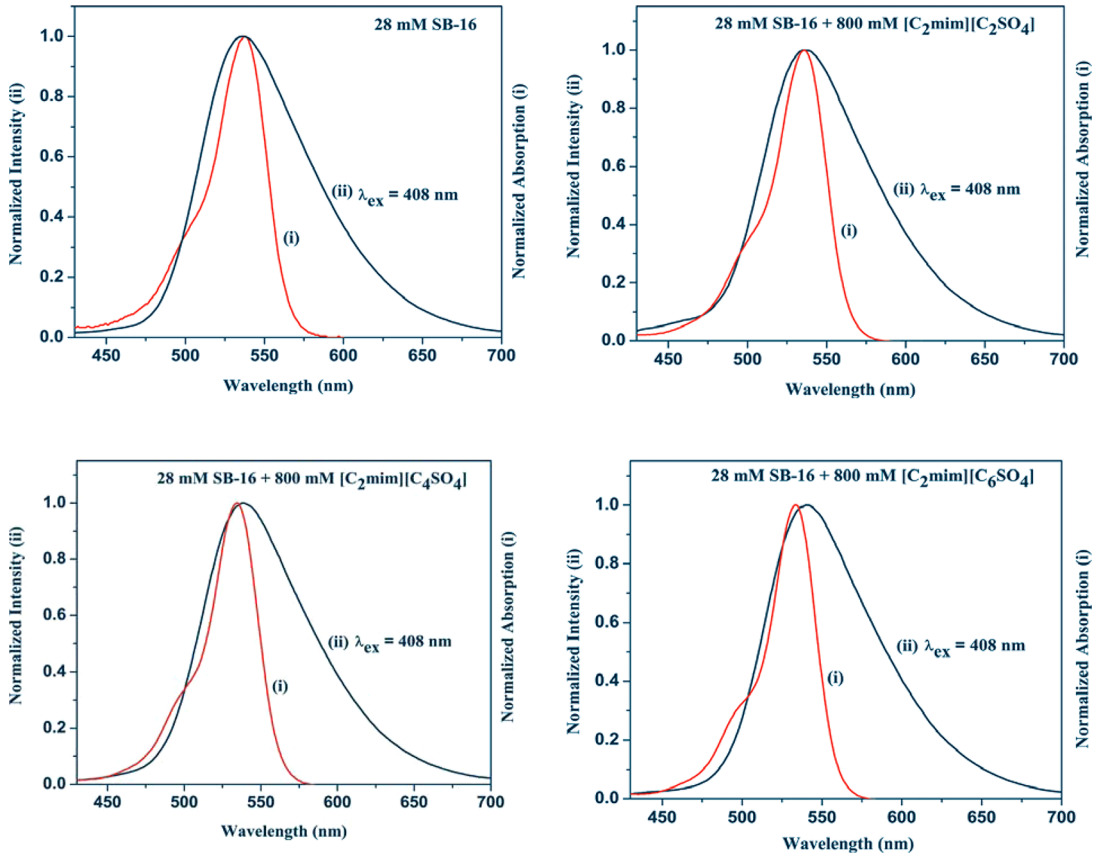

Figure 1. Spectral overlap of donor (C-153, 28 μM) emission (black line) and acceptor (R6G, 40 μM) absorption (red line) in different systems.

and 536 nm, respectively. So, the micellar environment causes a red shift in the absorption maxima of R6G by 12 nm and blue shift in emission maxima of C-153 by 14 nm. This opposite effect of micelle on acceptor and donor energy causes a huge increase in spectral overlap of acceptor (R6G) and donor (C-153). The representative overlap of emission spectra of donor and absorption spectra of the acceptor in 28 mM aqueous SB-16 micelles is shown in Figure 1. Following this, we have also observed the effect of IL addition on the behavior of C-153 molecule. A gradual red shift in emission spectra of C-153 is observed with the addition of ILs to the micellar system. The maximum red shift is observed in the case of $[C_2mim][C_6SO_4]$ addition. The emission maximum is red-shifted from 536 to 541 nm with the addition of 800 mM $[C_2mim][C_6SO_4]$ to 28 mM aqueous SB-16 micelles. The increased micellar hydration is responsible for the observed red shift with the addition of RTILs.[60,61] We have also observed the absorption and emission behavior of R6G in the 28 mM aqueous SB-16 micelles. With the addition of the acceptor (R6G, 40 μM) to a 28 mM aqueous SB-16 solution containing donor (C-153, 28 μM), we observed a significant decrease in the fluorescence intensity of the donor (Figure 2). At the same time we have also compared the emission intensity of R6G (40 μM) observed in the presence and absence of donor (Figure 2), which clearly indicates a pronounced increase in acceptor emission. So, the marked decrease in the fluorescence intensity of donor and similar increase in acceptor emission clearly indicates FRET from C-153 to R6G.

