

Förster Resonance Energy Transfer between Acridinedione and Rhodamine-B

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Abstract—The Förster Resonance Energy Transfer (FRET) studies between acridinedione dyes as donors and rhodamine dyes as acceptors are explored by spectroscopic techniques like absorption, fluorescence and lifetime measurements. Enhancement of the acceptor photoluminescence intensity and a decrease of the donor decay time are observed in solutions containing acridinedione dye with Rhodamine B (Rh-B). The Förster parameters were calculated and were found to well correlate with the experimental findings. The evidence for the emergence of acridinedione dyes as good energy donors was observed from the high efficiency of energy transfer obtained from fluorescence lifetime measurements and high rate constants obtained from Stern-Volmer calculations.

Keywords: Acridinedione, Rhodamine B, FRET, lifetime.

I. INTRODUCTION

The fluorescence resonance energy transfer between potential energy donors and organic dyes as acceptor molecules are of profound interest for a long time. Especially, the interest in fluorescence dyes for qualitative and quantitative assays has considerably increased during the last 20 years [1]. Since FRET is usually a technique which occurs over distances realized in the interaction between most biomolecules, that is typically in the range of 1-10 nm, it is a powerful spectroscopic tool to interrogate changes in molecular conformation, association and the assembly or disassembly of biomolecular machinery. In recent years, numerous advances have been achieved in FRET investigations, regarding both fundamental theoretical analyses and biological applications, including the design of biosensors and the analysis of biomolecular conformation and interaction [2]. The emphasis is on a system containing two types of randomly distributed molecules that can interact with each other. One type of molecule (the "donor") is endowed initially with excess energy that either dissipates spontaneously or can be transferred irreversibly to the other type of molecule (the "acceptor") by virtue of their mutual interaction. The interaction is distance dependent, causing the energy transfer rate to fall off with increasing donor-acceptor separation [3]. Very recently, FRET is visualized as a widely prevalent photophysical process that involves the transfer of excitation energy of an electronically excited "donor" (D) molecule to an "acceptor" (A) molecule via non radiative routes. A quantitative interpretation of an efficient energy transfer is studied as follows: According to Förster theory, the rate of energy transfer (k_{FRET}) from a donor to an acceptor, separated by a distance 'r' is given by [4],

$$k_{\text{FRET}} = \frac{1}{\tau_D^0} \left(\frac{R^0}{r} \right)^6 \quad (1)$$

Where τ_D^0 is the lifetime of the donor in the absence of acceptor, R^0 is the distance (Å) between the donor and acceptor and is given by the equation (2)

$$R^0 = 0.211 \left[\kappa^2 \eta^{-4} Q_D J(\lambda) \right]^{1/6} \quad (2)$$

where Q_D is the quantum yield of the donor in the absence of acceptor[5], η is the refractive index of the medium, κ^2 is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor, $J(\lambda)$ is the spectral overlap between donor emission and acceptor absorption and is given by the expression[6],

$$J(\lambda) = \frac{\int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d(\lambda)}{\int F_D(\lambda) d(\lambda)}$$

The value of κ^2 may vary from 0 (mutually perpendicular transition dipoles) to 4 (collinear dipoles). For random orientation of D and A dipoles, value of κ^2 is used as 2/3 for R^0 calculations [7].

The efficiency (E) of FRET process can be calculated from the steady-state and time resolved data as given by equations 3 & 4.

$$E = 1 - \frac{I_D}{I_{0D}} \quad (3)$$

$$E = 1 - \frac{\tau_D}{\tau_{0D}} \quad (4)$$

Where I_{0D} , I_D , τ_{0D} , τ_D are the intensities and lifetimes of the donor in the absence and presence of acceptors, respectively[5].

