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The fluorescence resonance energy transfer between dye compounds in micellar media

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ABSTRACT

The fluorescence resonance energy transfer from fluorescein to merocyanine 540 in aqueous sodium dodecyl sulfate, cetyltrimethylammonium bromide and Triton X-100 micellar solutions as well as deionized water was investigated at room temperature using steady-state and time-resolved fluorescence spectroscopy techniques. Fluorescence resonance energy transfer rate constants (k_T), obtained using both Stern–Volmer and Förster theories, were in good agreement. Moreover, energy transfer efficiency values of 0.14, 0.38, 0.77 and 0.85 for deionized water, sodium dodecyl sulfate, TX-100 and cetyltrimethylammonium bromide micellar solution were obtained at the highest acceptor concentration at which fluorescence resonance energy transfer was achieved. The data obtained from steady-state absorption, fluorescence spectral studies and time-resolved lifetime measurements indicated that the fluorescence resonance energy transfer from fluorescein to merocyanine 540 occurred most effectively in aqueous cetyltrimethylammonium bromide micellar solutions.

1. Introduction

Fluorescence resonance energy transfer (FRET) is a fundamental phenomenon in fluorescence spectroscopy with applications for biological systems, including photosensitization [1,2]. For instance, fluorescence energy transfer studies are common to investigate protein-folding phenomena and to examine distances between fluorescent tags in order to determine structural and conformational properties of proteins [1]. FRET can be described by the following equation [1,3]:

$$D^* + A \xrightarrow{k_T} D + A^* \quad (1)$$

where D^* is an excited donor and A is an acceptor molecule at the ground state. k_{ET} is the rate constant of energy transfer from donor to acceptor. According to Förster's theory, the rate of energy transfer is based on the overlap of emission spectrum of the donor and absorption spectrum of the acceptor, relative orientations of the donor and acceptor transition dipoles, the distance between the donor and acceptor transition dipoles, and fluorescence quantum yield of the donor [1].

Fluorescein (FL) and merocyanine 540 (MC540) were chosen as donor and acceptor dye molecules, respectively; fluorescein is an anionic dye that is used as a photosensitizer in photochemistry, as a fluorophore in the biosciences and in medical treatments such as fluorescein angiography [4,5]. Although the dye can exist in a number of prototropic forms, each of which possesses its own distinct spectral properties [6,7], it is the dianionic species that is usually observed under neutral conditions and which has both a large extinction coefficient and high fluorescence quantum yield. MC540 is an anionic lipophilic polymethine dye that binds to micelles and biological membranes [8]. The fluorescence quantum yield of MC540 in aqueous solution is low because it has the ability to form non-fluorescent aggregates [9]; MC540 also has a tendency to bind micelles, liposomes or vesicles [10]. Hence, aggregates of MC540 dissociate to fluorescent monomers when the dissolving medium contains micelles or vesicles [11].

The FRET process between dye molecules in vesicles or micelle solutions has been studied extensively because vesicle or micelle systems are usually assumed as model membrane systems for the biological applications [10,12–16]. But the FRET between FL and MC540 dyes in micellar media has not been investigated although the photophysical properties of the dye compounds in different media have been investigated [11,17–21]. The understanding of the FRET between these dyes in micellar media is important for the spectroscopic applications. Therefore, we studied the fluorescence resonance energy transfer from FL dye to MC540 dye in anionic sodium dodecyl sulfate (SDS), cationic cetyltrimethylammonium bromide (CTAB), nonionic Triton X-100 (TX-100) micelle solutions and in deionized water for the comparison.

