EFFECT OF CONFINEMENT IN ENERGY TRANSFER DYNAMICS WITHIN MOLECULAR AGGREGATES

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A dissertation submitted for the partial fulfilment of BS-MS dual degree in science

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CERTIFICATE OF EXAMINATION

This is to certify that the dissertation titled "**Effect of confinement in energy transfer dynamics within molecular aggregates**" submitted by Ms. Anusree P V (Reg No: MS12125) for the partial fulfillment of BS MS dual degree program of Indian Institute of Science Education and Research Mohali, has been examined by the thesis committee duly appointed by the institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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Dated: April 21, 2017

DECLARATION

The work presented in this dissertation has been carried out by me under the guidance of Dr. Arijit Kumar De at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussion. This thesis is a bona-fide record of original work done by me and all sources listed within have been detailed in the bibliography.

Anusree P V
(Candidate)
Dated: April 21, 2017

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Arijit K. De
(Supervisor)

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CONTENT

LIST OF ABBREVIATIONS

FRET: Förster resonance energy transfer

Flu : Fluorescein

R6G : Rhodamine 6G

DLS : Dynamic light scattering

TCSPC : Time correlated single photon counting

AOT : Aerosol-OT, sodium bis(2-ethylhexyl) sulfosuccinate

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ABSTRACT:

Förster resonance energy transfer (FRET) happening between two dyes Fluorescein and Rhodamine 6G in aqueous solution and within the confinement of reverse micelle of fixed diameter was investigated. Energy transfer is occurring from Fluorescein to Rhodamine 6G, i.e. Fluorescein acts as a donor and Rhodamine 6G as an acceptor. pH variation of solutions from 7 to 9.2 is not affecting the energy transfer efficiency. Used reverse micelle as a confinement and tried to study the energy transfer from donor to acceptor. AOT in n-hexane and TritonX-100 in cyclohexane are used for making reverse micelles.

Chapter1. INTRODUCTION

Förster resonance energy transfer (FRET) or Fluorescence resonance energy transfer has wide applications in medical diagnostics, DNA analysis and optical imaging. This is because of the distance for energy transfer is of the size of a protein, or the thickness of a membrane[1]. FRET is spreading its wings in sensing applications other than biosensors, like ion sensor[2], environmental sensors[3]. The extent of FRET is predictable from the spectral properties. Mostly FRET will not be affected by the biomolecules.

Photosynthesis is the process by which plants, algae, cyanobacteria, and anoxygenic photosynthetic bacteria convert light energy into chemical energy and this is initiated by a sequence of photophysical and photochemical reactions.

Photosynthetic pigments utilized in light harvesting process are chlorophyll, carotenoids and phycobilins. Energy absorbed by the pigment molecules in the photosynthetic unit transferred to the reaction center, where photoreactions get started. Pigment aggregates act as an antenna, which harvest the light energy and deliver to the reaction center. Typical PSU consists of two reaction centres photosystems I (PS I) and II (PS II). Photosynthetic pigments are examples of conjugated π electron systems with high molar extinction coefficient ~10^5 $M^{-1}cm^{-1}$. Light harvesting complex contains chromophores in very high concentration up to 0.6M[4]. Our motivation is the energy transfer happening within the pigment protein complex. There are two limits of energy transfer- coherent and incoherent. FRET is coming under incoherent energy transfer.

FRET is a phenomenon that occurs between a donor molecule (D) in the excited state and an acceptor molecule (A) in the ground state. This energy transfer occurs without the emission of a photon and is the result of a long range dipole-dipole interaction between the donor and acceptor[1, 5]. Energy transfer from donor to acceptor leads to reduction in the fluorescence intensity and excited state lifetime of donor, while that of acceptor increases.

This technique is good at measuring structural changes in protein. Even though the resolution of FRET spectroscopy is lower than X-ray diffraction, absolute distance measured remains problematic, because calculation of FRET distance assumes that the probes are able to undergo free isotropic motion[6].

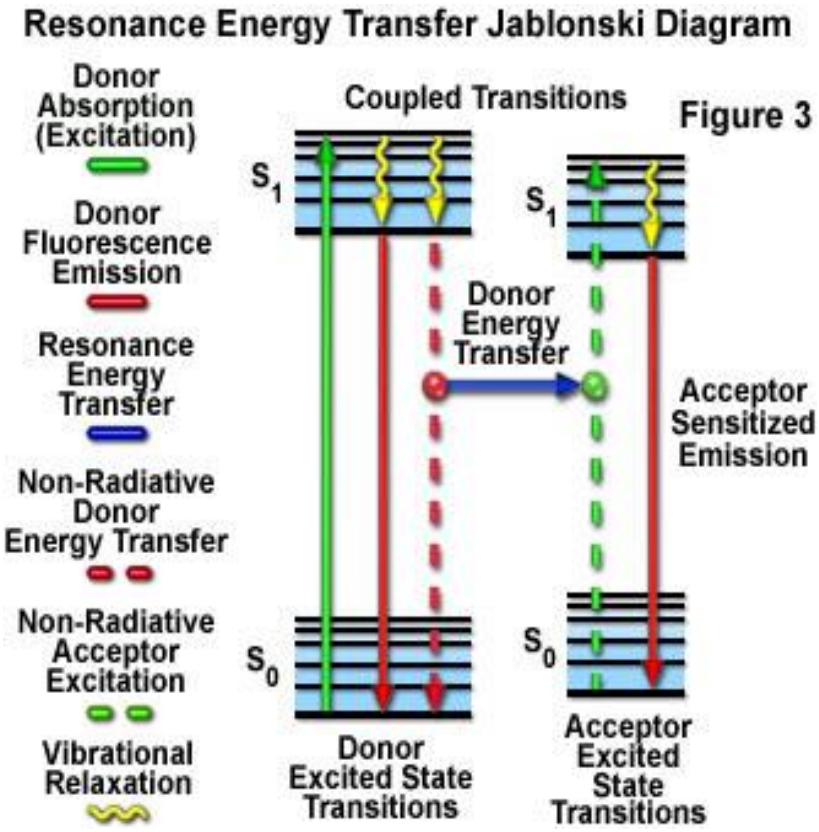


Fig1: Jablonski diagram illustrating the coupled transition between donor and acceptor

Ref: Olympusmicro.com

The rate of energy transfer depends on -

- Fluorescence quantum yield of donor
- Refractive index of the medium
- Relative orientation of the donor and acceptor dipoles
- Spectral overlap of the emission of donor and absorption of acceptor.

Quantum yield is the number of emitted photons to the absorbed photons.

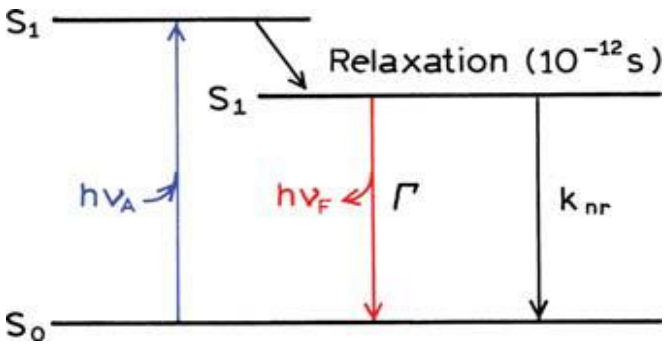


Fig2: Example of a Jablonski diagram

Ref: Principles of Fluorescence Spectroscopy, Lakowicz J.R

Where Γ is the rate of fluorescence and k_{nr} is the rate of non-radiative decay.

$$Q = \frac{\Gamma}{\Gamma + k_{nr}} \quad (1)$$

The lifetime of the excited state is defined by the average time of the molecule spends in the excite state before returning to the ground state, and it is given by

$$\tau = \frac{1}{\Gamma + k_{nr}} \quad (2)$$

The rate constant for energy transfer is inversely proportional to the sixth power of the distance between the groups[5].

$$k_T(r) = \frac{1}{T_D}\left(\frac{R_0}{r}\right)^6 \quad (3)$$

Here r is the distance between donor and acceptor and $\mathbf{T_D}$ is the lifetime of the donor in the absence of acceptor. R_0 is called Förster distance.

The distance at which energy transfer efficiency is 50% efficient is called the Förster distance R_0. i.e., at this distance half of the donor molecules decay by energy transfer and half decay by the usual radiative and non-raidative rates[1]. The distance over which energy can be transferred is in the range 10-100 A^0.

$$R_0^6 = \left[\frac{9000\,(\ln 10)K^2\,\Phi_D}{128\,\pi^5 N n^4}\right]\int_0^\alpha F_D(\lambda)\,\eta\,\varepsilon_A(\lambda)\,\lambda^4\,d\lambda$$

$$R_0 = 0.2108\left(K^2 n^{-4}\,\Phi_D J(\lambda)\right)^{1/6} \quad (4)$$

Where J (**λ**) is

$$J(\lambda) = \int_0^\alpha F_D(\lambda)\,\varepsilon_A(\lambda)\,\lambda^4\,d\lambda$$

Above expression says that the Förster distance to be calculated from the quantum yield of donor and spectral properties of the donor and acceptor. The term K^2 describes the relative orientation of the transition dipoles of donor and acceptor, is usually assumed to be 2/3.