We have calculated the spectral overlap integral, $J(\lambda)$, between the emission spectrum of the donor (C-153) and the absorption spectrum of the acceptor (R6G) in 28 mM

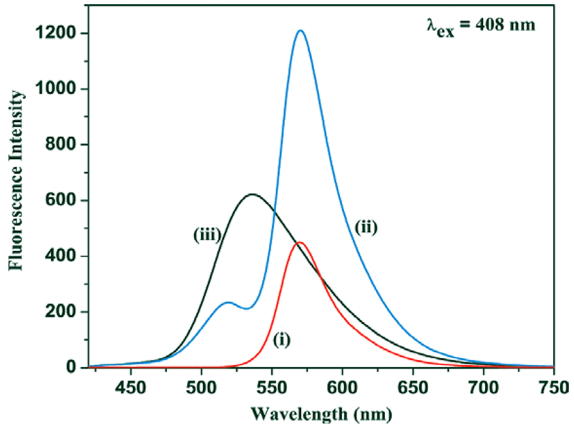

Figure 2. Steady-state fluorescence spectra of (i) acceptor (R6G, 40 μM), (ii) acceptor (R6G, 40 μM) + donor (C-153, 28 μM), and (iii) donor (C-153, 28 μM) in 28 mM aqueous SB-16 micelles.

aqueous SB-16 solution in the presence of different amounts of ILs. As shown in Figure 1, the overlap between the absorption spectrum of the acceptor (R6G) and the emission spectrum of the donor (C-153) in 28 mM aqueous SB-16 micelles varies with the nature of the added ionic liquid. The numerical values of spectral overlap integral, $J(\lambda)$, are given in Table 1. The magnitude of $J(\lambda)$ was found to be 7.05×10^{14} M^{-1} cm^{-1} nm^4 in 28 mM aqueous SB-16 micelles. With the addition of 800 mM $[C_2mim][C_6SO_4]$, $J(\lambda)$ decreases to 6.50×10^{14} M^{-1} cm^{-1} nm^4. This decrease in the $J(\lambda)$ arises due to the red shift in emission maximum from 536 to 541 nm with the addition of 800 mM $[C_2mim][C_6SO_4]$ to 28 mM aqueous SB-16 micelles.

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Table 1. Energy Transfer Parameters for the C-153−R6G Pair in 28 mM Aqueous SB-16 Solution in the Presence of Different Amounts of ILs

concn of additive (mM)	additive	$J(\lambda)^a \times 10^{-14}$ (M^{-1} cm^{-1} nm^4)	$R_0{}^a$ (Å)	$\Phi^0{}_D{}^a$
0	no additive	7.05	37.2	0.24
20	$[C_2mim][C_2SO_4]$	10.03	44.5	0.33
	$[C_2mim][C_4SO_4]$	7.93	37.3	0.22
	$[C_2mim][C_6SO_4]$	6.96	38.1	0.28
100	$[C_2mim][C_2SO_4]$	9.98	40.1	0.27
	$[C_2mim][C_4SO_4]$	7.93	38.3	0.26
	$[C_2mim][C_6SO_4]$	6.85	37.6	0.27
400	$[C_2mim][C_2SO_4]$	9.87	37.7	0.19
	$[C_2mim][C_4SO_4]$	8.77	37.9	0.22
	$[C_2mim][C_6SO_4]$	6.54	35.0	0.18
800	$[C_2mim][C_2SO_4]$	9.77	39.3	0.25
	$[C_2mim][C_4SO_4]$	7.67	37.3	0.23
	$[C_2mim][C_6SO_4]$	6.50	35.4	0.20