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2. Experimental

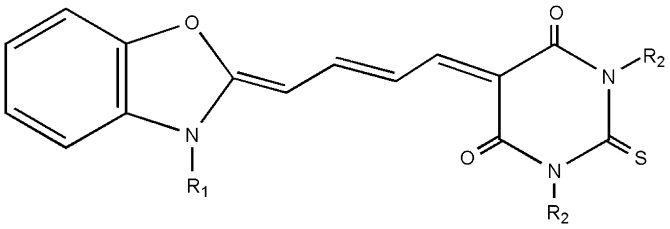
FL was purchased from Sigma and used without further purification. MC540, SDS, CTAB and TX-100 were purchased from Fluka. Molecular structures of dye compounds are given in Fig. 1. Deionized water was used for the preparation of solutions. The concentrations of surfactants were 25.0×10^{-3} M, 3.0×10^{-3} M and 1.0×10^{-3} M for SDS, CTAB, and TX-100, respectively. These values are much higher than the critical micelle concentrations (CMC) corresponding to 8.1×10^{-3} M, 1.0×10^{-3} M and 0.3×10^{-3} M for SDS, CTAB and TX-100, respectively, to ensure complete micellisation. FL and MC540 were stored in the dark as a concentrated stock solution of 5.0×10^{-3} M in methanol. For the measurements, the donor concentration was 1.0×10^{-5} M and was kept constant for all experiments whereas acceptor concentration was variable starting from 10^{-7} M to 10^{-5} M. Absorption spectra of the samples were taken with a Shimadzu UV-3101PC UV–VIS–NIR spectrophotometer and fluorescence spectra were taken with a PTI spectrofluorophotometer and converted to the corrected fluorescence spectra. Measurements were carried out by using magic angle geometry of the cuvette to the excitation to minimize the effect of reabsorption of donor emission by the acceptor. The emission was observed perpendicular to the direction of the exciting beam. Thus the errors due to fluorescence reabsorption were reduced. Temperature of the samples was controlled by using a Grant W14 circulating water bath during the absorption and fluorescence spectra measurements and, therefore, ambient temperature was used. The pH values of samples were also determined and it was seen that all samples had about neutral pH values.

Fluorescence decay lifetime measurements were carried out with a LaserStrobe Model TM-3 lifetime fluorophotometer from Photon Technology International [22]. The excitation source consisted of a pulsed nitrogen laser/tunable dye laser combination. All samples were excited at 500 nm in which optical density of each sample was approximately 0.10 to eliminate inner filter effects. The decay curves were collected over 200 channels using a nonlinear time scale with the time increment increasing according to arithmetic progression. This approach enhances temporal resolvability of heterogeneous decays by providing higher data density (shorter time increments) at shorter times, whereas the time increments gradually increase at longer times. The fluorescence decays were analyzed with the lifetime distribution analysis software from the instrument supplying company. The quality of fits was assessed by χ^2 values and weighed residuals.

Fluorescence quantum yields (Φ_f) were determined by comparison with a reference solution. For this purpose, the following relation has been applied to calculate relative fluorescence quantum yields [23].

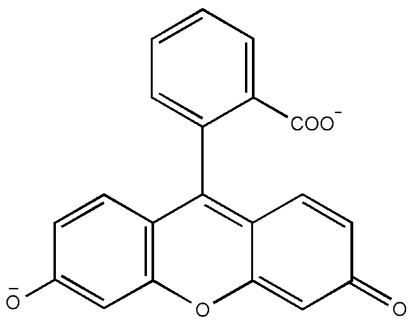
$$\Phi_s = \Phi_r \left(\frac{D_s}{D_r}\right)\left(\frac{n_s}{n_r}\right)^2 \left(\frac{1-10^{-OD_r}}{1-10^{-OD_s}}\right) \quad (2)$$

where D_s and D_r are the integrated area under the corrected fluorescence spectra for the sample and reference, n_s and n_r are the refractive indexes of the sample and reference, respectively. OD_s and OD_r are the optical densities for the sample and reference at the excitation wavelength, respectively. The reference used for this study is fluorescein of 1.0×10^{-6} M in 0.01 N NaOH. This reference has a known fluorescence quantum yield of 0.92 in this condition [19].



$R_1 = (CH_2)_3SO_3^-Na^+$; $R_2 = (CH_2)_3CH_3$

Merocyanine 540



Fluorescein-Na (Dianion)

Fig. 1. Molecular structures of dye compounds.

