Study of energy transfer from 7-amino coumarin donors to rhodamine 6G acceptor in non-aqueous reverse micelles

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Abstract

The energy transfer using 7-amino coumarin dyes as donor and rhodamine 590 (Rh6G) as acceptor were investigated in methanol and acetonitrile solubilized non-aqueous reverse micelles using steady state and time resolved fluorescence spectroscopy. For all donors and acceptor we have obtained higher value of overlap integral ($J(k)$) and higher value of Förster distance ($R_0$). Although we have got higher value of $J(k)$ but the rate constant of energy transfer ($k_{ET}$) in almost all systems are small. Coumarin153–Rh6G pair is the most efficient donor acceptor pair as reflected by the value of $k_{ET}$. The different value of energy transfer rate constant in case of different donors indicate the dependence of energy transfer on the structure of donors.

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1. Introduction

Fluorescence resonance energy transfer (FRET) is a technique – half a century old, but due to recent development, it is under going renaissance [1–26]. FRET is often cited as ‘spectroscopic ruler’ in the modern day fluorescence spectroscopy. FRET is unique in its capacity to supply accurate spatial measurements and to detect molecular complexes over distances from 10 to 100 Å, which makes this method well established for studying various biological system [1–8] and in bichromophoric molecular system [9]. FRET, which relies on the distance dependent transfer of energy from donor fluorophore to an acceptor fluorophore, is one of the few tools available to measure distances in Å scale and change in distances, both in vitro and in vivo [10]. The uncertainty in distance measurement arises mainly due to orientation factor, which is a major problem of using this technique but several methods were proposed to overcome this problem [7,11–13].

Among several molecular mechanisms FRET from the excited donor molecule (D) to the acceptor molecule (A) provide us about the structural information of a complex media.

The mechanism of energy transfer is given below, in which D* is an excited donor and A is an acceptor to be excited

$$D^* + A \rightarrow D + A^*$$

The above mechanism requires that the rate of excitation energy transfer depend upon (i) on the quantum yield ($\phi_D$) of emission of D*, (ii) on the extent of overlap of the emission spectrum of D* and the absorption spectrum of A, (iii) on the light absorbing ability of A. (iv) on the concentration of the acceptor molecule and (v) on the relative orientation of the donor and acceptor transition dipoles. An elegant theory relating experimental observables to the mechanism of FRET was first proposed by Förster [14]. Recently some theoretical studies based on the limitation or validity of Förster theory was also done [15–17].

Energy transfer phenomena have wide application in energy conversion process like photosensitization and...
photosynthesis [18]. FRET has also been used to study protein folding [19] and measurement of distances between fluorescent tags in a protein [1, 6] and the observation of kinetics of conformational changes in RNA by time resolved FRET [20]. Similar kinds of experiments were employed to elucidate the structure and dynamics of polymer–polymer interfaces [21, 22]. FRET can also be used to analyze conformation of carbohydrate [23]. It is also used to investigate restricted molecular geometries [26]. Keeping in mind the versatile uses of energy transfer we would like to study energy transfer phenomena in non-aqueous reverse micelles formed by surfactant. Reverse micelles are used as elegant model of biological membrane [27]. The characterization of non-aqueous reverse micelles was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27].

Reverse micelles were employed to elucidate the structure and dynamics of reverse micelles [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27]. The characterization of non-aqueous reverse micelles formed by surfactants was done by Levinger et al. [28, 30]. The solvation dynamics in non-aqueous reverse micelles was done by Levinger et al. [27].

Interaction:

Between fluorescent tags in a protein [1, 6] and the observation of kinetics of conformational changes in RNA by time resolved FRET [20]. Similar kinds of experiments were employed to elucidate the structure and dynamics of polymer–polymer interfaces [21, 22]. FRET can also be used to analyze conformation of carbohydrate [23]. It is also used to investigate restricted molecular geometries [26]. Keeping in mind the versatile uses of energy transfer we would like to study energy transfer phenomena in non-aqueous reverse micelles formed by surfactant. Reverse micelles are used as elegant model of biological membrane [27].