Alternatively, energy transfer efficiency can also be calculated as:

$$E = \frac{1}{1 + \left(\frac{r}{R_0}\right)^6} \quad (5)$$

Where 'r' is the distance between D & A chromophores, ' R_0 ' is the so-called Förster distance, at which the energy transfer efficiency is 50%. Thus R_0 is the critical distance, at which energy transfer and spontaneous decay of the excited donor are equally probable, and it may be experimentally determined from the spectroscopic data and is given by equation 6 as

$$R_0^6 = \frac{9000(\ln 10) k^2 W_D}{128 f^5 N_A Y^4} J(\lambda) \quad (6)$$

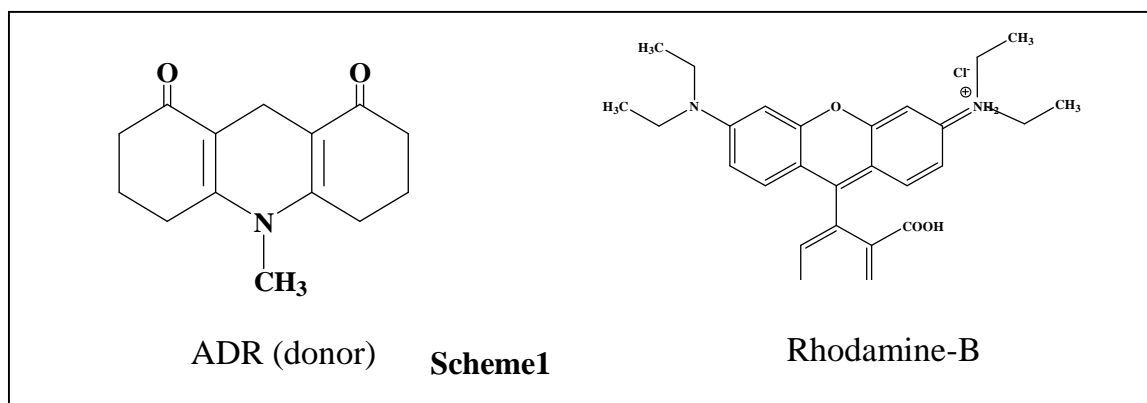
where k^2 is an orientation factor and has a value of 2/3 for randomly oriented donor-acceptor dipoles, W_D is the quantum yield of the donor in the absence of acceptor, N_A is the Avogadro number, f is the refractive index of the medium and $J(\lambda)$ is a quantitative measure of the donor-acceptor overlap[7]. Among the factors appearing in the equation, it is the spectral overlap integral $J(\lambda)$ which makes a difference with regard to efficiency of the process since all the others remain the same for a typical system.

II. MATERIALS AND METHODS

Methanol used was of HPLC grade and was obtained from Qualigens India Ltd. Rhodamine B obtained from Sigma-Aldrich used without any further purification. Acridinedione dye was synthesized in the lab following reported procedures[8]. Absorption spectra were recorded in an Agilent 8453 diode array spectrophotometer. Fluorescence spectra were recorded using a Horiba Jobin Yvon Fluoromax 4P Spectrofluorimeter. Fluorescence quantum yields were obtained from the corrected fluorescence spectrum using quinine sulphate in 0.1N H_2SO_4 [9]. Time resolved fluorescence decays were obtained by the Time-Correlated-Single-Photon-Counting (TCSPC) method. The excitation source used was a 375 nm LED (model N-375 LH) with a pulse width of 600 ps purchased from Horiba Jobin-Yvon. The emission intensity were counted by a MCP PMT (Hamamatsu R 3809) and processed through CFD, TAC and MCA. The fluorescence decay was analyzed by using the software provided by IBH (DAS-6).

III. RESULTS AND DISCUSSION

The structures of the donor (ADR) and the acceptor (Rhodamine-B) are as shown in Scheme 1. This D-A pair was chosen based on the effective spectral overlap observed between the donor emission and acceptor absorption. The spectral parameters calculated for the above D-A pair is as shown in **Table 1**.



For all photophysical measurements, the concentration of ADR was maintained as $1 \times 10^{-5} \text{M}$ and that of the Rh-B acceptor was varied from 2 to 8 μM levels.

