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Investigation of Fluorescence Resonance Energy Transfer between Fluorescein and Rhodamine 6G

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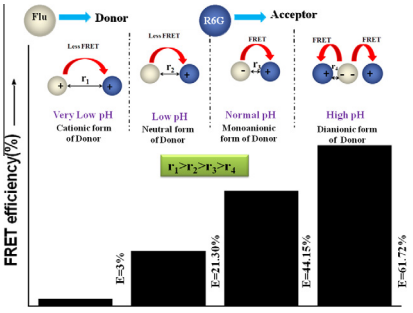
HIGHLIGHTS

- Energy transfer occurred between Fluorescein and Rhodamine 6G.
- Incorporation of nanoclay laponite enhances the energy transfer efficiency.
- Energy transfer was pH sensitive.
- Energy transfer between these dyes can be used to design pH sensor.

GRAPHICAL ABSTRACT

Separation between the FRET pair (Flu + R6G) at different solvent pH and variation of FRET efficiency (*E*%).



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ABSTRACT

Fluorescence Resonance Energy Transfer between two organic dyes Fluorescein and Rhodamine 6G was investigated in aqueous solution in presence and absence of synthetic clay laponite. Spectroscopic studies suggest that both the dyes were present mainly as monomer in solution. Fluorescence Resonance Energy Transfer occurred from Fluorescein to Rhodamine 6G in solutions. Energy transfer efficiency increases in presence of laponite and the maximum efficiency was 72.00% in aqueous laponite dispersion. Energy transfer efficiency was found to be pH sensitive. It has been demonstrated that with proper calibration it is possible to use the present system under investigation to sense pH over a wide range from 1.5 to 8.0.

Introduction

FRET is a non-radiative electro dynamical phenomenon having wide range of applications [1,2]. Biosensors based on FRET open up a new window to low cost and user friendly treatment in medical science. These sensors are useful to study the structure, conformation, hybridization and auto sequencing of nucleic acids [3]. FRET is used to diagnose some common diseases like cancer, Alzheimer's etc based on change in pH values inside the cell [4,5]. FRET is also spreading its wings in sensing applications other than biosensors, like hard water sensor [6], ion sensor [7], several environmental sensors [8] etc.

FRET is the fundamental phenomenon between two dye molecules in which excited state energy is transferred from one molecule (donor) to another molecule (acceptor) without emission

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of a photon. The transfer of energy leads to a reduction in the donor's fluorescence intensity and excited state lifetime accompanied with an increase in the acceptor's emission intensity. The rate of energy transfer depends on a number of factors including fluorescence quantum yield of donor in absence of acceptor, refractive index of the solution, dipole angular orientation of each molecule and spectral overlap integral of the donor emission and acceptor absorption [9]. If any of these factors changes due to the presence of any external agency, energy transfer efficiency also changes. This makes FRET process a useful tool in sensor technology [6,7].

Fluorescent sensing is one of the important methods for sensing of various chemical as well as biological materials. Of late fluorescence spectroscopy has become a powerful tool for the detection of transition and heavy metal ions [10,11]. However, these sensors use only one signal (fluorescence intensity) for detection, which could be easily perturbed by the environmental and instrumental conditions [12]. FRET based ratiometric sensors can overcome or minimize these external factors as it measures the ratio between two fluorescence intensities [13,14]. In this regard FRET can be an interesting tool to design ratiometric sensors with high selectivity [13]. Another significant advantage of FRET based sensors is that it simplifies the design of the fluorophore. Therefore, it is extremely important to identify new FRET pairs, study and quantify FRET process between them.

In the present paper the results of our investigations on FRET between two dyes Fluorescein (Flu) and Rhodamine 6G (R6G) have been reported. To the best of our knowledge, FRET between Flu and R6G has never been reported earlier. Among the molecules under investigation absorption and fluorescence spectra of Flu are highly pH sensitive [15]. This may in turn affect the FRET process between Flu and other dye molecules. Therefore, it is extremely important to study the FRET between Flu and other dyes under different conditions. It has been demonstrated that our results can be used to design pH sensor.

Material and methods

Material

Both the dyes Flu and R6G were purchased from Sigma Chemical Co., USA and were used as received. Ultrapure Milli-Q water (resistivity 18.2 MΩ-cm) was used as solvent. The dyes used in our study were cationic (R6G) and anionic (Flu) in nature in ambient condition. The clay mineral used in the present work was synthetic Laponite, obtained from Laponite Inorganic, UK and used as received. The laponite dispersion was prepared by using Millipore water and stirred for 24 h with a magnetic stirrer followed by 30 min ultrasonication before use. To check the effect of laponite on the spectral characteristics, the dye solutions (Flu and R6G) were prepared in the laponite suspensions.

UV–Vis absorption and fluorescence spectra measurement

UV–Vis absorption and fluorescence spectra of various solutions were recorded by a Perkin Elmer Lambda-25 Spectrophotometer and Perkin Elmer LS-55 Fluorescence Spectrophotometer, respectively. For fluorescence measurement the excitation wavelength was monitored at 430 nm (close to the absorption maximum of Flu).