According to Förster the rate of transfer for a donor and acceptor separated by a distance r is given by

\[ k_T(r) = \frac{Q_D \kappa^2}{\tau_D \epsilon_A} \left( \frac{9000(\ln 10)}{128 \pi^3 N n^4} \right) \int_0^\infty F_D(\lambda) \epsilon_A(\lambda) \lambda^4 \, d\lambda, \]  

(1)

where \( Q_D \) is the quantum yield of the donor in the absence of acceptor, \( N \) is Avogadro’s number, \( n \) is the refractive index of the medium between donor and acceptor, \( \tau_D \) is the lifetime of the donor in the absence of acceptor; \( F_D(\lambda) \) is the corrected fluorescence intensity in the donor in the wavelength range \( \lambda \) to \( \lambda + \Delta \lambda \), with the total intensity normalized to unity; \( \epsilon_A(\lambda) \) is the extinction coefficient of the acceptor at \( \lambda \), which is typically in units of \( M^{-1} \, \text{cm}^{-1} \); \( \kappa^2 \) is the well-known orientation factor of two dipoles interacting and is usually assumed to be equal to 2/3, which is appropriate for dynamic random averaging of the donor and acceptor.

The overlap integral \( J(\lambda) \) expresses the degree of spectral overlap between the donor emission and the acceptor absorption.

\[ J(\lambda) = \int_0^\infty \frac{F_D(\lambda) \epsilon_A(\lambda) \lambda^4}{\int_0^\infty F_D(\lambda) \, d\lambda} \, d\lambda. \]  

(2)

\( F_D(\lambda) \) is dimensionless. If \( \epsilon_A(\lambda) \) is expressed in units of \( M^{-1} \, \text{cm}^{-1} \) and \( \lambda \) is in nanometers, then \( J(\lambda) \) is in units of \( M^{-1} \, \text{cm}^{-1} \, \text{nm}^{-4} \). Eq. (1) can be expressed as

\[ k_T(r) = \frac{1}{\tau_D} \left( \frac{R_0}{r} \right)^6, \]  

(3)

where \( R_0 \) is defined as

\[ R_0 = \frac{9000(\ln 10) \kappa^2 Q_D}{128 \pi^3 N n^4} \int_0^\infty F_D(\lambda) \epsilon_A(\lambda) \lambda^4 \, d\lambda. \]

The above equation reduced to

\[ R_0 = 9.78 \times 10^3 [\lambda^2 \, \text{nm}^4 \epsilon_D J(\lambda)]^{1/6} \]  

(4)

\( R_0 \) is known as Förster distance, it is the distance at which the transfer rate \( k_T(r) \) is equal to the decay rate of the donor in the absence of acceptor (\( \tau_D^{-1} \)). That is the separation distance that yields 50% energy transfer efficiency.

The efficiency of energy transfer (E) is the fraction of photons absorbed by the donor that are transferred to the acceptor. This fraction is given by

\[ E = \frac{k_T}{\tau_D^{-1} + k_T}, \]  

(5)

which is the ratio of the transfer rate to the total decay rate of the donor. Recalling that \( k_{ET} = \tau_D^{-1}(R_0/r)^6 \), one can easily rearrange Eq. (5) to yield

\[ E = \frac{R_0^6}{R_0^6 + r^6}. \]  

(6)