Table 1: Spectral parameters of Donor and Acceptor in methanol

Donor/ Acceptor	ϵ ($\text{M}^{-1} \text{cm}^{-1}$)	$J(\lambda)$ ($\text{mol}^{-1} \text{cm}^{-1} \text{nm}^4$)	R^0 (\AA)	R_{DA} (\AA)	τ (ns)	Φ_D
Rhodamine - B	1.06×10^5	1.2×10^{-13}	54.22	62.12	-	-
ADR	-	-	-	-	4.92	0.91

A. Steady-state measurements:

Fig 1 shows the absorption spectrum for ADR for increasing concentrations of Rh-B acceptor. No significant change was observed at the absorption maximum (396 nm) for ADR with successive additions of acceptor solution. This confirms the absence of acceptor absorbance at the λ_{max} of the donor. Hence the steady-state emission studies were carried out by exciting the donor-acceptor combined solutions at this wavelength.

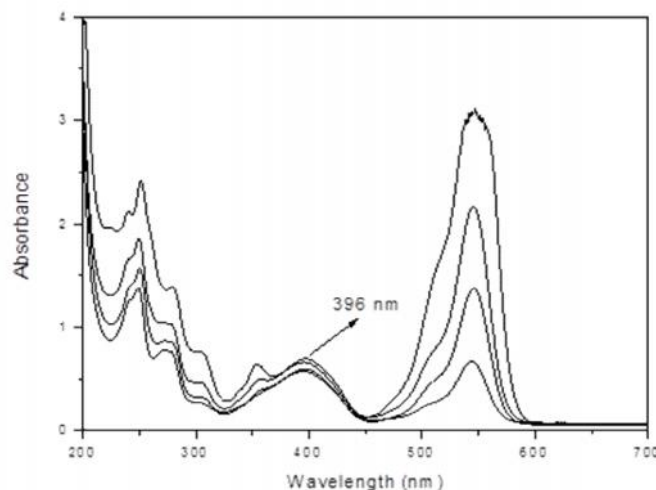


FIGURE 1: Absorption spectrum of ADR in the presence of increasing concentration of Rh-B. [ADR]- $1 \times 10^{-5} \text{M}$, [Rh-B] : (a) 0M b) $2 \times 10^{-6} \text{M}$ (c) $4 \times 10^{-6} \text{M}$ (d) $6 \times 10^{-6} \text{M}$ e) $8 \times 10^{-6} \text{M}$.

Fig.2 shows the steady-state emission of ADR for increasing concentrations of the acceptor. The excitation wavelength of 396 nm was absorbed dominantly by the donor. Upon increasing the concentration of the acceptor, a decrease in intensity of the donor emission peak at 465 nm was observed with increase in concentration of Rh-B solution. Also a new acceptor emission peak appeared at 575 nm and its emission intensity was found to increase with continuous additions of the Rh-B solution. This gives the evidence for energy transfer from ADR as there is no significant contribution of Rh-B fluorescence by direct excitation at 375nm. A red shift of 6 nm and a blue shift of 4 nm in the acceptor fluorescence maximum and donor fluorescence maximum, respectively, were observed with increase in the acceptor concentration. The red shift is attributed to radiative migration due to the effect of self-absorption among acceptors and the blue shift is due to the radiative transfer from donor to acceptor[10]. From the intensity values of the ADR in the presence of different concentrations of Rh-B, the rate constant of the FRET process was calculated as $4.58 \times 10^{12} \text{M}^{-1} \text{s}^{-1}$ using Stern-Volmer plots (Inset in Fig 2). Also from the fluorescence intensity of ADR in the presence and absence of Rh-B, the FRET efficiency is calculated as 31%.