Theoretical consideration for FRET measurements

The donor–acceptor distance at which energy transfer is 50% efficient is referred to as the Förster radius (R_0). The magnitude of the R_0 is dependent on the overlap integral of the emission spectrum of donor with the absorption spectrum of acceptor and their mutual molecular orientation as expressed by the following equation [16,17].

$$R_0^6 = \left[\frac{9000\,(\ln 10)\,K^2\,\Phi_D}{128\,\pi^5\,Nn^4}\right]\int_0^{\alpha} F_D\,(\lambda)\,\eta\,\varepsilon_A\,(\lambda)\,\lambda^4\,d\lambda \qquad (1)$$

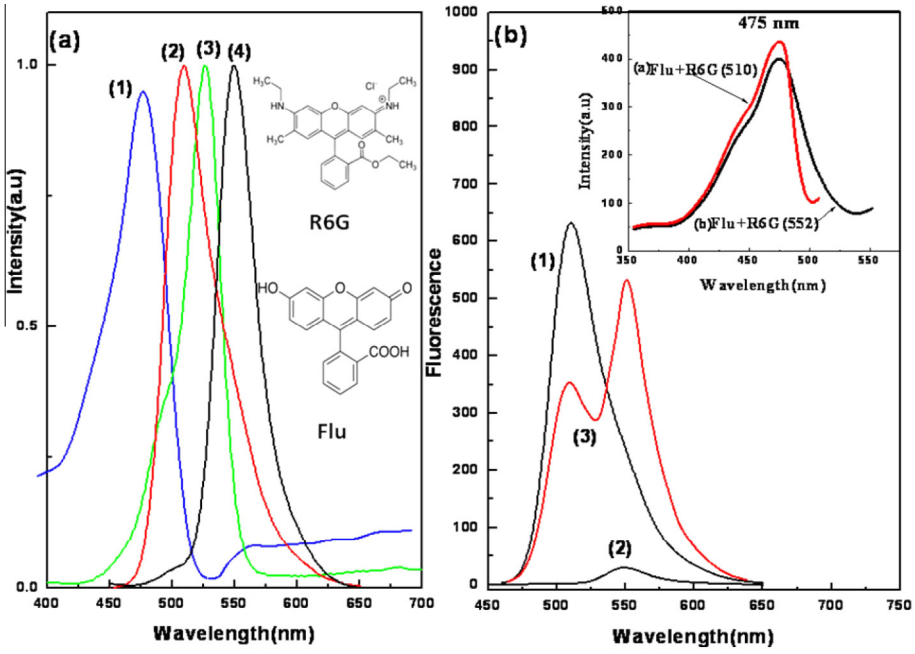

Fig. 1. (a) Normalized absorption (curve 1 and 3) and emission (curve 2 and 4) spectra of Flu and R6G in aqueous solution. Inset shows molecular structure of Flu and R6G (b) fluorescence spectra of pure Flu (curve 1), pure R6G (curve 2) and Flu–R6G (1:1 volume ratio) mixture in aqueous solution (curve 3). Excitation wavelength was 430 nm. The dye concentration was 10^{-6} M and 0.5×10^{-5} M for Flu and R6G, respectively. Inset shows the excitation spectra for Flu–R6G mixture with monitoring emission wavelengths at (a) 510 nm (Flu emission maximum) and (b) 552 nm (R6G emission maximum).

Table 1
Values of spectral overlap integral ($J(\lambda)$), Förster radius (R_0), donor–acceptor distance (r) and energy transfer efficiency (E%) for FRET between Flu and R6G with different acceptor (R6G) concentration in aqueous solution. The donor (Flu) concentration was fixed at 10^{-6} M (these are calculated from the spectral characteristics of Fig. S1 of Supporting information).

Acceptor (R6G) concentration (in M)	$J(\lambda) \times 10^{15}$ m^{-1} cm^{-1} nm^4	R_0 (nm)	r (nm)	FRET efficiency (%)
0.5×10^{-5}	4.852	6.58	7.13	44.15
0.539×10^{-5}	4.971	6.61	6.74	47.30
0.63×10^{-5}	5.062	6.62	6.4	55.39
0.67×10^{-5}	5.081	6.63	6.28	58.41
0.75×10^{-5}	5.117	6.64	6.07	63.00

where F_D is the normalized emission spectrum of donor and ε_A is the molar extinction coefficient (in M^{-1} cm^{-1}) of acceptor; λ, the wavelength (in nm); Φ_D, the fluorescence quantum yield of the donor in absence of acceptor; n is the refractive index of the intervening medium; K^2, orientation factor of transition dipole moment between donor (D) and acceptor (A); N, Avogadro number per mole.

The integral part of Eq. (1) is known as the spectral overlap integral $J(\lambda)$ and is given by

$$J(\lambda) = \int_0^{\alpha} F_D(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4\, d\lambda \tag{2}$$

Therefore the above definition of R_0 in Eq. (1) can be rewritten in units of Å with the scaling constant 0.02108 as follows

$$R_0 = 0.2108 \left(K^2 n^{-4} \Phi_D J(\lambda)\right)^{1/6} \tag{3}$$

where the unit of $J(\lambda)$ is M^{-1} cm^{-1} nm^4.