2. Experimental

All the coumarin dyes and Rh6G were obtained from the Exciton (laser grade) and used as received. The structure of the acceptor and donors are shown in Scheme 1 (abbreviations are also shown there). AOT (Dioctyl sulfosuccinate, sodium salt, Aldrich) was purified by standard procedure [31]. The purified AOT was dried and kept in vacuo for 12–13 h before use. n-Heptane was purchased from Spectrochem, were freshly distilled over calcium hydride before use. The solution was prepared using the procedure used by Levinger et al. [30]. The concentration of AOT was kept at 0.09 M for all the measurements. The steady state absorption and emission spectra were recorded using a Shimadzu (model no: UV-1601) UV–Vis absorption spectrophotometer and a Spex Fluorolog-3 (model no: FL3-11) spectrofluorimeter. The details of the pico-second time resolved fluorescence spectrophotometer was described elsewhere [31]. Briefly we have used a picosecond laser diode (IBH, UK) at 408 nm. The signal has been detected at magic
angle ($54.7^\circ$) polarization using Hamamatsu MCP PMT (3809U). The time resolution of our experimental set up is $\sim 90$ ps. The temperature was kept $298 \pm 1$ K for all the measurements.

3. Results

3.1. Steady state results

We used 7-amino coumarin dyes as the donor molecule and Rh6G as the acceptor molecule. Since these pair shows extensive overlap zone (Fig. 1). For all coumarin dyes absorption and emission spectra have been red shifted with increase in $w$ value ($w = 2$–$6$) for both MeOH and ACN reverse micelle [31]. We have observed the spectral feature of acceptor dye Rh6G in AOT–MeOH and AOT–ACN RM. Rh6G exhibit emission peak in pure MeOH at 556 nm. On going from $w = 2$ to 6 for AOT–MeOH RM and from $w = 2$ to 4 for AOT–ACN RM Rh6G exhibit more or less same emission and absorbance peak.

With successive addition of micromolar amount of Rh6G the fluorescence intensity of the donor C-151, C-153, C-480 in RM at different $w$ value are gradually decreasing. This decrease in donor intensity is due to non-radiative energy transfer from donor to acceptor. During addition of micromolar amount of Rh6G, for all coumarin dyes emission maximum ($\lambda_{max}$) is shifted toward the blue end of the donor spectrum, with slight red shift of Rh6G emission peak (Fig. 2a,b). For C-153–Rh6G pair, emission peak of C-153 is blue shifted to maximum 20 nm, but for C-151 and C-480 emission peak is blue shifted to $\sim 10$ nm and $\sim 5$ nm, respectively. Among the coumarin–Rh6G pairs C-153–Rh6G pair was found more efficient with respect to energy transfer. Efficiency of energy transfer ($E$) was calculated as

$$E = 1 - \frac{F_{DA}}{F_D},$$

where $F_{DA}$ is the fluorescence intensity of the donor in the presence of acceptor, $F_D$ is the fluorescence intensity of the donor in the absence of acceptor. This equation is equivalent to Eq. (5). As we increase ‘$w$’ from $w = 2$ to 6 for AOT/$n$-heptane/MeOH RM, the efficiency of energy transfer gradually increases for C-153–Rh6G pair. But the efficiency of energy transfer is more or less same in AOT/$n$-heptane/ACN RM. Again for C-480–Rh6G pair efficiency of energy transfer decreases as we move from $w = 2$ to 6 for AOT/$n$-heptane/MeOH RM and it is also true for AOT/$n$-heptane/ACN RM. But in case of C-151–Rh6G pair efficiency of energy transfer in AOT/$n$-heptane/MeOH RM increases from $w = 2$ to 4 after that energy transfer efficiency remain constant with increase in ‘$w$’ value. We also calculate $J$ and $R_0$ for different system and are listed in Table 1. The $R_0$ value for the D–A pairs in non-aqueous reverse micelles is found to be larger than neat solvent methanol. The distance $r$ between the donor and acceptor can be calculated from
Eq. (6) from the value of $E$ and $R_0$. The calculated $r$ values have been shown in Table 1.

### 3.2. Time-resolved analysis

In addition to steady state measurements we have also performed time-resolved experiments to find out the energy transfer rate constant between donor and acceptor molecule. The rate of energy transfer depends on the lifetime of donor molecule by Eq. (3). Lifetime measurements were performed by exciting the donor molecule at 410 nm because donor molecules have sufficient absorption at 410 nm. With gradual addition of micromolar amount of acceptor dye solution lifetime of donor decreases, which are listed in Table 2 and shown in Fig. 4. We can calculate efficiency of energy transfer ($\varepsilon$), Förster distances ($R_0$) and $r$ from quenching of lifetime of donor, but it would be better to calculate those parameter form steady state data due to nonexponential behaviors of donor decay. Now we can be able to calculate energy transfer rate constant ($k_{ET}$) by knowing lifetime of donor in the absence of acceptor. The values of $k_{ET}$ for different system are shown in Table 1.