Energy transfer efficiency for a single donor-acceptor pair at a fixed distance is given by the equation,

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

The transfer efficiency is measured using the relative fluorescence intensity as

$$E = 1 - \frac{F_{DA}}{F_D} \quad (6)$$

Where F_D is the fluorescence intensity of the donor in the absence of acceptor and F_{DA} is the fluorescence intensity of the donor in the presence of acceptor[1].

From the fluoscence intensity of acceptor, relative energy transfer efficiency

$$E = \frac{F_A}{F_A + F_D} \quad (7)$$

Transfer efficiency can also be calculated from the lifetime of D:

$$E = 1 - \frac{T_{DA}}{T_D} \quad (8)$$

From the lifetime of acceptor

$$E = 1 - \frac{T_A}{T_{AD}} \quad (9)$$

INSTRUMENTATION

UV-Vis spectrometer: Used for measuring the absorbance of the samples and in turn concentration based on Beer-Lambert law.

Absorbance, A = εcl

Where ε is the molar extinction coefficient C is the concentration of sample and l is the pathlength.

Fluorescence spectrophotometer: Used for measuring steady state intensity of fluorophore molecules.

Dynamic light scattering (DLS):

This is an optical technique used for analyzing dynamic properties and size distribution of a variety of physical, chemical and biological systems. Technique is based on the

extraction of spectral information derived from time-dependent fluctuations of the light from the sample. From the scattered light one can obtain the diffusion coefficient D and by using the Stokes-Einstein equation the hydrodynamic radius R is

$$R = \frac{kT}{6\pi\eta D} \qquad 10$$

With k the Boltzmann constant, T the temperature in Kelvin, and η the viscosity of the suspending medium[7].

Time correlated single photon spectroscopy (TCSPC):

Present day most of the time domain measurements are performed by using time correlated single photon counting. This instrument uses high repetition rate mode-locked picosecond (ps) laser light sources. Here the sample is excited with a pulse of light and conditions are adjusted so that less than one photon is detected per laser pulse. The detection rate is typically one photon per 100 excitation pulses. The response of the instrument to a zero lifetime sample is the instrument response function (IRF). This can be collected using a dilute scattering solution of Ludox (colloidal silica). This time profile represents the shortest time profile that can be measured by instrument.[1]

The lifetime changes can be due to changes in the overlapping emission and absorption spectra of two dyes used or changes in the quenching intensity. TCSPC can be used to measure lifetime and anisotropy fluctuations[8].

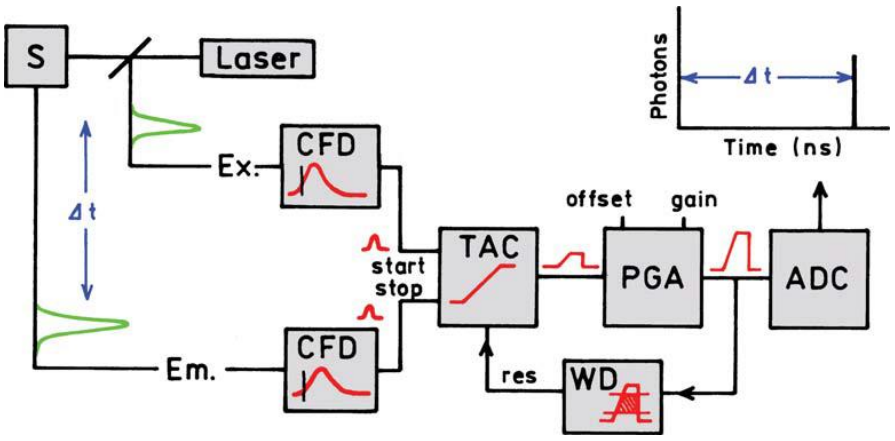
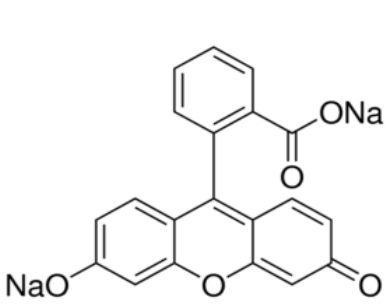


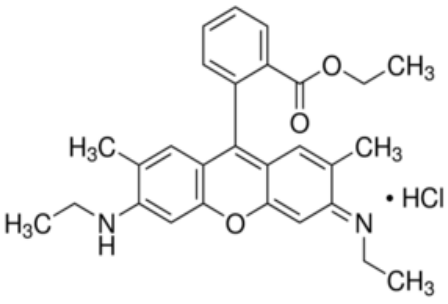
Fig3: Schematic diagram for TCSPC [Principles of Fluorescence Spectroscopy, Lakowicz J.R]

- Excitation pulse excites the sample and sends signal to the electronics. Laser diodes (LD) and Light emitting diodes (LED) can be used as light source.
- Signal gets passed through constant function discriminator (CFD) measures the arrival time of signal.
- Time to amplitude converter (TAC) generates a voltage ramp against the time. This voltage is proportional to the time delay between the excitation and emission signals.
- Programmable gain amplifier (PGA) is used to amplify the voltage and converted to a numerical value by the analog to digital converter (ADC).
- Almost all TCSPC measurements are taken in the reverse mode in which emission signal is used to start TAC and excitation signal to stop.

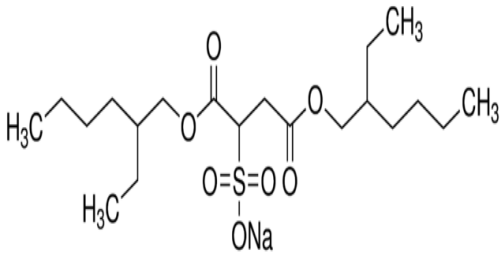
My investigation deals with the FRET between two dyes Fluorescein (Flu) and Rhodamine6G (R6G). These two molecules exist as anion and cation in alkaline pH, so that there will be a strong non covalent interaction which holds them closer. This closeness results in an increase in the energy transfer efficiency. Among the molecules under investigation absorption and fluorescence emission spectra are highly pH sensitive[15]. This will affect the process of FRET between Flu and R6G.

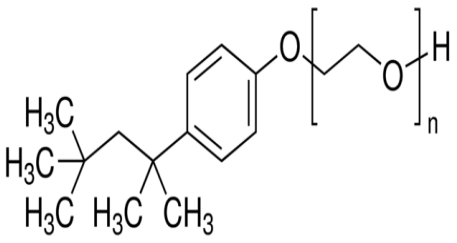


Fluorescein sodium salt



Rhodamine 6G





AOT [Aerosol-OT, sodium bis(2-ethylhexyl) sulfosuccinate] TritonX-100

Fig4: Structure of Fluorescein, Rhodamine 6G dye molecules, AOT and TritonX-100

Ref: sigmaaldrich.com

PROCEDURE

For this particular FRET pair, Flu acts as donor and R6G as acceptor, also electrostatic force of attraction plays an important role in bringing them closer for an efficient energy transfer.

Molecular weight of Fluorescein sodium salt = 376.27 g/mol

Molecular weight of Rhodamine6G =479.01 g/mol

For maintaining pH=7.0, we dissolved one buffer tablet in 100ml ultra pure water and this was used as solvent for dissolving dye molecules. We prepared 50ml solution of each dye. Prepared the same concentration of solutions for pH=9.2 also.

6mg of Flu in 50ml water gives a concentration of

$$M = \frac{6\times10^{-3}}{376.27\times50} \times 1000$$

$$= 3.189\times10^{-4}\text{M}$$

Then prepared 2×10^{-4}M, 10^{-4}M, 5×10^{-5}M, 2×10^{-5}M, 10^{-5}M, 5×10^{-6}M, 2×10^{-6}M, 10^{-6}M, 5×10^{-7}M, 2×10^{-7}M and 10^{-7}M solutions for Flu by using dilution formula $M_1V_1=M_2V_2$