[a]Experimental error of ±10%

3.2. Time-Resolved Studies. In addition to steady-state measurements, we have also performed time-resolved experiments to determine the energy transfer rate constant between the donor and acceptor molecule in different systems. We collected the time-resolved decays of donor (C-153, 28 μM), both in 28 mM aqueous SB-16 micelles and SB-16 micelles containing acceptor (R6G, 40 μM). With our time-resolved fluorescence setup we did not observe any change in the lifetime of the donor (C-153) with the addition of the acceptor (R6G) (Figure 3). This is in contrast to the observed decrease in the emission intensity of the donor. It seems that the donor emission is dominated by unquenched non-FRET donors, and hence, no shortening of the donor lifetime is detected in our picosecond setup. This behavior is well-supported by similar observations of Bhattacharyya et al.[19−23] Since the picosecond decay of the donor does not accurately describe FRET, we determined the rate of FRET from the rise of the acceptor (R6G) emission. For this purpose we have excited the R6G probes using 408 nm laser light, and the emission was collected at 570 nm. The concentrations of donor (C-153, 28 μM) and acceptor (R6G, 40 μM) are selected in such a way that the contribution of the quenched donor emission is negligible at 570 nm.

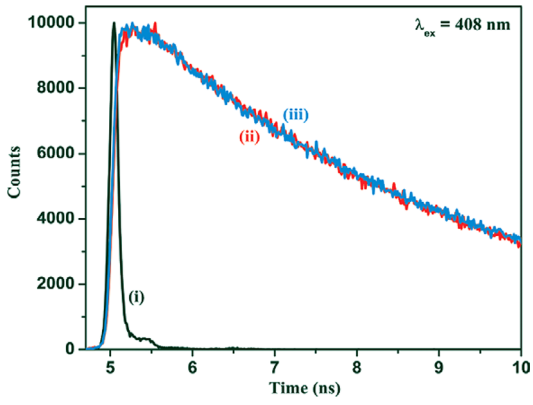

Figure 3. Time-resolved picosecond transients of (ii) donor (C-153, 28 μM) and (iii) donor (C-153, 28 μM) + acceptor (R6G, 40 μM) in 28 mM aqueous SB-16 solution (λ_{em} = 536 nm). (i) Instrument response function in all cases.

From the steady-state absorption and emission spectra we have gathered information regarding some useful parameters, namely, fluorescence quantum yield of donor in the absence of acceptor (Q_D), spectral overlap integral [$J(\lambda)$] between the emission spectrum of the donor (C-153) and the absorption spectrum of the acceptor (R6G), and the Förster distance (R_0) between donor and acceptor in the presence of different concentrations of ILs (Table 1). To get information regarding the rate of fluorescence energy transfer and to determine the donor−acceptor distance (R_{DA}) we have used eq 1.

The average lifetime of the donor molecule (τ_0^D) in 28 mM aqueous SB-16 was found to be 4.10 ns (Table 2). With the addition of RTILs the τ_0^Ddecreases, and the decrease is more pronounced in the case of $[C_2mim][C_6SO_4]$ compared to that for $[C_2mim][C_4SO_4]$ and $[C_2mim][C_2SO_4]$ (Table 2). With the addition of 800 mM $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, and $[C_2mim][C_6SO_4]$ in 28 mM aqueous SB-16, the average lifetime changes from 4.10 to 3.66, 3.61, and 3.16 ns, respectively. The relatively huge change of average lifetime in the case of $[C_2mim][C_6SO_4]$ addition can be explained by our earlier observations, where we have shown that with the addition of 800 mM $[C_2mim][C_6SO_4]$ the microfluidity increases drastically.[60,61] This increased microfluidity around the C-153 molecule opens up an additional pathway for nonradiative decay, which causes reduction in the average lifetime.