### 3.3. Discussion

In all systems we have studied, we find value of $J(k)$ is order of $10^{-11}$ to $10^{-12}$ M$^{-1}$ cm$^3$. But the rate of energy transfer ($k_{ET}$) is not large enough for all systems. In all systems we have got Förster distance ($R_0$) greater than 100 Å and donor acceptor distance also greater than 100 Å in most of the cases. We calculate $J(\lambda)$ from donor emission and acceptor absorption spectra. Due to high value of extinction coefficient of acceptor ($a_24$), we get a high value of $J(\lambda)$. This high value of $J(\lambda)$ and high

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**Table 1**

Energy transfer parameters for different coumarin–Rh6G pairs in methanol and acetonitrile RM$^a$

<table>
<thead>
<tr>
<th>System</th>
<th>$J(\lambda)$ M$^{-1}$ cm$^3$</th>
<th>$R_0$ (Å)</th>
<th>$E$</th>
<th>$r$ (Å)</th>
<th>$\varepsilon_0^2$</th>
<th>$k_{ET} \times 10^{-7}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-151 in MeOH RM at w = 2</td>
<td>$1.09 \times 10^{-11}$</td>
<td>118</td>
<td>15</td>
<td>157</td>
<td>1.314</td>
<td>2.17</td>
</tr>
<tr>
<td>C-151 in MeOH RM at w = 4</td>
<td>$1.04 \times 10^{-11}$</td>
<td>114</td>
<td>20</td>
<td>144</td>
<td>1.146</td>
<td>3.02</td>
</tr>
<tr>
<td>C-151 in MeOH RM at w = 6</td>
<td>$1.42 \times 10^{-11}$</td>
<td>121</td>
<td>19</td>
<td>154</td>
<td>1.199</td>
<td>2.88</td>
</tr>
<tr>
<td>C-153 in MeOH RM at w = 2</td>
<td>$2.62 \times 10^{-11}$</td>
<td>121</td>
<td>23</td>
<td>148</td>
<td>0.725</td>
<td>6.34</td>
</tr>
<tr>
<td>C-153 in MeOH RM at w = 4</td>
<td>$2.68 \times 10^{-11}$</td>
<td>121</td>
<td>38</td>
<td>131</td>
<td>0.637</td>
<td>12.80</td>
</tr>
<tr>
<td>C-153 in MeOH RM at w = 6</td>
<td>$2.50 \times 10^{-11}$</td>
<td>118</td>
<td>45</td>
<td>122</td>
<td>0.580</td>
<td>21.40</td>
</tr>
<tr>
<td>C-480 in MeOH RM at w = 2</td>
<td>$8.56 \times 10^{-12}$</td>
<td>101</td>
<td>38</td>
<td>109</td>
<td>0.671</td>
<td>15.45</td>
</tr>
<tr>
<td>C-480 in MeOH RM at w = 4</td>
<td>$7.18 \times 10^{-12}$</td>
<td>104</td>
<td>30</td>
<td>120</td>
<td>0.921</td>
<td>9.00</td>
</tr>
<tr>
<td>C-480 in MeOH RM at w = 6</td>
<td>$6.99 \times 10^{-12}$</td>
<td>103</td>
<td>26</td>
<td>122</td>
<td>0.908</td>
<td>7.7</td>
</tr>
<tr>
<td>C-480 in Methanol</td>
<td>$1.18 \times 10^{-11}$</td>
<td>107</td>
<td>18</td>
<td>138</td>
<td>0.692</td>
<td>2.80</td>
</tr>
<tr>
<td>C-153 in Methanol</td>
<td>$2.49 \times 10^{-11}$</td>
<td>106</td>
<td>77</td>
<td>87</td>
<td>0.308</td>
<td>116.73</td>
</tr>
<tr>
<td>C-151 in Methanol</td>
<td>$1.52 \times 10^{-11}$</td>
<td>117</td>
<td>30</td>
<td>135</td>
<td>0.909</td>
<td>6.07</td>
</tr>
<tr>
<td>C-153 in ACN RM at w = 2</td>
<td>$9.04 \times 10^{-12}$</td>
<td>103</td>
<td>24</td>
<td>125</td>
<td>0.724</td>
<td>5.37</td>
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<tr>
<td>C-153 in ACN RM at w = 4</td>
<td>$1.66 \times 10^{-11}$</td>
<td>107</td>
<td>26</td>
<td>127</td>
<td>0.497</td>
<td>5.93</td>
</tr>
<tr>
<td>C-480 in ACN RM at w = 2</td>
<td>$2.61 \times 10^{-12}$</td>
<td>82</td>
<td>39</td>
<td>88</td>
<td>0.649</td>
<td>17.13</td>
</tr>
<tr>
<td>C-480 in ACN RM at w = 4</td>
<td>$4.52 \times 10^{-12}$</td>
<td>60</td>
<td>22</td>
<td>74</td>
<td>0.058</td>
<td>5.59</td>
</tr>
</tbody>
</table>