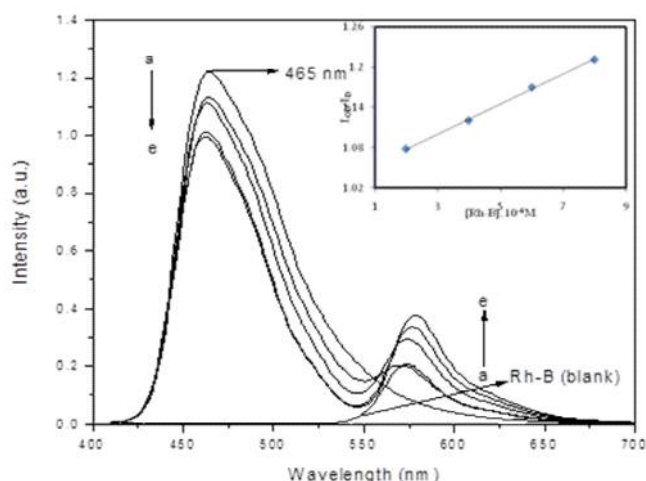


FIGURE 2: Steady state emission spectrum of ADR in the presence of increasing concentrations of Rh-B. [ADR]- $1 \times 10^{-5} \text{M}$, [Rh-B] : (a) 0M (b) $2 \times 10^{-6} \text{M}$ (c) $4 \times 10^{-6} \text{M}$ (d) $6 \times 10^{-6} \text{M}$ (e) $8 \times 10^{-6} \text{M}$. $\lambda_{\text{exc}} = 396 \text{ nm}$. Inset:: Intensity based Stern-Volmer plot of ADR/Rh-B system. $\lambda_{\text{exc}} = 396 \text{ nm}$.

IV. LIFETIME MEASUREMENTS

Time Correlated single Photon Counting measurements were carried out by exciting the donor-acceptor combinations at 375 nm and recording the decay profiles at donor emission wavelength (465 nm) and acceptor emission wavelength (575 nm). With successive additions of Rh-B solutions, the decay profiles at 465 nm were found to be single exponential (Fig.3) but the lifetime decreased continuously. Whereas, the decay recorded at the acceptor wavelength (575 nm) were bi-exponential (Fig.4) and one of the component was found to show a –ve amplitude. The negative component was indicative of the FRET process [11] and the lifetime of this component was found to increase with increasing concentrations of the acceptor. This confirmed the fact that with increase in concentration of the acceptor, there was a rise in the acceptor molecules coming in close proximity of the donor and hence there occurs higher chances of long range resonance energy transfer.

The lifetime and amplitude obtained from fluorescence lifetime analysis is as shown in Table 2. From the lifetime of ADR in the presence and absence of Rh-B, the efficiency of FRET was obtained as 18% and the rate constant was calculated as $9.58 \times 10^{12} \text{ M}^{-1} \text{ s}^{-1}$ by Stern-Volmer plots (Fig 6). It was observed that the efficiency of the FRET process obtained from lifetime measurements was only around 50% of that obtained from steady state measurements. Such a decrease in FRET efficiency is explained by the fact that the interference due to cross-talks and background noise which are more prevalent in steady-state measurements are completely ruled out in the case of lifetime measurements and hence the efficiency obtained is more reliable even though it is less.