3. Results and discussion

3.1. Absorption and fluorescence spectral properties

The electronic absorption spectra of fluorescein and merocyanine 540 in deionized water and various surfactant media are given in Figs. 2(a) and (b). Two absorption maxima are observed for both FL and MC540 dyes in deionized water although diluted concentrations of the samples are used. This is because of aggregate formation of the dye compounds in deionized water. The aggregation of the dye compounds in water was prevented by addition of the surfactants above the critical micelle concentration to the medium. As seen in Fig. 2(a) and (b), aggregation bands, blue-shifted, of the dye molecules disappear in case of micellar solutions and only monomeric species of the dye molecules become available in micelles containing aqueous solutions. The absorption and fluorescence maxima, fluorescence lifetime and quantum yield values are listed in Table 1 for FL and MC540 in different medium. From Table 1, one can see that the quantum yield value of FL in deionized water is lower than that of FL in micellar solutions. The same behavior was also observed for MC540. This indicates that although both donor and acceptor molecules are anionic, interactions between dye compound and micelles take place in micellar solutions. Therefore, there is a greater red shift in the absorption and fluorescence spectra maxima of the probes, FL and MC540, in cationic CTAB micelle solution relative to other micellar solutions and deionized water systems as seen in Fig. 2(a) and (b). This is because polarity of the probe environment increases and excited states of FL and MC540 strongly interact with micelles in solution compared to the ground states of the probes [24]. MC540 has almost the same spectral properties in TX-100 and CTAB micelle solutions as shown in Table 1. But fluorescence lifetime and quantum yield values are higher for MC540 in TX-100 micelle solution. The increase in fluorescence lifetime and quantum yield values mainly originated from the strong interactions of hydrophobic part of the probe and vicinity of the micelle to decrease free motions of the probe and lowering the non-radiative transition rate constant. For this reason, we observe an increase in fluorescence

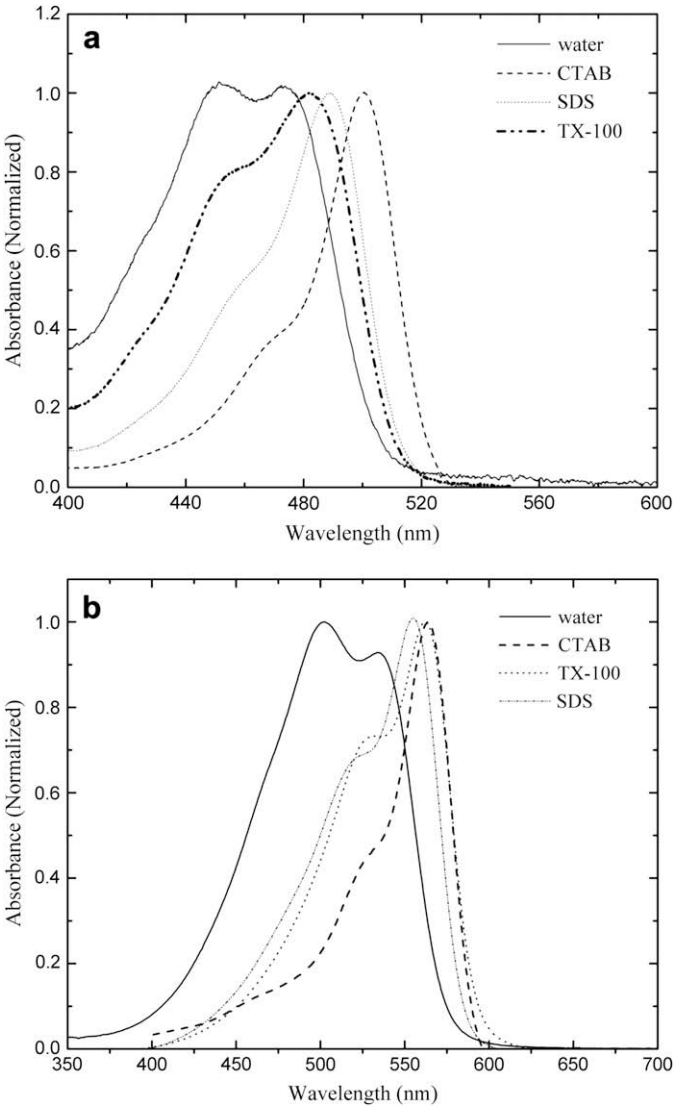


Fig. 2. Normalized absorption spectra of dyes in deionized water and micellar solutions. (a) Fluorescein and (b) merocyanine 540 at ambient temperature. [FL] = 1.0×10^{-5} M and [MC540] = 1.0×10^{-5} M.

lifetime and quantum yield values of the probes by varying the micelle solutions.