For preparing 2×10^{-4}M from 3.1895×10^{-4}M,

$$3.189\times10^{-4}\text{M}\times V=2\times10^{-4}\text{M}\times50$$

$$=31.358\text{ml}$$

For preparing 10^{-4}M from 2×10^{-4}M,

$$2\times10^{-4}\text{M}\times V=10^{-4}\text{M}\times50$$

$$=25\text{ml}$$

For preparing 5×10^{-5}M from 10^{-4}M,

$$10^{-4}\text{M}\times V=5\times10^{-5}\text{M}\times50$$

$$=25\text{ml}$$

For preparing 2×10^{-5}M from 10^{-4}M,

$$10^{-4}M\times V=2\times10^{-5}M\times50$$

$$=10ml$$

For preparing 10^{-5}M from 10^{-4}M,

$$10^{-4}M\times V=10^{-5}M\times50$$

$$=5ml=5000\mu l$$

For preparing 5×10^{-6}M from 10^{-4}M,

$$10^{-4}M\times V=5\times10^{-6}\times50$$

$$=2.5ml=2500\mu l$$

2×10^{-6}M from 10^{-5}M,

$$10^{-5}M\times V=2\times10^{-6}M\times50$$

$$=1ml=1000\mu l$$

10^{-6}M from 10^{-4}M,

$$10^{-4}M\times V=10^{-6}M\times50$$

$$=0.5ml=500\mu l$$

5×10^{-7}M from 2×10^{-5}M,

$$2\times10^{-5}M\times V=5\times10^{-7}M\times50$$

$$=1.25ml=1250\mu l$$

2×10^{-7}M from 2×10^{-6}M,

$$2\times10^{-6}M\times V=2\times10^{-7}M\times50$$

$$=5ml=5000\mu l$$

10^{-7}M from 2×10^{-6}M,

$$2\times10^{-6}M\times V=10^{-7}M\times50$$

=2.5ml=2500µl

8mg of R6G in 50ml water gives a concentration of

$$M = \frac{8 \times 10^{-3}}{479.01 \times 50} \times 1000$$

$= 3.34\times10^{-4}$M

For preparing 2×10^{-4}M from 3.34×10^{-4}M,

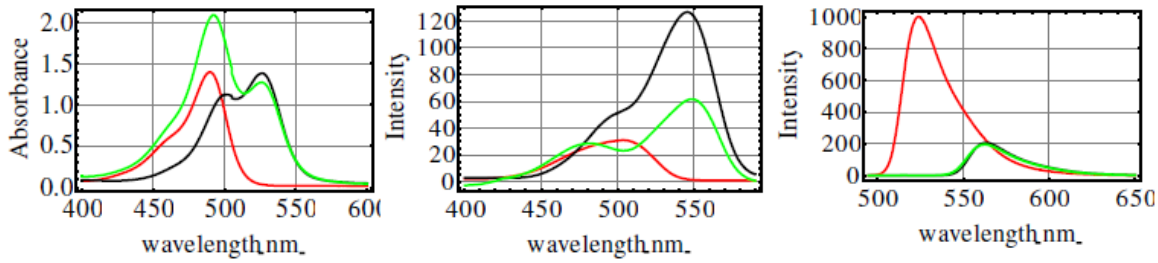
$3.34\times10^{-4}\text{M}\times V = 2\times10^{-4}\text{M}\times50$

=29.941ml

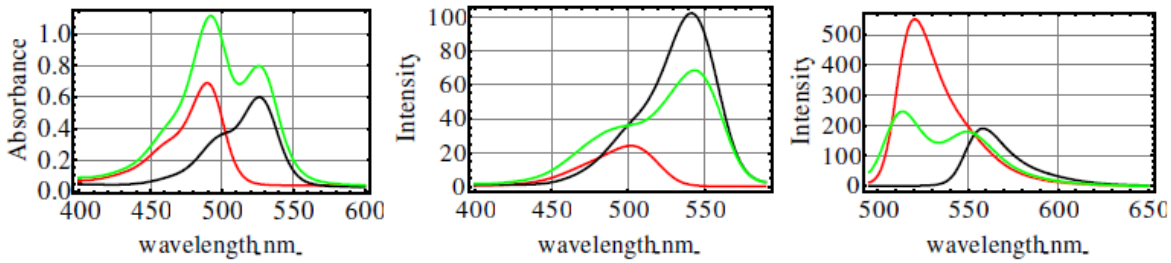
Prepared 10^{-4}M, 5×10^{-5}M, 2×10^{-5}M, 10^{-5}M, 5×10^{-6}M, 2×10^{-6}M, 10^{-6}M and 5×10^{-7} M solutions for R6G as calculated above. Solutions of mix are prepared by mixing double concentrated solutions of each component i.e, 2×10^{-4}M, 2×10^{-5}M, 2×10^{-6}M, which gives a concentration of 10^{-4}M, 10^{-5}M and 10^{-6}M. Similarly mixing 10^{-4}M of Flu and R6G gives a solution of 5×10^{-5}M concentration.

UV-VIS absorption spectra, Fluorescence excitation spectra and Fluorescence emission spectra of pH =7.0 for different concentrations are given below.

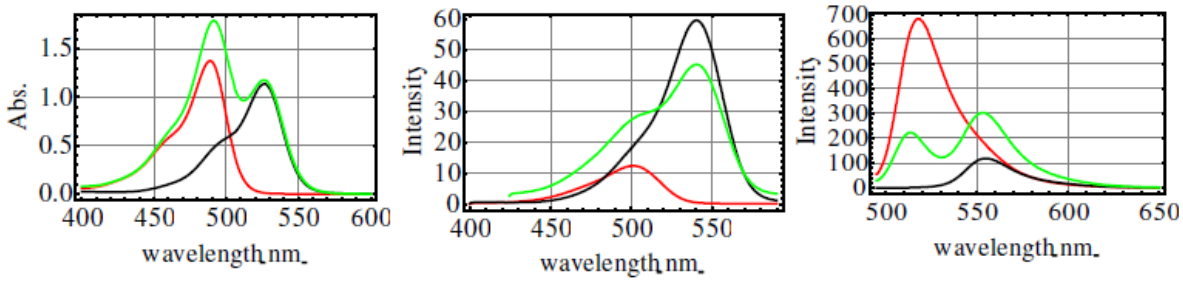
10^{-4}M



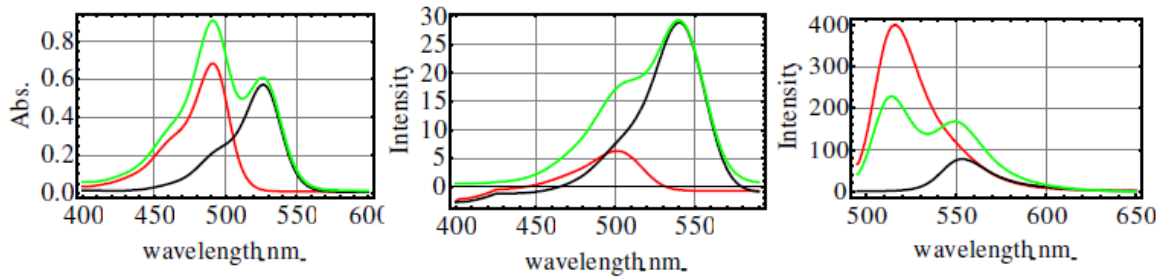
$5\text{x}10^{-5}$M



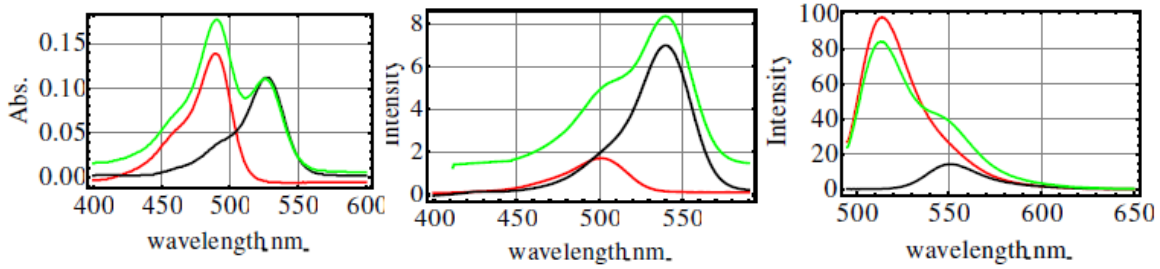
10^{-5}M



$5x10^{-6}$M



10^{-6}M



$5x10^{-7}$M

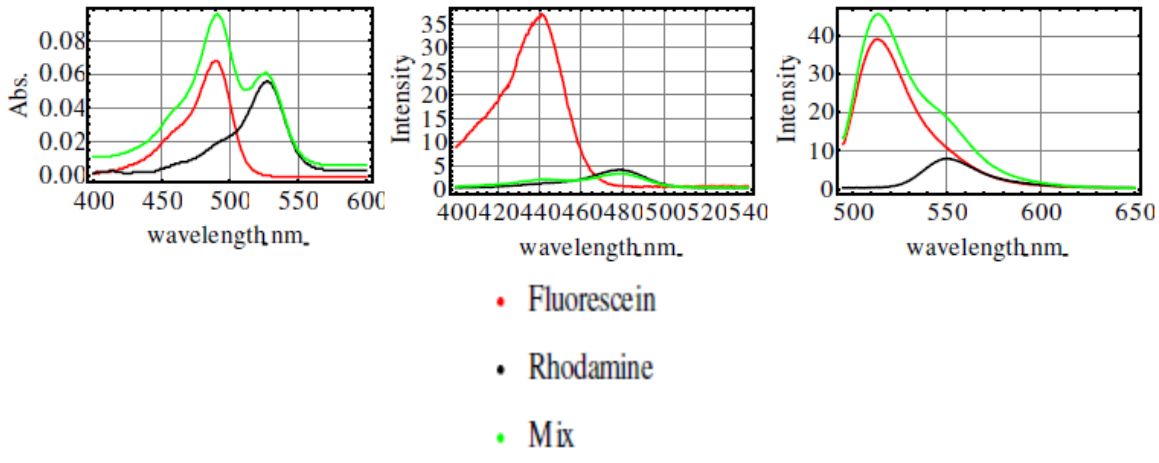


Fig5: Absorption, fluorescence excitation, fluorescence emission spectra for different concentration at pH=7

Measured the excited state lifetime of Flu and R6G in pure solutions and in mixture for different emission wavelengths.