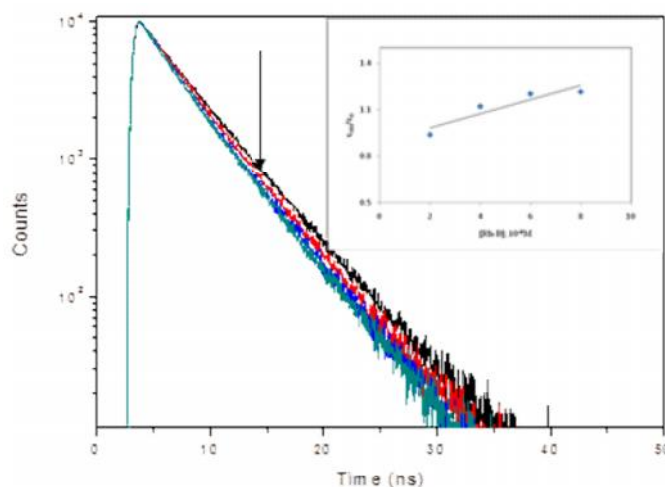


FIGURE 3: Lifetime decay profile of ADR/Rh-B system at 465 nm. [ADR] : $1 \times 10^{-5} \text{M}$, [Rh-B] : (a) $2 \times 10^{-6} \text{M}$ (b) $4 \times 10^{-6} \text{M}$ (c) $6 \times 10^{-6} \text{M}$ (d) $8 \times 10^{-6} \text{M}$. $\lambda_{\text{exc}} = 375 \text{ nm}$. Inset: Lifetime decay profile of ADR/Rh-B system at 575 nm. [ADR] : $1 \times 10^{-5} \text{M}$, [Rh-B] : (a) $2 \times 10^{-6} \text{M}$ (b) $4 \times 10^{-6} \text{M}$ (c) $6 \times 10^{-6} \text{M}$ (d) $8 \times 10^{-6} \text{M}$. $\lambda_{\text{exc}} = 375 \text{ nm}$.

Table 2: Lifetime measurements of ADR/Rhodamine-B system at 465 nm and 575 nm

[Rh-B], 10^{-6} M	Donor lifetime at 465 nm			Acceptor lifetime at 575 nm		
	τ_D (ns)	Relative Amplitude (%)	τ^2	τ_A (ns)	Relative Amplitude (%)	τ^2
0	4.81	100.00	1.06	2.07	-199.50	1.04
				3.38	75.75	
2	4.51	100.00	1.1	2.38	223.75	1.3
				1.975	-125.81	
4	4.29	100.00	1.3	2.95	225.81	1.2
				2.204	-240.54	
6	4.00	100.00	1.2	2.459	5.97	1.12
				2.77	334.57	
8	3.94	100.00	1.15	2.07	-122.27	1.12
				2.74	-316.94	
				2.86	539.21	

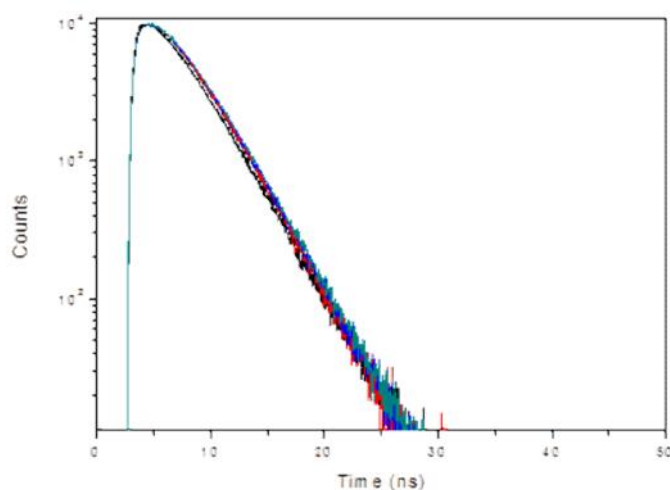


Figure 4: Lifetime decay profile of ADR/Rh-B system at 575 nm. [ADR] : 1×10^{-5} M, [Rh- B] : (a) 2×10^{-6} M (b) 4×10^{-6} M (c) 6×10^{-6} M (d) 8×10^{-6} M. $\lambda_{exc} = 375$ nm.

V. CONCLUSION

We have successfully carried out Förster Resonance Energy Transfer studies with acridinedione dye as donor and Rh-B as acceptor. The FRET studies were quantified with the help of steady-state and lifetime measurements. A FRET efficiency of 18% was obtained when the studies were carried out in a neat solution like methanol. This study sheds more light on the possibilities of further FRET studies with other similar donor and acceptor combinations in neat solutions, polymer solutions or matrices.

VI. ACKNOWLEDGEMENT

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VII. REFERENCES

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