3.2. Steady-state fluorescence quenching and FRET

The fluorescence intensity quenching of FL has been studied in micelles and deionized water by using MC540 as a quencher. Fig. 3

Table 1
Photophysical and spectral properties of fluorescein and merocyanine 540 in deionized water and micellar solutions.

Medium	λ_{abs} (nm)	λ_{fl} (nm)	τ_f (ns)	Φ_f
Fluorescein				
In deionized water	453 and 475	512	3.78 ± 0.05	0.29 ± 0.02
In CTAB	501	527	5.19 ± 0.01	0.82 ± 0.01
In SDS	487	516	4.49 ± 0.15	0.61 ± 0.01
In TX-100	481	515	4.38 ± 0.16	0.42 ± 0.03
Merocyanine 540				
In deionized water	506 and 539	575	0.19 ± 0.01	0.06 ± 0.01
In CTAB	564	589	1.38 ± 0.01	0.32 ± 0.01
In SDS	553	583	0.56 ± 0.02	0.16 ± 0.01
In TX-100	563	588	1.47 ± 0.02	0.37 ± 0.01

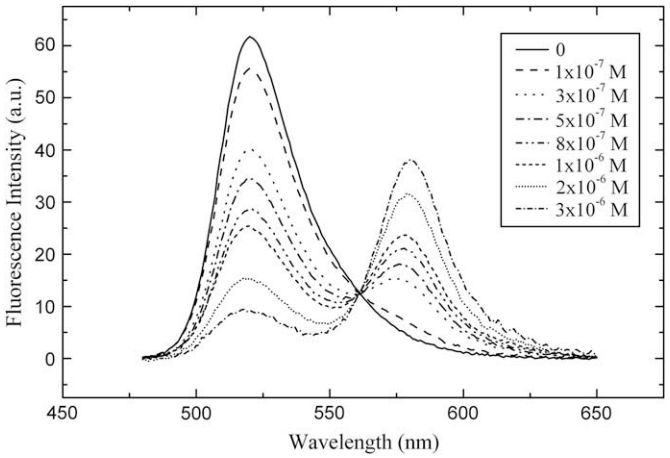


Fig. 3. Fluorescence intensity quenching of FL with increasing MC540 concentration in CTAB.

shows the fluorescence spectra containing different concentrations of MC540 in CTAB micelle solutions. The concentration of FL was kept constant at 1.0×10^{-5} M for all samples. When MC540 concentrations are increased, the fluorescence intensity of FL decreased as the fluorescence intensity of MC540 increased with a maximum at 585 nm. This behavior is the same in SDS, TX-100 micelle solutions and in deionized water. As a result, an isosbestic point for the fluorescence intensity quenching studies was observed at 561, 560, 565 and 540 nm for CTAB, SDS, TX-100 micellar systems and deionized water, respectively, which is evidence for the absence of exciplex formation between the excited donor and ground state acceptor [25].

The fluorescence energy transfer can be regarded as a bimolecular process. Therefore, the rate constant of fluorescence energy transfer ($k_{T(I)}$) can be calculated by using the Stern–Volmer (SV) relation [1]

$$\frac{I_0}{I} = 1 + K_{SV}[Q] = 1 + k_{T(I)}\tau_D[Q] \tag{3}$$

where I_0 and I are the fluorescence intensity of donor in the absence and in the presence of the acceptor, respectively, [Q] is the acceptor or quencher concentration, τ_D is the fluorescence lifetime of the donor in the absence of quencher, and K_{SV} is the SV quenching rate constant.

A plot of I_0/I vs. [Q] should yield a straight line with a slope of K_{SV}. The SV plots of fluorescence intensity quenching of FL by using MC540 as acceptor in micellar systems and deionized water is shown in Fig. 4. The values of K_{SV} and $k_{T(I)}$ for FL-MC540 molecular pair are listed in Table 2. Fluorescence lifetimes of the donor (FL) in Table 1 were used to determine the $k_{T(I)}$. Moreover, absorption spectrum studies of donor show no changes upon the addition of acceptor, which indicates the absence of a ground state complex between the donor and acceptor molecules. This is a typical property of dynamic quenching [1].