Intensity decay follows a form as

$$I(t) = \sum_{k=1}^{n} \alpha_i \exp(-t/\tau_i)$$

α_i is the fraction of molecules in each conformation at t=0, n is the number of decay times and τ_i are the decay times.

For calculating the energy transfer efficiency with respect to donor, we measured the excited state lifetime at emission wavelength 510,515,520nm.

10^{-4}M	Flu						Mix			
	α_1	T_1(ns)	α_2	T_2(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	-0.49	4.798	0.51	4.927	4.864	0.977	1	4.258	4.258	1.055
515	-0.49	4.807	0.51	4.927	4.868	0.915	1	4.274	4.274	1.055
520	-0.49	4.8	0.51	4.914	4.858	0.978	1	4.337	4.337	1.043

$5x10^{-5}$M	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	4.975	4.975	1.019	1	4.469	4.469	1.038
515	1	4.964	4.964	0.986	1	4.572	4.572	1.009
520	1	4.964	4.964	1.021	1	4.802	4.802	1.018

10^{-5}M	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	4.328	4.328	1.089	1	4.258	4.258	1.055
515	1	4.294	4.294	1.119	1	4.274	4.274	1.054
520	1	4.259	4.259	1.009	1	4.337	4.337	1.043

$5x10^{-6}$M	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	4.133	4.133	0.92	1	4.124	4.124	0.907
515	1	4.134	4.134	0.917	1	4.142	4.142	0.864
520	1	4.129	4.129	1.011	1	4.164	4.164	1.051

10^{-6}M	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	3.97	3.97	0.962	1	3.968	3.968	0.992
515	1	3.971	3.971	0.915	1	3.962	3.962	0.992
520	1	3.977	3.977	1.006	1	3.974	3.974	0.982

$5x10^{-7}M$	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	3.951	3.951	1.027	1	3.929	3.929	1.107
515	1	3.941	3.941	0.858	1	3.943	3.943	1.007
520	1	3.945	3.945	0.973	1	3.941	3.941	1.113

Table1: Measured lifetime of Flu and Mix. at 510,515,520nm

For calculating energy transfer efficiency w.r.t. acceptor, measured the lifetime of R6G at 590,595,600,610,620nm.

$10^{-4}M$	R6G				Mix					
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	α_2	T_2	T(ns)	X^2
590	1	4.957	4.957	1.006	-0.48	4.146	0.52	4.266	4.208	1.062
595	1	4.95	4.95	1.061	-0.49	4.16	0.51	4.269	4.216	1.044
600	1	4.935	4.935	1.062	-0.49	4.155	0.51	4.235	4.196	1.011
610	1	4.941	4.941	1.045	-0.48	4.132	0.52	4.26	4.198	1.064
620	1	4.826	4.826	0.896	-0.49	4.169	0.51	4.266	4.219	1.081

$5x10^{-5}M$	R6G				Mix					
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	α_2	T_2(ns)	T(ns)	X^2
590	1	4.485	4.485	1.061	-0.5	4.483	0.5	4.52	4.502	1.079
595	1	4.497	4.497	1.065	-0.5	4.5	0.5	4.551	4.526	1.108
600	1	4.473	4.473	1.042	-0.49	4.316	0.51	4.572	4.447	0.987
610	1	4.471	4.471	1.051	-0.5	4.408	0.5	4.493	4.451	0.976
620	1	4.473	4.473	1.089	-0.48	4.255	0.52	4.646	4.459	1.08

$10^{-5}M$	R6G				Mix					
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	α_2	T_2(ns)	T(ns)	X^2
590	1	4.147	4.147	1	-0.48	4.146	0.52	4.266	4.208	1.062
595	1	4.167	4.167	1.049	-0.49	4.16	0.51	4.269	4.216	1.044
600	1	4.139	4.139	1.054	-0.49	4.155	0.51	4.235	4.196	1.01
610	1	4.143	4.143	1.023	-0.48	4.132	0.52	4.26	4.198	1.064
620	1	4.134	4.134	1.086	-0.49	4.169	0.51	4.267	4.219	1.081

$5x10^{-6}M$	R6G				Mix					
	α_1	T_1	T	X^2	α_1	T_1	α_2	T_2	T	X^2
590	1	4.063	4.063	1.029	-0.44	4.007	0.56	4.242	4.139	1.039
595	1	4.062	4.062	1.076	-0.44	4.037	0.56	4.259	4.162	1.043
600	1	4.056	4.056	1.081	-0.44	4.025	0.56	4.25	4.152	1.026
610	1	4.062	4.062	1.067	-0.43	4.002	0.57	4.237	4.135	1.03
620	1	4.055	4.055	1.058	-0.47	4.074	0.53	4.189	4.135	1.092

10^{-6}M	R6G				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
590	1	3.917	3.917	1.082	1	4.106	4.11	1.056
595	1	3.911	3.911	1.115	1	4.105	4.11	1.073
600	1	3.907	3.907	1.108	1	4.105	4.11	1.092
610	1	3.85	3.85	0.968	1	4.108	4.11	1.108
620	1	3.852	3.852	0.987	1	4.072	4.07	0.828

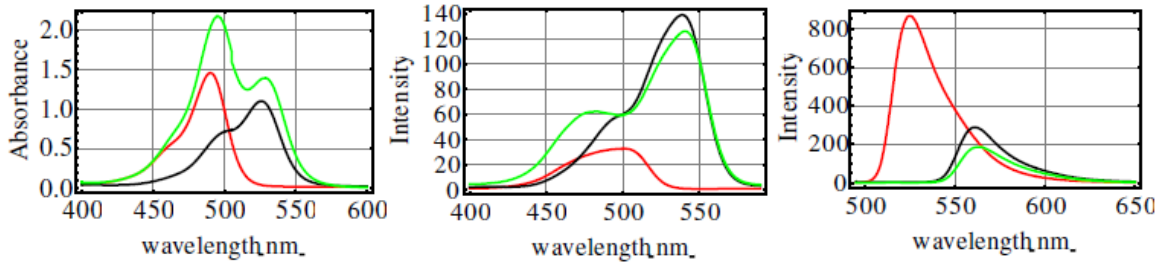
$5x10^{-7}$M	R6G				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
590	1	3.874	3.874	1.062	1	3.955	3.955	0.906
595	1	3.882	3.882	0.999	1	3.96	3.96	0.916
600	1	3.884	3.884	1.082	1	3.956	3.956	0.789
610	1	3.886	3.886	1.012	1	3.956	3.956	0.864
620	1	3.839	3.839	0.975	1	4.089	4.089	0.917

Table2: lifetime measured for R6G and mix at 590,595,600,610,620nm

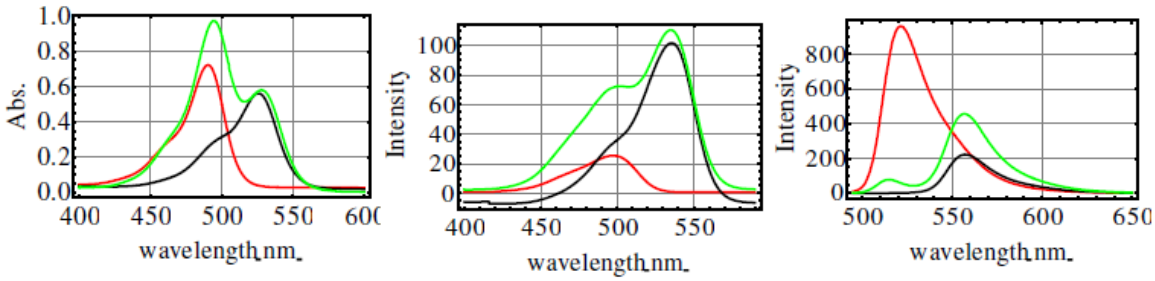
pH = 9.2

UV-VIS absorption spectra, Fluorescence excitation spectra and Fluorescence emission spectra of pH =9.2 for different concentrations are given below.