Table 2. Average Lifetime of Donor (C-153, 28 μM) Molecule (in the Absence of Acceptor) in 28 mM Aqueous SB-16 in the Presence of Different Amounts of ILs

concn of additive (mM)	additive	τ_0^D (ns)[a]
0	no additive	4.10
20	$[C_2mim][C_2SO_4]$	4.01
	$[C_2mim][C_4SO_4]$	4.02
	$[C_2mim][C_6SO_4]$	4.02
100	$[C_2mim][C_2SO_4]$	3.90
	$[C_2mim][C_4SO_4]$	3.79
	$[C_2mim][C_6SO_4]$	3.50
400	$[C_2mim][C_2SO_4]$	3.80
	$[C_2mim][C_4SO_4]$	3.65
	$[C_2mim][C_6SO_4]$	3.30
800	$[C_2mim][C_2SO_4]$	3.66
	$[C_2mim][C_4SO_4]$	3.61
	$[C_2mim][C_6SO_4]$	3.16

[a]Experimental error of ±5%

To get the information about the rise component of acceptor emission (τ_{rise}^A) in the presence of donor molecule, we have recorded the fluorescence transients of R6G in 28 mM aqueous SB-16 solutions in the absence and presence of the donor (C-153) (Figure 4A). We found that in the absence of donor (C-153) the acceptor (R6G) exhibits a single-exponential decay with an average lifetime of 4.77 ns. On the other hand, in the

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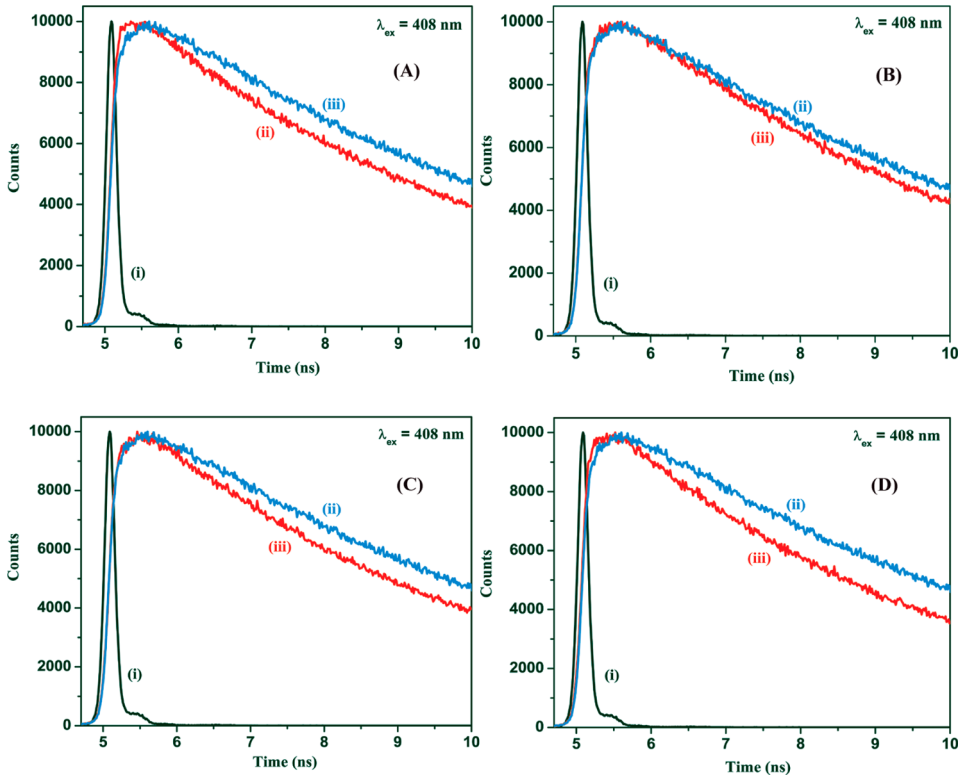

Figure 4. (A) Time-resolved picosecond transients of (ii) acceptor (R6G, 40 μM) and (iii) acceptor (R6G, 40 μM) + donor (C-153, 28 μM) in 28 mM aqueous SB-16 solution, indicating the rise of the acceptor emission in the presence of the donor (λ_{em} = 570 nm in all the cases). (B–D) (ii) Time-resolved picosecond transients of acceptor (R6G, 40 μM) + donor (C-153, 28 μM) in 28 mM aqueous SB-16 solution and (iii) effect of 800 mM RTILs [C_2mim][C_2SO_4] (B), [C_2mim][C_4SO_4] (C), and [C_2mim][C_6SO_4] (D) addition on the time-resolved picosecond transients of acceptor (R6G, 40 μM) + donor (C-153, 28 μM) in 28 mM aqueous SB-16 solution (λ_{em} = 570 nm in all the cases). (i) Instrument response function in all cases.