$^a$ The size of the RM lying between 2 and 4 nm [28,29].
value of donor quantum yield are responsible for high value of $R_0$ or donor acceptor distance. Moreover, the low value of $k_{ET}$ may be arising due to higher donor–acceptor distance or strong coupling between donor and acceptor. If the coupling between donor and acceptor is very weak then we can use Förster type pair wise energy transfer model, but under strong coupling between donor and acceptor Förster relation becomes inadequate [15–17]. In the strong coupling limit the energy transfer from a single donor to more than one acceptor is possible. In case of Rh6G dimer formation is verified from the absorption spectra [33]. If the energy transfer will take place from one donor to two acceptors it will reduce the expected energy transfer time. This concept is verified in several theoretical papers [16,17]. Energy transfer efficiency calculated from steady state and time resolved methods do not match for all systems. For C-151 and C-480 time resolved method give low value of energy transfer efficiency, which confirmed that in a mixture donor radiative rates perturbed by acceptor (Rh6G). The possible reason for low value of time resolved energy transfer efficiency for C-151 and C-480 is due to the trivial (radiative) energy transfer takes place in these systems [32]. But for C-153–Rh6G system time resolved method give higher value of energy transfer efficiency than steady state studies. It may be due to weak coupling between C-153 and Rh6G.

As we increase ‘w’ value, the distance between donor acceptor increases for C-480–Rh6G pair due to increase in the size of the RM, so $k_{ET}$ decreases. We have also performed energy transfer experiment using the same donor acceptor but in pure methanol and found very high value of $J(\lambda)$ (∼$10^{-11}$). So we get very high value of $R_0$ (107 Å) and low value of energy transfer efficiency and $k_{ET}$. So with the increase in methanol contain in RM the $k_{ET}$ value goes to that of pure solvent. For C-151–Rh6G pair the energy transfer rate increases from $w = 2$ to 4 after that energy transfer rate remain more or less same. It may be possible that C-151 form hydrogen bond with the sulphonate ($SO_3^-$) group of AOT, so with increase in ‘w’ energy transfer rate remain more or less same. In this case we also get high value of $J(\lambda)$ but low value of $k_{ET}$ due to high value of $R_0$. In pure methanol we get very low value of $k_{ET}$ between C-151 and Rh6G. The $k_{ET}$ value is lower than pure methanol in RM for this pair due to increase in donor acceptor distance in RM. For C153–Rh6G system we have observed result opposite to that of C-480 system i.e., with increase in ‘w’ energy transfer efficiency and $k_{ET}$ value increases due to decrease in D–A distance. Again with increase in ‘w’ value more C-153 has been shifted from bulk n-heptane to RM core. It may be due to higher solubility of C-153 in MeOH and we have got higher value of energy transfer efficiency at $w = 6$. This is also reflected in time resolved studies that we get single exponential decay of C-153 in AOT–MeOH RM at $w = 6$. In this case we also get high value of $J(\lambda)$ and also high value of donor acceptor distance. We have also performed energy transfer experiments between C-153 and Rh6G in pure methanol solution. Here we have obtained high value of $k_{ET} \sim 10^9$ s⁻¹ and probably it is due to high value of energy transfer efficiency and low value of donor acceptor distance (87 Å) compared to other system but $R_0$ value is again higher than 100 Å and it may be due to high value of $J(\lambda)$. So with increase in methanol contain of RM $k_{ET}$ value reaches to that of pure solvent. Difference between energy transfer rate or efficiency between RM and pure solvent is due to the fact that we take an average value of $\kappa^2 = 2/3$, which is not same for RM and in methanol.