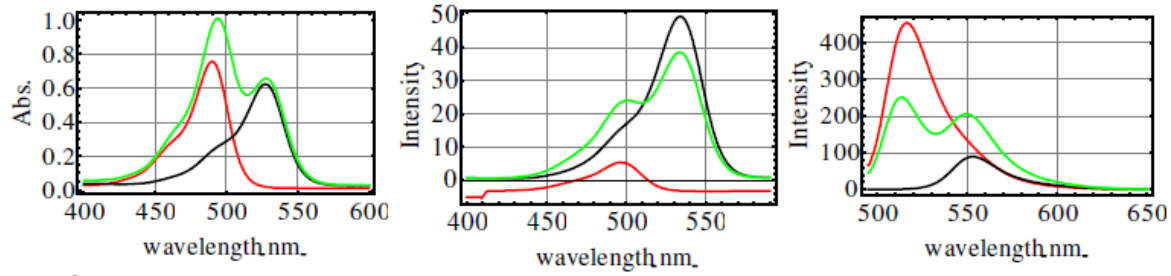
10^{-4}M



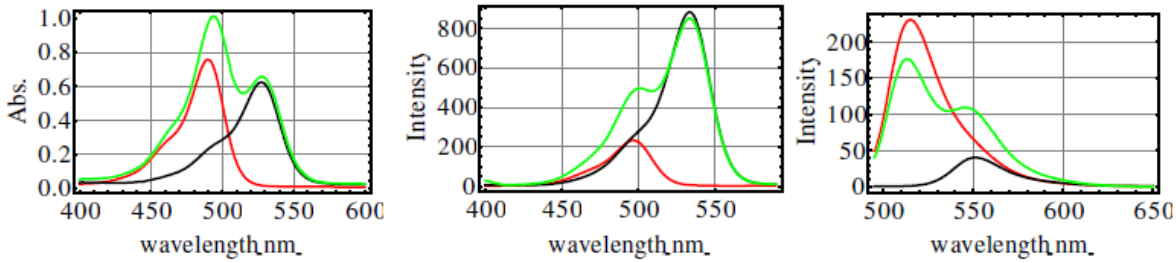
$5x10^{-5}$M



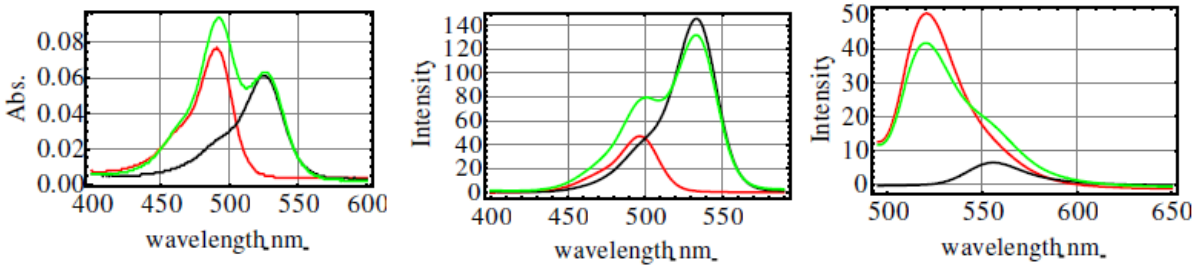
10^{-5}M



$5x10^{-6}$M



10^{-6}M



$5x10^{-7}$M

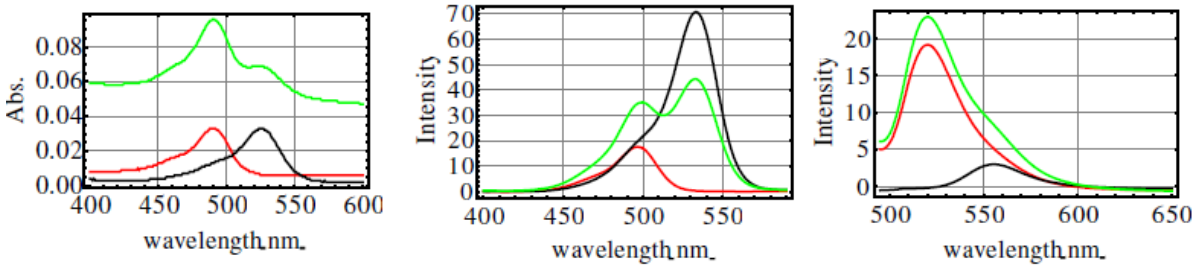


Fig6: Absorption, fluorescence excitation, fluorescence emission spectra for different concentration

Lifetime measured for Flu and Mix at 510, 515, 520nm

10^{-4}M	**Flu**						**Mix**					
	α_1	T_1(ns)	α_2	T_2(ns)	T(ns)	X^2	α_1	T_1(ns)	α_2	T_2(ns)	T(ns)	X^2
510	-0.49	4.919	0.51	4.997	4.959	0.905	-0.13	1.596	0.87	5.145	4.695	1.115
515	-0.5	4.949	0.5	4.986	4.968	0.915	-0.18	2.421	0.82	5.353	4.827	1.065
520	-0.49	4.916	0.5	5.013	4.966	0.956	-0.35	3.82	0.65	5.424	4.859	1.081

$5x10^{-5}M$	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	5.072	5.072	0.989	1	4.695	4.695	1.09
515	1	5.067	5.067	0.977	1	4.774	4.774	1.087
520	1	5.066	5.066	0.979	1	4.932	4.932	1.114

$10^{-5}M$	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	4.164	4.164	1.057	1	4.049	4.049	0.934
515	1	4.165	4.165	0.966	1	4.071	4.071	0.891
520	1	4.161	4.161	0.949	1	4.094	4.094	0.887

$5x10^{-6}M$	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	4.061	4.061	0.839	1	4.043	4.043	0.856
515	1	4.068	4.068	0.881	1	4.056	4.056	0.772
520	1	4.081	4.081	0.799	1	4.066	4.066	0.83

$10^{-6}M$	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	3.971	3.971	0.894	1	3.975	3.975	0.828
515	1	3.97	3.97	0.891	1	3.974	3.974	0.888
520	1	3.966	3.966	0.873	1	3.976	3.976	0.881

$5x10^{-7}M$	Flu				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
510	1	3.956	3.956	0.981	1	3.973	3.973	0.938
515	1	3.956	3.956	1.041	1	3.972	3.972	0.995
520	1	3.963	3.963	1.115	1	3.959	3.959	0.995

Table3: lifetime measured for Flu and mix. at 510,515,520nm

Lifetime measured w.r.t acceptor

$10^{-4}M$	R6G				Mix					
	α_1	T_1	T	X^2	α_1	T_1	α_2	T_2	T	X^2
590	1	4.765	4.765	1.075	-0.5	4.956	0.5	4.959	4.957	1.102
595	1	4.75	4.75	1.045	-0.5	4.964	0.5	4.968	4.966	1.095
600	1	4.739	4.739	1.024	-0.5	4.967	0.5	4.972	4.969	1.068
610	1	4.739	4.739	1.064	-0.5	4.974	0.5	4.983	4.978	1.027
620	1	4.729	4.729	1.066	-0.5	4.973	0.5	4.976	4.975	1.092

$5x10^{-5}M$	R6G				Mix					
	α_1	T_1	T	X^2	α_1	T_1	α_2	T_2	T	X^2
590	1	4.419	4.419	1.05	0.5	4.517	-0.5	4.449	4.484	0.9
595	1	4.421	4.421	1.047	-0.49	4.408	0.51	4.549	4.479	0.894
600	1	4.416	4.416	0.998	-0.49	4.395	0.51	4.554	4.476	0.933
610	1	4.411	4.411	1.091	-0.46	3.971	0.54	4.943	4.501	0.953
620	1	4.414	4.414	1.002	-0.47	4.134	0.53	4.813	4.495	1.061

$10^{-5}M$	R6G				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
590	1	4.146	4.146	1.038	1	4.762	4.762	0.964
595	1	4.143	4.143	1.053	1	4.775	4.775	1.097
600	1	4.138	4.138	1.057	1	4.777	4.777	0.963
610	1	4.144	4.144	1.061	1	4.814	4.814	1.059
620	1	4.124	4.124	1.075	1	4.772	4.772	1.098

$5x10^{-6}M$	R6G				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
590	1	3.991	3.991	0.891	1	4.491	4.491	1.087
595	1	3.991	3.991	0.859	1	4.491	4.491	1.072
600	1	3.996	3.996	0.887	1	4.484	4.484	1.118
610	1	4.003	4.003	0.892	1	4.379	4.379	1.084
620	1	3.997	3.997	0.872	1	4.406	4.406	0.907

$10^{-6}M$	R6G				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
590	1	3.935	3.935	0.919	1	4.061	4.061	0.799
595	1	3.937	3.937	0.861	1	4.069	4.069	0.893
600	1	3.935	3.935	0.946	1	4.063	4.063	0.758
610	1	3.933	3.933	0.844	1	4.064	4.064	0.806
620	1	3.935	3.935	1.083	1	4.067	4.067	0.877

$5x10^{-7}M$	R6G				Mix			
	α_1	T_1(ns)	T(ns)	X^2	α_1	T_1(ns)	T(ns)	X^2
590	1	3.923	3.923	0.885	1	3.979	3.979	0.862
595	1	3.926	3.926	0.925	1	3.974	3.974	0.936
600	1	3.928	3.928	0.924	1	3.979	3.979	0.806
610	1	3.923	3.923	1.086	1	3.969	3.969	0.934
620	1	3.918	3.918	1.018	1	3.975	3.975	1.035

Table4: lifetime measured for different concentrations of R6G and mix at 590, 595, 600, 610,620nm

Reverse micelle as a confinement:

We prepared reverse micelle of w=3 with surfactant AOT in n-hexane. The solution prepared is 50ml with a concentration of 0.1M