presence of donor (C-153) the acceptor (R6G) exhibits a biexponential profile having a distinct rise component of 0.94 ns and decay component of 5.16 ns (Table 3). So, the average lifetime increases from 4.77 ns (in absence of donor) to 5.88 ns (in presence of donor). The appearance of the rise component and the increase in average lifetime of acceptor in presence of donor clearly indicate that the energy transfer is taking place from donor (C-153) to acceptor (R6G). One may argue that this rise component may originate from the contribution of the quenched donor emission at 570 nm. As we have already mentioned, in order to rule out this possibility, we have chosen the concentration of donor (C-153, 28 μM) and acceptor (R6G, 40 μM) in such a way that the contribution of the quenched donor emission is negligible at 570 nm. Further, we have recorded the fluorescence transients of C-153 in 28 mM aqueous SB-16 micelles at 570 nm. The fluorescence transient shows a rise component of 0.39 ns and decay component of 4.24 ns. The overlap of fluorescence transients of C-153 (28 μM) and R6G (40 μM) + C-153 (28 μM) in 28 mM aqueous SB-16 micelles (λ_{em} = 570 nm) is shown in Figure 5. This clearly proves that the rise component of acceptor (R6G, 0.94 ns) in the presence of donor (C-153) arises due to energy transfer from donor to acceptor. Interestingly, the observed rise component of R6G in SB-16 micelle (0.94 ns) is small compared to the rise component observed in the case of P123 micelles by Bhattacharyya et al.[19] (rise component of 1.00 ns using picosecond setup). This difference is certainly due to the difference in donor–acceptor distance owing to the different micellar size (the difference in donor–acceptor distance is compared in the following section).

It has been observed that in the presence of added RTILs ([C_2mim][C_6SO_4] and [C_2mim][C_4SO_4]) the rise component gradually decreases. Figure 4B–D clearly indicates the observed fluorescence transients of the R6G–C-153 pair in the presence of 800 mM of different RTILs. The observed components obtained from the fitting parameters of fluorescence transients

Table 3. Time-Resolved Decay Parameters of R6G (40 μM, λ_{em} = 570 nm) in the Presence of C-153 (28 μM) with Different Amounts of Ionic Liquids in 28 mM Aqueous SB-16 Micelles

concn of additive (mM)	additive	$\tau_1{}^a$ (ns) (a_1)	$\tau_2{}^a$ (ns) (a_2)
0	no additive	0.94 (−0.17)	5.16 (1.17)
20	[C_2mim][C_2SO_4]	0.74 (−0.14)	4.99 (1.14)
	[C_2mim][C_4SO_4]	0.62 (−0.19)	4.87 (1.19)
	[C_2mim][C_6SO_4]	0.47 (−0.19)	4.83 (1.19)
100	[C_2mim][C_2SO_4]	0.71 (−0.15)	4.91 (1.15)
	[C_2mim][C_4SO_4]	0.54 (−0.18)	4.74 (1.18)
	[C_2mim][C_6SO_4]	0.29 (−0.17)	4.67 (1.17)
400	[C_2mim][C_2SO_4]	0.65 (−0.13)	4.81 (1.13)
	[C_2mim][C_4SO_4]	0.39 (−0.17)	4.56 (1.17)
	[C_2mim][C_6SO_4]	–	4.49 (1.00)
800	[C_2mim][C_2SO_4]	0.53 (−0.15)	4.76 (1.15)
	[C_2mim][C_4SO_4]	0.22 (−0.17)	4.39 (1.17)
	[C_2mim][C_6SO_4]	–	4.37 (1.00)