![Table 2](image_url)

<table>
<thead>
<tr>
<th>System</th>
<th>Acceptor (Rh6G) concentration (µM)</th>
<th>$b_1$</th>
<th>$r_1$ (ns)</th>
<th>$b_2$</th>
<th>$r_2$ (ns)</th>
<th>$\zeta$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-151 in MeOH RM at $w = 2$</td>
<td>(a) 0</td>
<td>0.16</td>
<td>1.63</td>
<td>0.84</td>
<td>5.26</td>
<td>4.68</td>
</tr>
<tr>
<td></td>
<td>(b) 19</td>
<td>0.14</td>
<td>1.56</td>
<td>0.86</td>
<td>5.21</td>
<td>4.69</td>
</tr>
<tr>
<td>C-151 in MeOH RM at $w = 4$</td>
<td>(a) 0</td>
<td>0.07</td>
<td>1.59</td>
<td>0.93</td>
<td>5.37</td>
<td>5.105</td>
</tr>
<tr>
<td></td>
<td>(b) 19</td>
<td>0.08</td>
<td>1.69</td>
<td>0.92</td>
<td>5.4</td>
<td>5.103</td>
</tr>
<tr>
<td>C-151 in MeOH RM at $w = 6$</td>
<td>(a) 0</td>
<td>0.08</td>
<td>1.39</td>
<td>1.25</td>
<td>5.34</td>
<td>4.99</td>
</tr>
<tr>
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<td>0.11</td>
<td>1.19</td>
<td>0.89</td>
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<td>4.91</td>
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<td>C-153 in MeOH RM at $w = 2$</td>
<td>(a) 0</td>
<td>0.44</td>
<td>1.88</td>
<td>0.56</td>
<td>4.13</td>
<td>3.14</td>
</tr>
<tr>
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<td>0.53</td>
<td>1.51</td>
<td>0.47</td>
<td>3.47</td>
<td>2.43</td>
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<tr>
<td>C-153 in MeOH RM at $w = 4$</td>
<td>(a) 0</td>
<td>0.49</td>
<td>3.29</td>
<td>0.51</td>
<td>4.95</td>
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<td></td>
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<td>0.57</td>
<td>1.35</td>
<td>0.43</td>
<td>3.79</td>
<td>2.399</td>
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<td>(a) 0</td>
<td>1</td>
<td>4.52</td>
<td>–</td>
<td>–</td>
<td>4.52</td>
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<td></td>
<td>(b) 19</td>
<td>0.61</td>
<td>1.04</td>
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<td>2.08</td>
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<td>C-480 in MeOH RM at $w = 2$</td>
<td>(a) 0</td>
<td>0.20</td>
<td>1.25</td>
<td>0.78</td>
<td>3.95</td>
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<td>(b) 19</td>
<td>0.48</td>
<td>1.35</td>
<td>0.52</td>
<td>3.92</td>
<td>2.69</td>
</tr>
<tr>
<td>C-480 in MeOH RM at $w = 4$</td>
<td>(a) 0</td>
<td>0.22</td>
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<td>0.78</td>
<td>3.97</td>
<td>3.37</td>
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<td>0.27</td>
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<td>C-480 in MeOH RM at $w = 6$</td>
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<td>0.72</td>
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</tbody>
</table>
Again it is also possible that due to distribution of coumarin dye between bulk n-heptane and RM we get an average value of energy transfer parameter in RM. We have obtained same trend in energy transfer parameter for AOT–ACN RM but difference in energy transfer parameter in these two systems is due to difference in polarity of methanol and acetonitrile. Rh6G is not soluble in n-heptane but in presence of AOT it solubilizes in n-heptane. AOT is (−ve) charge and Rh6G is (+ve) charge so here an electrostatic attractive force is operating between AOT and Rh6G, which solubilizes Rh6G in AOT/n-heptane mixture. It is expected that Rh6G be localized at the vicinity of the polar solvent/surfactant interface. The location of Rh6G molecule is more or less fixed within the reverse micelle for different w/C212/C213 value. So variation of energy transfer efficiency is due to only location of donor molecules. Again if we examine the absorption spectra of Rh6G at different w we see that Rh6G exhibit a shoulder at around 510 nm, it is may be due to dimer formation [33] (Fig. 3). Moreover, the different value of energy transfer rate constant in case of different donors indicate that the energy transfer is very much dependent on the structure of the donors.