Molecular weight of AOT = 444.56g/mol

Mass of AOT taken = 2.2228g

Molarity of 1000g of water in 1000ml is 55.56M

w=1, 55.56MxV= 50mlx0.1M

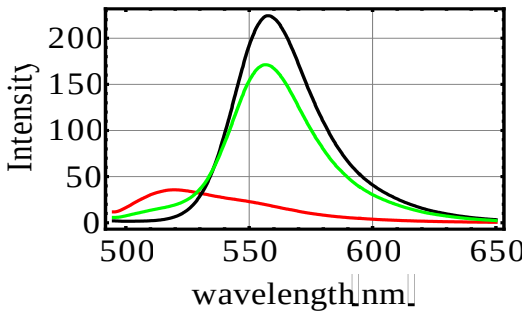
V= 90.9µl

w=2, volume of aqueous solution taken V=181.8µl

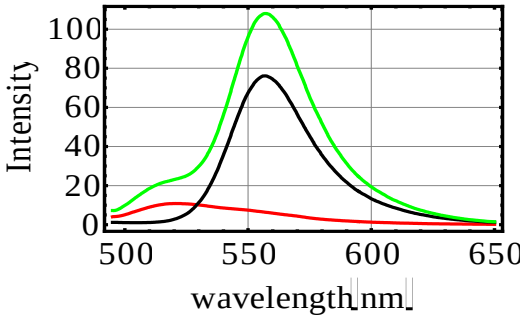
For preparing w=3, volume V=272.7µl

Fluorescence emission spectra for pH=7

10^{-4}M

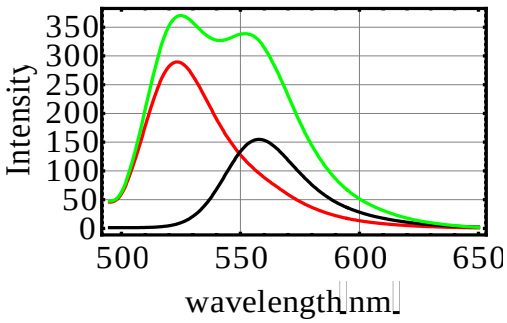


$5x10^{-5}$M



Fluorescence emission spectra for pH= 9.2

10^{-4}M



$5x10^{-5}$M

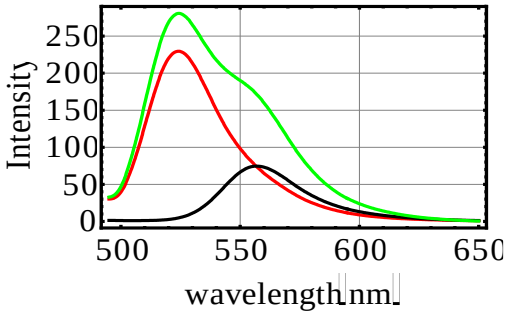


Fig7: Fluorescence emission spectra with reverse micelle (AOT) at pH=7 and 9.2

Prepared reverse micelle with TritonX-100, a size of w=3

Density of TritonX-100 =1.07g/ml

Molecular weight =625g

Molarity of the given surfactant= $\frac{1.07\times1000}{625}$

=1.712M

For preparing 0.2M, 25ml of surfactant solution in cyclohexane, volume of surfactant needed

1.712M×V=0.2×25ml =2.92ml

For preparing reverse micelle of size w=1, volume solution needed V is,

0.2M x 25ml = 55.5M x V

V= 0.2ml x 25ml

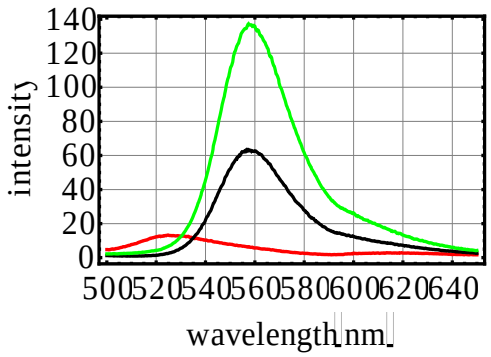
=90µl

For w=3, Volume of solution needed V=270 µl

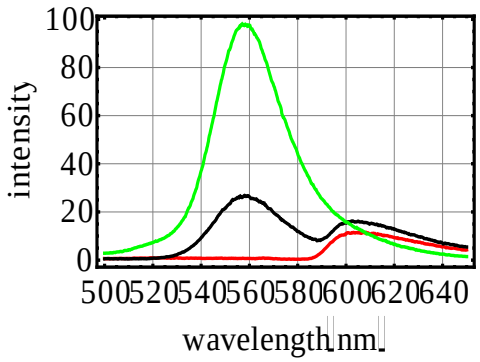
Fluorescence emission spectra:

pH=7

10^{-4}M



$5x10^{-5}$M



pH = 9.2

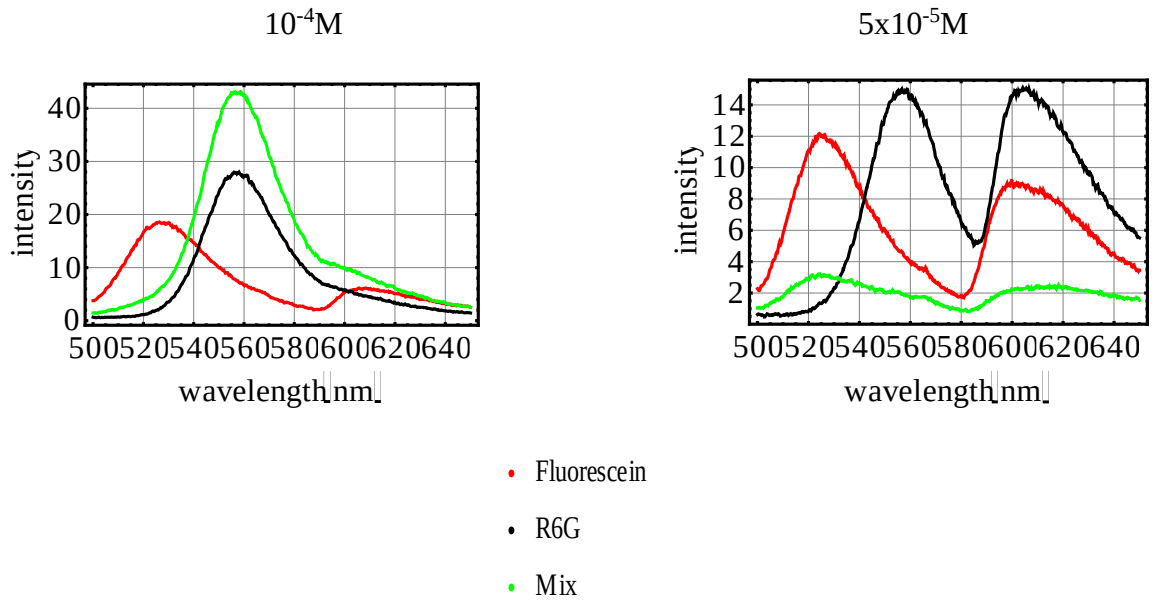


Fig8: Fluorescence emission spectra with reverse micelle (TritonX-100) at pH=7 and 9.2

Chapter2. RESULTS AND CONCLUSION

For **pH=7**

Energy transfer efficiency calculated by using equation (6) from steady state fluorescence emission spectrum by using the intensity in donor channel that is 510, 515,520 nm for different concentrations are given in table.

510 nm			
Conc.	Intensity (Fluorescein)	Intensity (Mix)	Efficiency
10^{-4}M	213.578	1.606	0.992
$5x10^{-5}$M	305.182	232.063	0.239
10^{-5}M	518.703	205.186	0.604
$5x10^{-6}$M	351.5	212.077	0.396
10^{-6}M	93.539	81.056	0.133
$5x10^{-7}$M	37.81	47.954	_

515 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	587.575	2.448	0.995
$5x10^{-5}$M	481.302	245.167	0.490
10^{-5}M	657.657	220.061	0.665
$5x10^{-6}$M	398.88	228.002	0.428
10^{-6}M	97.624	83.729	0.142
$5x10^{-7}$M	38.775	49.425	_

520 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	918.885	2.039	0.997
$5x10^{-5}$M	551.016	214.668	0.610
10^{-5}M	671.344	184.26	0.725
$5x10^{-6}$M	384.035	203.73	0.469
10^{-6}M	89.735	75.945	0.153
$5x10^{-7}$M	35.617	45.108	_

Table5: shows fluorescence intensity and energy transfer efficiency at 510,515,520nm

Energy transfer efficiency calculated by using equation (7) from steady state fluorescence emission spectrum by using the intensity in acceptor channel that is 590, 595, 600, 610, 620nm for different concentrations are given in table.