[a]Experimental error of ±5%

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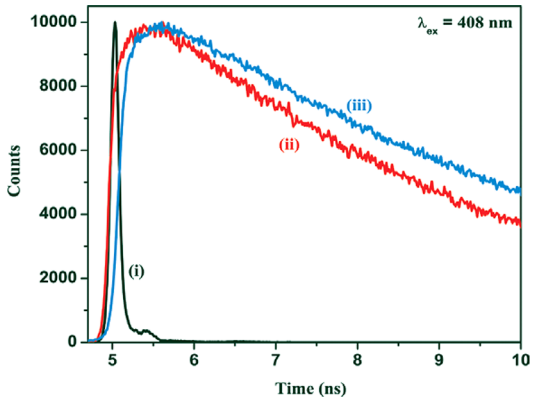

Figure 5. Time-resolved picosecond transients of (ii) donor (C153, 28 μM) and (iii) donor (C153, 28 μM) + acceptor (R6G, 40 μM) in 28 mM aqueous SB-16 solution (λ_{em} = 570 nm). (i) Instrument response function.

of the R6G−C-153 pair are tabulated in Table 3. Table 3 clearly indicates that the decrease in rise component is more pronounced in the case of $[C_2mim][C_6SO_4]$ compared to that for $[C_2mim][C_4SO_4]$ and $[C_2mim][C_2SO_4]$. With the addition of 100 mM of $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, and $[C_2mim][C_6SO_4]$ to 28 mM aqueous SB-16 solution containing R6G−C-153, the rise component changes from 0.94 to 0.71, 0.54, and 0.29 ns, respectively. In fact, with further increase in concentration of $[C_2mim][C_6SO_4]$, we did not observe any rise component. This disappearance of rise component is due to the ultrafast FRET which we are unable to detect from our instrumental setup, since the instrument response function is ~0.090 ns. Following the rise component values we have calculated the rates of fluorescence energy transfer (k_{FRET}), which are tabulated in Table 4. The k_{FRET} in 28 mM aqueous SB-16 solution containing R6G−C-153 was found to be 1.06×10^9 s^{-1}. The k_{FRET} being inversely proportional to τ^A_{rise} indicates that since the rise component decreases with the addition of RTILs, the k_{FRET} will increase. Table 4 clearly indicates that the increase in k_{FRET} is more pronounced in the case of $[C_2mim][C_6SO_4]$ compared to that for $[C_2mim][C_4SO_4]$ and $[C_2mim][C_2SO_4]$. Moreover, with the addition of 100 mM of $[C_2mim][C_2SO_4]$, $[C_2mim][C_4SO_4]$, and $[C_2mim][C_6SO_4]$ to 28 mM aqueous SB-16 solution containing R6G−C-153, the k_{FRET} changes from 1.06×10^9 to 1.41×10^9, 1.85×10^9, and 3.45×10^9 s^{-1}, respectively. This rapid decrease of rise component or rapid increase of rate of fluorescence energy transfer (k_{FRET}) in the case of $[C_2mim][C_6SO_4]$ addition can be explained by results obtained in our earlier paper.[60,61] We have already shown there that with the addition of $[C_2mim][C_6SO_4]$ the penetration of water molecule increases to a great extent,[60] so the probe in the palisade layer undergoes a relative migration toward the micellar surface.[61] This migration causes reduction in distance between donor and acceptor, which results in the observed increase in rate of fluorescence energy transfer (k_{FRET}) or decrease of rise component. In order to lend support to this reasoning, the distances between donor and acceptor (R_{DA}) were determined. The R_{DA} in 28 mM aqueous SB-16 solution was found to be 29.1 Å. With the addition of 100 mM $[C_2mim][C_6SO_4]$ and 800 mM $[C_2mim][C_4SO_4]$ to 28 mM aqueous SB-16 solution containing R6G−C-153, R_{DA} decreases to 24.8 and 23.4 Å, respectively (Table 4). This further confirms that the migration of donor (C-153) toward the micellar surface causes the reduction in distance between donor and acceptor. Interestingly, the observed donor (C-153)−acceptor (R6G) distance in SB-16 micelle (29.1 Å) is found to be smaller than that in P123 micelle (44.0 Å), as reported by Bhattacharyya et al.[19] This further supports the observed lower value of rise component in the case of SB-16 micelles compared to P123 micelles (vide supra).