Förster theory was also employed to calculate the fluorescence energy transfer rate constant ($k_{T(II)}$) by the following relations [1,3]

$$k_{T(II)} = \frac{9000(\ln 10)\kappa^2\Phi_D}{128\pi^5 n^4 N r^6 \tau_D}\int_0^\infty \frac{F_D(\bar{\nu})\varepsilon_a(\bar{\nu})}{\bar{\nu}^4}d\bar{\nu} \tag{4}$$

or

$$k_{T(II)} = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 \tag{5}$$

where κ^2 is the orientation factor determined by the angle between the donor and acceptor dipoles and is equal to 2/3 for isotropic

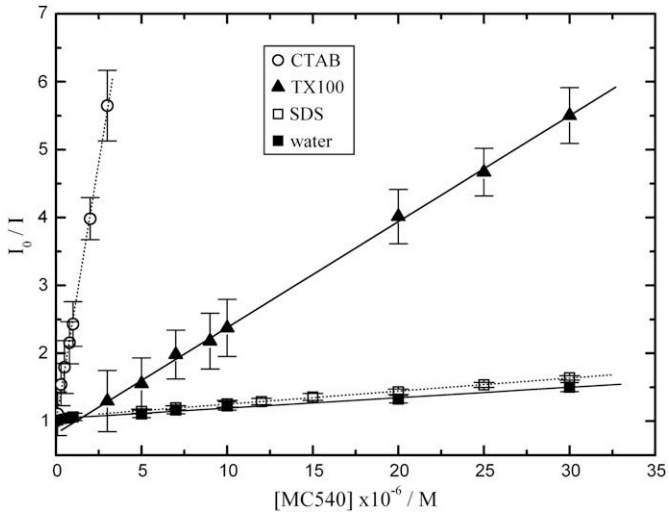


Fig. 4. Stern–Volmer plots for FL-MC540 molecular pair in deionized water and micellar solutions.

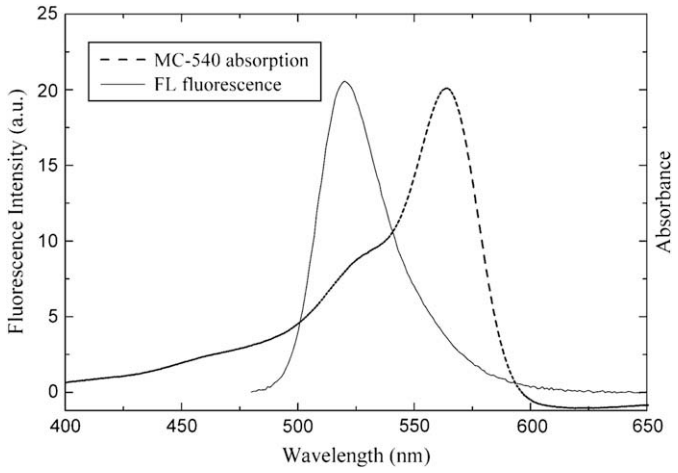


Fig. 5. Spectral overlap for FL-MC540 molecular pair in CTAB micellar solution.

media. Φ_D is the fluorescence quantum yield of the donor in the absence of the acceptor, n is the refractive index of the solvent, N is Avogadro's number, τ_D is the fluorescence lifetime of donor in the absence of acceptor, ε_a is the molar absorption coefficient of the acceptor, $\bar{v}$ is the wave number, and F_D is the spectral distribution of donor normalized to unity. R_0 and r are the critical energy transfer distance (or Förster critical distance) and distance between donor and acceptor, respectively. $k_{T(II)}$ values are given in Table 2. In our calculations, one can see that both SV relation and Förster theory give almost the same energy transfer rate constant values for the FRET studies as shown in Table 2. Förster type fluorescence energy transfer depends on the overlap between the donor fluorescence and the acceptor absorption spectra. For instance, Fig. 5 shows the overlap between the fluorescence spectrum of FL and the absorption spectrum of MC540 in CTAB micellar solution. R_0, which is an important parameter in the Förster theory that is called as the Förster critical distance at which 50% of the excitation energy is transferred to the acceptor, is calculated from the overlap according the following equation [1,3]

$$R_0 = 9.79 \times 10^3 \left[\kappa^2 n^{-4} \Phi_D J\right]^{1/6} \left(A\right) \tag{6}$$

where J is the normalized spectral overlap integral given by

$$J = \int_0^\infty \frac{F_D(\bar{v})\varepsilon_a(\bar{v})}{\bar{v}^4} d\bar{v} \tag{7}$$