So we can conclude that large J(λ) is not enough to guarantee a significant contribution to the energy transfer rate. The limit of donor acceptor distance, i.e., less than 100 Å may not be true. Recently Wong et al. [34] shown that the distance at which Förster rate is recovered is to be rather large i.e., greater than 100 Å which is also observed in our experiments. Hesegawa et al. [35] have shown that at high concentration of AOT, RM is not randomly dispersed in n-heptane but form clusters through intermicellar flocculation in aqueous RM and consequently facilitate energy transfer. We have also performed the above experiment by increasing concentration of AOT = 0.2 M. But in our case increasing concentration of AOT has no effect on energy transfer rate. The calculated value of R0 and donor acceptor distance is greater than 100 Å using Förster relation for all systems we have studied. It may be possible that Förster relation is not valid in these systems. We have only considered the energy transfer between one donor and one acceptor. However, since this situation comes about by increasing the concentration of dye molecules, there must be similar contribution from donor–donor and acceptor–acceptor energy transfer. We can ignore the donor–donor energy transfer because we have used very low concentration of donor (∼10−6 M) for our experiment. We have excited the probes at ∼410 nm where the acceptor absorption is negligible. So the possibilities of acceptor–acceptor energy transfer can also be ruled out. The energy transfer is possible from one donor to more than one acceptor. Because in case of Rh6G dimer formation is verified. If we consider the diameter of the core of the RM (2–4 nm) [28, 29] and the size of the donors ∼0.8 nm and size of the acceptor ∼1 nm.
(calculated by using Edward molecular volume methods) it is more probable that one donor and either one or two acceptors may reside in the core of the RM. The energy transfer from the donor to the dimer acceptor reduces the energy transfer rate constant.

4. Conclusion

To the best of our knowledge this is the first report of intermolecular energy transfer in non-aqueous reverse micelles. In all the systems we have obtained a higher value of $J(\lambda)$ consequently we get a higher value of Förster distance ($R_0$) ($\geq 100$ Å) in most of the non-aqueous reverse micelles. Although we have obtained higher value of $J(\lambda)$ but energy transfer rate constant ($k_{ET}$) in most cases are smaller than it is expected from the value of $J(\lambda)$. We can conclude that higher value of $J(\lambda)$ is not the indicator to get higher value of energy transfer efficiency or higher value of $k_{ET}$. C-153–Rh6G pair provides better donor–acceptor pair for energy transfer as reflected by the value of energy transfer efficiency and $k_{ET}$. Finally we can conclude that Förster relation is not valid in these systems.

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