Table 4. Donor−Acceptor Distance in the 28 mM Aqueous SB-16 Micelles Containing Different Amounts of Ionic Liquids Calculated from the Rise of Acceptor Emission

concn of additive (mM)	additive	τ_{FRET}[a] (ns)	k_{FRET}[b] × 10^{-9} (s^{-1})	R_{DA}[b] (Å)	E[b] (%)
0	no additive	0.94	1.06	29.1	81.4
20	$[C_2mim][C_2SO_4]$	0.74	1.35	33.6	84.4
	$[C_2mim][C_4SO_4]$	0.62	1.61	27.3	86.6
	$[C_2mim][C_6SO_4]$	0.47	2.13	26.6	89.6
100	$[C_2mim][C_2SO_4]$	0.71	1.41	30.2	84.5
	$[C_2mim][C_4SO_4]$	0.54	1.85	27.7	87.5
	$[C_2mim][C_6SO_4]$	0.29	3.45	24.8	92.4
400	$[C_2mim][C_2SO_4]$	0.65	1.54	28.1	85.4
	$[C_2mim][C_4SO_4]$	0.39	2.56	26.1	90.3
	$[C_2mim][C_6SO_4]$	–	–	–	–
800	$[C_2mim][C_2SO_4]$	0.53	1.89	28.5	87.3
	$[C_2mim][C_4SO_4]$	0.22	4.55	23.4	94.3
	$[C_2mim][C_6SO_4]$	–	–	–	–

[a]Experimental error of ±5% [b]Experimental error of ±10%

Finally the FRET efficiency (E) is calculated using the following equation

$$E = \frac{{R_0}^6}{{R_0}^6 + {R_{DA}}^6} \quad (4)$$

The FRET efficiency (E) in 28 mM aqueous SB-16 solution containing R6G−C-153 was found to be 81.4%, which increases to 92.4 and 94.3% with the addition of 100 mM $[C_2mim][C_6SO_4]$ and 800 mM $[C_2mim][C_4SO_4]$, respectively (Table 4). This is once again attributed to the reduced distance between donor and acceptor (vide supra).

4. CONCLUSION

To the best of our knowledge, this is the first report of fluorescence resonance energy transfer in a zwitterionic micellar system where a comparative study of effect of ionic liquid addition on energy transfer has been carried out. We have studied FRET between Coumarin-153 (C-153, donor) and Rhodamine 6G (R6G, acceptor) by observing the rise time of the acceptor (R6G) emission. We have shown that the efficiency of FRET can be enhanced in the presence of different ILs. Herein, we have demonstrated that the increased efficiency of FRET is more pronounced in case of $[C_2mim][C_6SO_4]$ addition compared to that of $[C_2mim][C_4SO_4]$ and $[C_2mim][C_2SO_4]$ addition. The observed difference in the properties of three ionic liquids arises due to the difference in

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the location of the anions of the ILs. In our earlier publication, we have already stated that the hexyl chain on the hexylsulfate ion causes pronounced penetration of the hexylsulfate anion of $[C_2mim][C_6SO_4]$, whereas in case of $[C_2mim][C_2SO_4]$, the ethyl chain in the ethylsulfate ion is apparently unsuccessful in functioning similarly.[60] Due to this difference in the location of ILs, the donor molecule (C-153) moves toward the micellar surface with the addition of $[C_2mim][C_6SO_4]$, causing reduction in the distance between donor (C-153) and acceptor (R6G) (R_{DA}). The rapid decrease in R_{DA} with the addition of $[C_2mim][C_6SO_4]$ is responsible for the enhanced efficiency of FRET. So, we can convincingly say that our investigation has defined the unique concentration-dependent role of different ILs in modifying the distance between donor and acceptor and FRET efficiency. This will certainly increase the potential applications of these neoteric and environmentally friendly solvents (RTILs). Moreover, the combination of the zwitterionic micelles and ionic liquids may have enormous future application for conducting interfacial reactions and micellar catalysis.

AUTHOR INFORMATION

Corresponding Author

*E-mail: nilmoni@chem.iitkgp.ernet.in. Fax: 91-3222-255303.

Notes

The authors declare no competing financial interest.

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