Another useful parameter in resonance energy transfer is the energy transfer efficiency expressed as [1,3]

$$E = 1 - \frac{\tau_{DA}}{\tau} = 1 - \frac{I_{DA}}{I_D} \tag{8}$$

where I_{DA} and I_D are the donor fluorescence intensities in the presence and the absence of the acceptor, and τ_{DA} and τ_D are the donor lifetimes in the presence and absence of the acceptor, respectively. The distance r between the donor and acceptor can be calculated from Eq. (9) by using the values of E and R_0

$$E = \frac{R_o^6}{R_o^6 + r^6} \tag{9}$$

All energy transfer parameters have been calculated for the FL-MC540 molecular pair in micelles and in deionized water and reported in Table 2. The $k_{T(I)}$ and $k_{T(II)}$ values in Table 2 represent the energy transfer rate constants calculated from the SV relation and Förster theory, respectively. The high values of K_{SV}, k_T and R_0 indicate that fluorescence energy transfer is due to long-range dipole–dipole interactions between the excited donor and the ground state acceptor molecules for the studied systems [3]. R_0 values for the D–A pair in CTAB are found to be larger than in SDS, TX-100 micelles and deionized water systems since the r values for the D–A pair in CTAB are found to be smaller than those in SDS, TX-100 micellar solutions and deionized water. These result from the greater overlap between the donor fluorescence and acceptor absorption spectra in CTAB micellar solution and depend on the electrostatic interactions between dye molecules and CTAB micelles. Moreover, energy transfer efficiency (E) values show that FL and MC540 are good pair for the fluorescence energy transfer in CTAB micellar solution compared to the other SDS, TX-100 and deionized water systems. With this aim, energy transfer efficiency values were calculated as 0.14, 0.38 and 0.77 for deionized water, SDS and TX-100, respectively, as it is 0.85 for the CTAB micellar solution at the highest acceptor concentration at which FRET is completed. As seen from k_T values, fluorescence energy transfer rate constants in CTAB micelle solution are also higher than that of in SDS, TX-100 and deionized water systems. Therefore, one can state that CTAB micellar solution is the best for FL-MC540 molecular pair for FRET.

Table 2
Energy transfer parameters of fluorescein in the presence of merocyanine 540 in deionized water and micellar solutions.

Medium	K_{SV} (M^{-1})	$k_{T(I)}$ ($M^{-1}\,s^{-1}$)	$k_{T(II)}$ ($M^{-1}\,s^{-1}$)	R_0 (Å)	r (Å)
In CTAB	1.5×10^6	2.90×10^{14}	3.11×10^{14}	60.17	45.06
In TX-100	1.5×10^5	3.42×10^{13}	3.23×10^{13}	56.36	50.12
In SDS	1.9×10^4	4.23×10^{12}	4.32×10^{12}	56.48	61.50
In deionized water	0.8×10^4	2.17×10^{12}	1.65×10^{12}	41.68	56.73

4. Conclusions

Both methods used to evaluate the data, SV relation and Förster theory, indicated that organized environment caused the most efficient FRET even in SDS micelle solutions, containing negatively charged surfactants, compared to the free environment, in deionized water. When E and k_T values are taken into account, one can say that the efficiency of FRET phenomenon for FL-MC540 molecular pair has the order of deionized water < SDS < TX-100 < CTAB. Moreover, Φ_f values for FL increase from 0.29 ± 0.02 in deionized water to 0.82 ± 0.01 in CTAB micelle solution. The same trend was

also seen for MC540. In this case, Φ_f values for MC540 increase from 0.06 ± 0.01 in deionized water to 0.37 ± 0.01 in TX-100 micelle solution. MC540 has a Φ_f value of 0.32 ± 0.01 in CTAB micelle solution. These Φ_f values indicate that dye molecules are mostly located either on the surface of micelles or interior of the alkyl chains of the micelles compared the Φ_f values in deionized water. In addition, Förster critical distance, R_0, values and the distances between donor and acceptor, r, in different medium were calculated from Eqs. (6) and (9) by using Förster theory. This theory assumes that the distance between all the donor and acceptor pairs is constant. By taking into account r values in Table 2, one can say that the distance between dye molecules at which energy transfer takes place is shorter in case of CTAB micelle solution compared to the other studied media.

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