590 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	47.351	75.49	0.904
$5x10^{-5}$M	23.068	29.261	0.559
10^{-5}M	24.148	59.082	0.709
$5x10^{-6}$M	13.331	26.552	0.665
10^{-6}M	3.232	5.814	0.643
$5x10^{-7}$M	1.458	2.723	0.651

595 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	36.128	60.862	0.627
$5x10^{-5}$M	17.703	22.744	0.562
10^{-5}M	18.526	46.273	0.714
$5x10^{-6}$M	10.184	20.406	0.667
10^{-6}M	2.523	4.591	0.645
$5x10^{-7}$M	1.178	2.115	0.642

600 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	27.739	48.786	0.637
$5x10^{-5}$M	13.675	17.672	0.564
10^{-5}M	14.231	36.569	0.719
$5x10^{-6}$M	7.867	15.646	0.665
10^{-6}M	2.033	3.631	0.641
$5x10^{-7}$M	0.967	1.704	0.637

610 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	16.335	29.936	0.647
$5x10^{-5}$M	8.129	10.39	0.561
10^{-5}M	8.448	21.99	0.722
$5x10^{-6}$M	4.711	8.644	0.647
10^{-6}M	1.228	2.291	0.651
$5x10^{-7}$M	0.648	1.102	0.629

620 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	9.328	17.03	0.646
$5x10^{-5}$M	4.789	5.706	0.544
10^{-5}M	4.819	12.561	0.723
$5x10^{-6}$M	2.768	4.16	0.6004
10^{-6}M	0.794	1.469	0.649
$5x10^{-7}$M	0.521	0.644	0.553

Table6: shows fluorescence intensity and energy transfer efficiency at 590, 595, 600, 610,620 nm

Calculated the energy transfer efficiency from the lifetime of the excited state of donor by using equation (8)

510 nm			
Conc.	Fluorescein (ns)	Mix (ns)	Efficiency
10^{-4}M	4.864	4.258	0.124
$5x10^{-5}$M	4.975	4.469	0.102
10^{-5}M	4.328	4.258	0.0162
$5x10^{-6}$M	4.133	4.124	0.0022
10^{-6}M	3.97	3.968	0.0005
$5x10^{-7}$M	3.951	3.929	0.0056

515 nm			
Conc.	Fluorescein (ns)	Mix (ns)	Efficiency
10^{-4}M	4.868	4.274	0.122
$5x10^{-5}$M	4.964	4.572	0.078
10^{-5}M	4.294	4.274	0.0046
$5x10^{-6}$M	4.134	4.142	_
10^{-6}M	3.971	3.962	0.002
$5x10^{-7}$M	3.941	3.943	_

520 nm			
Conc.	Fluorescein (ns)	Mix (ns)	Efficiency
10^{-4}M	4.858	4.337	0.107
$5x10^{-5}$M	4.964	4.802	0.033
10^{-5}M	4.259	4.337	_
$5x10^{-6}$M	4.129	4.164	_
10^{-6}M	3.977	3.974	0.0007
$5x10^{-7}$M	3.945	3.941	0.0010

Table7: calculated energy transfer efficiency from lifetime for different concentrations

Energy transfer efficiency from the lifetime measurement of acceptor.

590 nm			
Conc.	Rhodamine6G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.957	4.208	
$5x10^{-5}$M	4.485	4.502	0.0037
10^{-5}M	4.147	4.208	0.014
$5x10^{-6}$M	4.063	4.139	0.018
10^{-6}M	3.917	4.106	0.046
$5x10^{-7}$M	3.874	3.955	0.0205

595 nm			
Conc.	Rhodamine6 G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.95	4.216	
$5x10^{-5}$M	4.497	4.526	0.0064
10^{-5}M	4.167	4.216	0.0116
$5x10^{-6}$M	4.062	4.162	0.024
10^{-6}M	3.911	4.105	0.047
$5x10^{-7}$M	3.882	3.96	0.019

600 nm			
Conc.	Rhodamine6 G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.935	4.196	
$5x10^{-5}$M	4.473	4.447	
10^{-5}M	4.139	4.196	0.013
$5x10^{-6}$M	4.056	4.152	0.023
10^{-6}M	3.907	4.105	0.048
$5x10^{-7}$M	3.884	3.956	0.018

610 nm			
Conc.	Rhodamine6 G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.941	4.198	
$5x10^{-5}$M	4.471	4.451	
10^{-5}M	4.143	4.198	0.013
$5x10^{-6}$M	4.062	4.135	0.017
10^{-6}M	3.85	4.108	0.062
$5x10^{-7}$M	3.886	3.956	0.017

620 nm			
Conc.	Rhodamine6 G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.826	4.219	
$5x10^{-5}$M	4.473	4.459	
10^{-5}M	4.134	4.219	0.020
$5x10^{-6}$M	4.055	4.135	0.019
10^{-6}M	3.852	4.072	0.054
$5x10^{-7}$M	3.839	4.089	0.061

Table8: energy transfer efficiency calculated for different concentrations at 590, 595, 600, 610, 620nm

For **pH=9.2**

Energy transfer efficiency calculated from steady state fluorescence emission spectrum by looking the donor channel that is 510, 515,520 nm for different concentrations are given in table.

510 nm			
Conc.	Intensity (Fluorescein)	Intensity (Mix)	Efficiency
10^{-4}M	152.041	0.642	0.995
$5x10^{-5}$M	435.935	60.258	0.862
10^{-5}M	388.58	236.256	0.392
$5x10^{-6}$M	211.793	168.732	0.203
10^{-6}M	35.923	30.758	0.144
$5x10^{-7}$M	14.042	16.808	_

515 nm			
Conc.	Intensity (Fluorescein)	Intensity (Mix)	Efficiency
10^{-4}M	460.286	1.034	0.997
$5x10^{-5}$M	776.939	78.087	0.899
10^{-5}M	449.697	248.775	0.446
$5x10^{-6}$M	230.634	175.129	0.241
10^{-6}M	46.2	38.799	0.160
$5x10^{-7}$M	17.803	21.307	_

520 nm			
Conc.	Intensity (Fluorescein)	Intensity (Mix)	Efficiency
10^{-4}M	770.879	0.857	0.998
$5x10^{-5}$M	953.843	64.45	0.932
10^{-5}M	438.362	217.609	0.503
$5x10^{-6}$M	217.456	156.224	0.281
10^{-6}M	50.423	41.734	0.172
$5x10^{-7}$M	19.195	22.969	_

Table9: intensity of fluorescein, mix and energy transfer efficiency at 510,515,520nm

Energy transfer efficiency calculated from steady state fluorescence intensity w.r.t. acceptor for different concentrations at 590, 595, 600, 610, 620nm are given below

590 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	43.9	73.53	0.626
$5x10^{-5}$M	39.446	109.443	0.735
10^{-5}M	15.003	33.811	0.693
$5x10^{-6}$M	7.464	15.106	0.669
10^{-6}M	1.026	2.644	0.720
$5x10^{-7}$M	0.326	0.929	0.740

595 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	33.488	59.698	0.641
$5x10^{-5}$M	29.993	86.457	0.742
10^{-5}M	11.374	26.441	0.699
$5x10^{-6}$M	5.708	11.561	0.669
10^{-6}M	0.432	1.84	0.809
$5x10^{-7}$M	0.101	0.553	0.845

600 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	25.763	47.834	0.649
$5x10^{-5}$M	23.001	67.976	0.747
10^{-5}M	8.715	20.661	0.703
$5x10^{-6}$M	4.445	8.831	0.665
10^{-6}M	0.667	1.243	0.651
$5x10^{-7}$M	0.223	0.272	0.549

610 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	15.248	29.321	0.658
$5x10^{-5}$M	13.424	40.864	0.753
10^{-5}M	5.097	12.432	0.709
$5x10^{-6}$M	2.599	4.884	0.653
10^{-6}M	0.232	0.421	0.647
$5x10^{-7}$M	0.211	0.320	0.602

620 nm			
Conc.	Intensity (Flu)	Intensity (Mix)	Efficiency
10^{-4}M	8.765	16.674	0.655
$5x10^{-5}$M	7.63	22.988	0.751
10^{-5}M	2.954	6.982	0.703
$5x10^{-6}$M	1.522	2.328	0.605
10^{-6}M	0.220	0.301	0.577
$5x10^{-7}$M	0.125	0.234	0.651

Table10: intensity of R6G, mix and energy transfer efficiency for different concentrations at 590,595,600,610,620nm

Calculated the energy transfer efficiency from the lifetime of the excited state of donor by using equation (3)

510 nm			
Conc.	Fluorescein (ns)	Mix (ns)	Efficiency
10^{-4}M	4.959	4.695	0.0532
$5x10^{-5}$M	5.072	4.695	0.0743
10^{-5}M	4.164	4.049	0.0276
$5x10^{-6}$M	4.061	4.043	0.0044
10^{-6}M	3.971	3.975	
$5x10^{-7}$M	3.956	3.973	

515 nm			
Conc.	Fluorescein (ns)	Mix (ns)	Efficiency
10^{-4}M	4.968	4.827	0.0283
$5x10^{-5}$M	5.067	4.774	0.0578
10^{-5}M	4.165	4.071	0.0225
$5x10^{-6}$M	4.068	4.056	0.0029
10^{-6}M	3.97	3.974	
$5x10^{-7}$M	3.956	3.972	

520 nm			
Conc.	Fluorescein (ns)	Mix (ns)	Efficiency
10^{-4}M	4.966	4.859	0.0215
$5x10^{-5}$M	5.066	4.932	0.0264
10^{-5}M	4.161	4.094	0.0161
$5x10^{-6}$M	4.081	4.066	0.0036
10^{-6}M	3.966	3.976	
$5x10^{-7}$M	3.963	3.959	

Table11: energy transfer efficiency calculated for different concentrations at 510,515,520nm

Energy Transfer efficiency calculated for samples w.r.t. acceptor

590 nm			
Conc.	Rhodamine 6G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.765	4.957	0.038
$5x10^{-5}$M	4.419	4.484	0.014
10^{-5}M	4.146	4.762	0.129
$5x10^{-6}$M	3.991	4.491	0.111
10^{-6}M	3.935	4.061	0.031
$5x10^{-7}$M	3.923	3.979	0.014

595 nm			
Conc.	Rhodamine 6G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.75	4.966	0.043
$5x10^{-5}$M	4.421	4.479	0.012
10^{-5}M	4.143	4.775	0.132
$5x10^{-6}$M	3.991	4.491	0.111
10^{-6}M	3.937	4.069	0.032
$5x10^{-7}$M	3.926	3.974	

600 nm			
Conc.	Rhodamine 6G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.739	4.969	0.046
$5x10^{-5}$M	4.416	4.476	0.013
10^{-5}M	4.138	4.777	0.133
$5x10^{-6}$M	3.996	4.484	0.108
10^{-6}M	3.935	4.063	0.031
$5x10^{-7}$M	3.928	3.979	0.012

610 nm			
Conc.	Rhodamine 6G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.739	4.978	0.048
$5x10^{-5}$M	4.411	4.501	0.019
10^{-5}M	4.144	4.814	0.139
$5x10^{-6}$M	4.003	4.379	0.085
10^{-6}M	3.933	4.064	0.032
$5x10^{-7}$M	3.923	3.969	0.011

620 nm			
Conc.	Rhodamine 6G (ns)	Mix (ns)	Efficiency
10^{-4}M	4.729	4.975	0.049
$5x10^{-5}$M	4.414	4.495	0.018
10^{-5}M	4.124	4.772	0.135
$5x10^{-6}$M	3.997	4.406	0.093
10^{-6}M	3.935	4.067	0.032
$5x10^{-7}$M	3.918	3.975	0.014

Table12: energy transfer efficiency calculated for different concentrations of R6G and Mix at 590, 595, 600, 610, 620nm

We chose high concentrations of sample that is 10^{-4}M and $5x10^{-5}$M for preparing reverse micelles. These two concentrations gave high energy transfer efficiency in aqueous solution.

For reverse micelle with TritonX-100:

pH = 7: Energy transfer efficiency calculated with respect to donor

10^{-4} M			
	Intensity (Flu)	Intensity (Mix)	Efficiency
510nm	7.729	2.584	0.665
515	10.052	3.198	0.682
520	12.253	4.31	0.648

With respect to acceptor

10^{-4} M			
	Intensity (Flu)	Intensity (Mix)	Efficiency
590nm	1.782	35.595	0.952
595	1.904	29.092	0.938
600	2.585	26.079	0.909
610	2.76	18.943	0.873
620	2.733	13.29	0.829

Table13: Energy transfer efficiency w.r.t. donor and acceptor at pH=7

pH = 9.2

10^{-4} M			
	Intensity (Flu)	Intensity (Mix)	Efficiency
510nm	9.042	2.283	0.747
515	12.957	2.961	0.771
520	16.616	4.044	0.756

With respect to acceptor

10^{-4} M			
	Intensity (Flu)	Intensity (Mix)	Efficiency
590 nm	2.089	11.534	0.846
595	3.297	10.679	0.764
600	5.337	9.926	0.650
610	6.026	8.011	0.571
620	5.092	6.069	0.543

Table14: Energy transfer efficiency w.r.t. donor and acceptor at pH=9.2

Comparison between steady state and Time resolved measurements

For pH=7

With respect to the donor

	Efficiency from steady state		
Conc.	510nm	515nm	520 nm
10^{-4}M	0.992	0.995	0.997
$5x10^{-5}$M	0.239	0.49	0.61
10^{-5}M	0.604	0.665	0.725
$5x10^{-6}$M	0.396	0.428	0.469
10^{-6}M	0.133	0.142	0.153
$5x10^{-7}$M	_	_	_

	TCSPC		
Conc.	510nm	515nm	520nm
10^{-4}M	0.124	0.122	0.107
$5x10^{-5}$M	0.102	0.078	0.033
10^{-5}M	0.0162	0.0046	_
$5x10^{-6}$M	0.0022	_	0.0007
10^{-6}M	0.0005	0.002	0.001
$5x10^{-7}$M	0.0056	_	_

Table15: Energy transfer efficiency from steady state and lifetime w.r.t. donor at pH=7

With respect to acceptor

	Efficiency from steady state				
Conc.	590nm	595nm	600nm	610nm	620nm
10^{-4}M	0.904	0.627	0.637	0.647	0.646
$5x10^{-5}$M	0.559	0.562	0.564	0.561	0.544
10^{-5}M	0.709	0.714	0.719	0.722	0.723
$5x10^{-6}$M	0.665	0.667	0.665	0.647	0.6004
10^{-6}M	0.643	0.645	0.641	0.651	0.649
$5x10^{-7}$M	0.651	0.642	0.637	0.629	0.553

	TCSPC				
Conc.	590	595	600	610	620
10^{-4}M	_	_	_	_	_
$5x10^{-5}$M	0.0037	0.0064	_	_	_
10^{-5}M	0.014	0.0116	0.013	0.013	0.02
$5x10^{-6}$M	0.018	0.024	0.023	0.017	0.019
10^{-6}M	0.046	0.047	0.048	0.062	0.054
$5x10^{-7}$M	0.0205	0.019	0.018	0.017	0.061

Table16: Energy transfer efficiency from steady state and lifetime w.r.t. acceptor at pH=7

For pH=9.2

With respect to the donor

	Efficiency from steady state		
Conc.	510	515	520
10^{-4}M	0.995	0.997	0.998
$5x10^{-5}$M	0.862	0.899	0.932
10^{-5}M	0.392	0.446	0.503
$5x10^{-6}$M	0.203	0.241	0.281
10^{-6}M	0.144	0.16	0.172
$5x10^{-7}$M	_	_	_

	TCSPC		
Conc.	510	515	520
10^{-4}M	0.053	0.028	0.021
$5x10^{-5}$M	0.074	0.057	0.026
10^{-5}M	0.027	0.022	0.016
$5x10^{-6}$M	0.004	0.003	0.003
10^{-6}M	_	_	_
$5x10^{-7}$M	_	_	_

Table17: Energy transfer efficiency from steady state and lifetime w.r.t. donor at pH=9.2

With respect to acceptor

	Efficiency from steady state				
Conc.	590nm	595nm	600nm	610 nm	620nm
10^{-4}M	0.626	0.641	0.649	0.658	0.655
$5x10^{-5}$M	0.735	0.742	0.747	0.753	0.751
10^{-5}M	0.693	0.699	0.703	0.709	0.703
$5x10^{-6}$M	0.669	0.669	0.665	0.653	0.605
10^{-6}M	0.72	0.809	0.651	0.647	0.577
$5x10^{-7}$M	0.74	0.845	0.549	0.602	0.651

	TCSPC				
Conc.	590nm	595nm	600nm	610nm	620nm
10^{-4}M	0.038	0.043	0.046	0.048	0.049
$5x10^{-5}$M	0.014	0.012	0.013	0.019	0.018
10-5M	0.129	0.132	0.133	0.139	0.135
$5x10^{-6}$M	0.111	0.111	0.108	0.085	0.093
10^{-6}M	0.031	0.032	0.031	0.032	0.032
$5x10^{-7}$M	0.014	_	0.012	0.011	0.014

Table18: Energy transfer efficiency from steady state and lifetime w.r.t. acceptor at pH=9.2

For reverse micelle with TritonX-100,

wavelength	pH=7	pH=9.2
510 nm	0.665	0.747
515	0.682	0.771
520	0.648	0.756
590	0.952	0.846
595	0.938	0.764
600	0.909	0.65
610	0.873	0.571
620	0.829	0.543

Table19: efficiency at different wavelength for pH 7 and 9.2

CONCLUSION: Variation in pH from 7 to 9.2 is not affecting the energy transfer efficiency when we compare the steady state calculation. For the aqueous solution, steady state data are showing that high concentration is giving high energy transfer efficiency like 10^{-4}M and $5x10^{-5}$M are giving more than 90% energy transfer efficiency for both pH 7 and 9.2 w.r.t. donor. With respect to acceptor, energy transfer efficiency for these two concentrations is more than 50%. Steady state energy transfer efficiency and energy transfer efficiency calculated from the lifetime measurement is not comparable directly; there is a large difference between these values.

In case of reverse micelle, that we prepared with AOT; AOT existing as anion at pH=7 and 9.2. R6G and Flu exist as cation and anion respectively at the same pHs. Electrostatic attraction which plays an important role in case of reverse micelle prepared with AOT. We think that R6G is going to the interfacial region of the reverse micelle and giving as observed; i.e., not giving FRET or decrement in intensity of Flu channel and increment in R6G channel.

In the case of reverse micelle that we prepared with TritonX-100 for both pH 7 and 9.2, from the steady state emission spectra; there is a decrement in the intensity of Flu channel and an increment in acceptor channel for the concentration 10^{-4}M. For reverse micelle prepared with concentration $5x10^{-5}$M, it was not giving FRET for both pHs. Presence of a second peak around 600nm is observed in the fluorescence emission spectra for $5x10^{-5}$M prepared with TritonX-100. Even though it is present in the higher concentration 10^{-4}M also, peak was not observed separately from the main peak that we got. This may be due to the dimer of each dye molecule is getting trapped in the reverse micelle and causing a shift in the dimer peak.

Near future we are planning to try some other neutral surfactant IGEPAL instead of TritonX-100. Use of some co-solvent like long chain alcohols with the hydrophobic phase cyclohexane in the preparation of reverse micelle has to be looked. It is also interesting to study the energy transfer efficiency with respect to size variation of reverse micelle.

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