The efficiency of FRET can be determined by steady state measurements and is expressed as [6]

$$E = 1 - \frac{F_{DA}}{F_D} \tag{4}$$

where F_{DA} and F_D are the donor fluorescence intensities with and without an acceptor, respectively.

The actual distance r between donor and acceptor is given by [6]

$$r = R_0[(1/E) - 1]^{1/6} \tag{5}$$

In the present case the values of $J(\lambda)$, R_0, E and r were calculated using Eqs. (2)–(5) respectively.

The fluorescence quantum yield of the donor in the absence of acceptor (Φ_D) has been calculated by using the standard theory [6,18] and the calculated value of Φ_D was 0.91 [19] for pure Flu in aqueous solution. The reported value of Φ_D for Flu is very close to this calculated value [19].

The orientation factor (K^2) of transition dipole moment between D and A mainly depends on the angle between the transition dipole moments of D and A molecules and the angles between each of these two dipole moments with the vector connecting their centers [6]. Theoretically K^2 has values from 0 to 4.

(i) $K^2 = 0$ (when dipoles are perpendicular to each other),
(ii) $K^2 = 4$ (when dipoles are collinear),
(iii) $K^2 = 2/3$ (when both dyes are freely rotating and can be considered to be isotropically oriented during the excited state lifetime),
(iv) $K^2 = 0.47$ (in case of solid films where the dipole moments of the individual molecules are orientational but they do not rotate by themselves).

In the present case, $K^2 = 2/3$ has been considered [6].

The value of refractive index (n) of the medium has also been used based on the references. For water solution it is 4/3 [20], for laponite dispersion it is 1.39, for NaOH solution it is 1.36 [6].

Results and discussions

Study of FRET in aqueous solution

Fig. 1a shows the normalized absorption and fluorescence spectra of Flu and R6G in aqueous solution. The absorption and emission maxima of Flu were centered at 475 and 510 nm respectively, which were assigned to be due to the Flu monomers [20,21]. On the other hand R6G absorption spectrum possess prominent intense 0–0 band at 525 nm along with a weak hump at 500 nm which was assigned to be due to the 0–1 vibronic transition [22]. The R6G fluorescence spectrum shows prominent monomer band at 552 nm [23].

A close look at Fig. 1a reveals sufficient overlapping of the fluorescence spectrum of Flu and absorption spectrum of R6G. Also both the dyes are highly fluorescent, fulfilling the requirement for FRET to occur [24]. This justifies the selection of these two dyes in order to study the energy transfer from Flu to R6G. Here Flu acts as a donor and R6G acts as an acceptor.

To study the energy transfer between Flu and R6G, the fluorescence spectra of Flu and R6G mixture (1:1 volume ratio) were measured with excitation wavelength fixed at 430 nm (close to the absorption monomer of Flu). Fig. 1b shows the fluorescence spectra of Flu, R6G and their mixture in aqueous solution. From figure it was observed that the fluorescence intensity of pure Flu (curve 1, of Fig. 1b) was much higher whereas that of pure R6G (curve 2, of Fig. 1b) is almost negligible. This is because the excitation wavelength (430 nm) was chosen in order to excite the Flu molecules directly and to avoid the direct excitation of the R6G molecules. However, the fluorescence spectrum of Flu–R6G mixture is very interesting. Here the Flu emission decreases with respect to pure Flu and on the other side R6G emission increases with respect to pure R6G (curve 3, of Fig. 1b). This may be due to the transfer of excited state energy from Flu molecules to R6G molecules via FRET. In order to confirm this, excitation spectra were recorded with monitoring emission wavelength at 510 nm (Flu emission maximum) and 552 nm (R6G emission maximum) for the aqueous solution of Flu–R6G mixture. It was observed that both the excitation spectra were very similar to the absorption spectrum of Flu monomer (shown in inset of Fig. 1b). This confirms that the sensitized R6G fluorescence is mainly due to the light absorption by Flu and corresponding transfer to R6G monomer. Thus FRET between Flu to R6G has been confirmed. In this case, FRET efficiency is 44.14% calculated by using Eq. (4) of the theoretical section.

It is well known that FRET efficiency largely depends on the molecular proximity of donor–acceptor in the mixture [6]. Therefore it is interesting to check the extent of FRET by varying acceptor concentration in the mixture. Accordingly we have measured the fluorescence spectra of Flu–R6G mixture with varying concentration of R6G.

Variation of FRET efficiency in case of Flu–R6G mixed aqueous solution at different concentration of R6G has also been studied (shown in Fig. S1(a) and (b) of the Supporting information). It has been observed that the FRET efficiency almost increases linearly with increase in acceptor (R6G) concentration in the mixture. This may be due to closer proximity of the Flu and R6G with increase in R6G concentration in the mixed solution. The values of spectral overlap integral $J(\lambda)$, energy transfer efficiency (E), Förster radius (R_0) and the donor acceptor distance (r) have been calculated from the fluorescence spectra of Flu–R6G mixture and listed in Table 1. It has been observed that the distance between

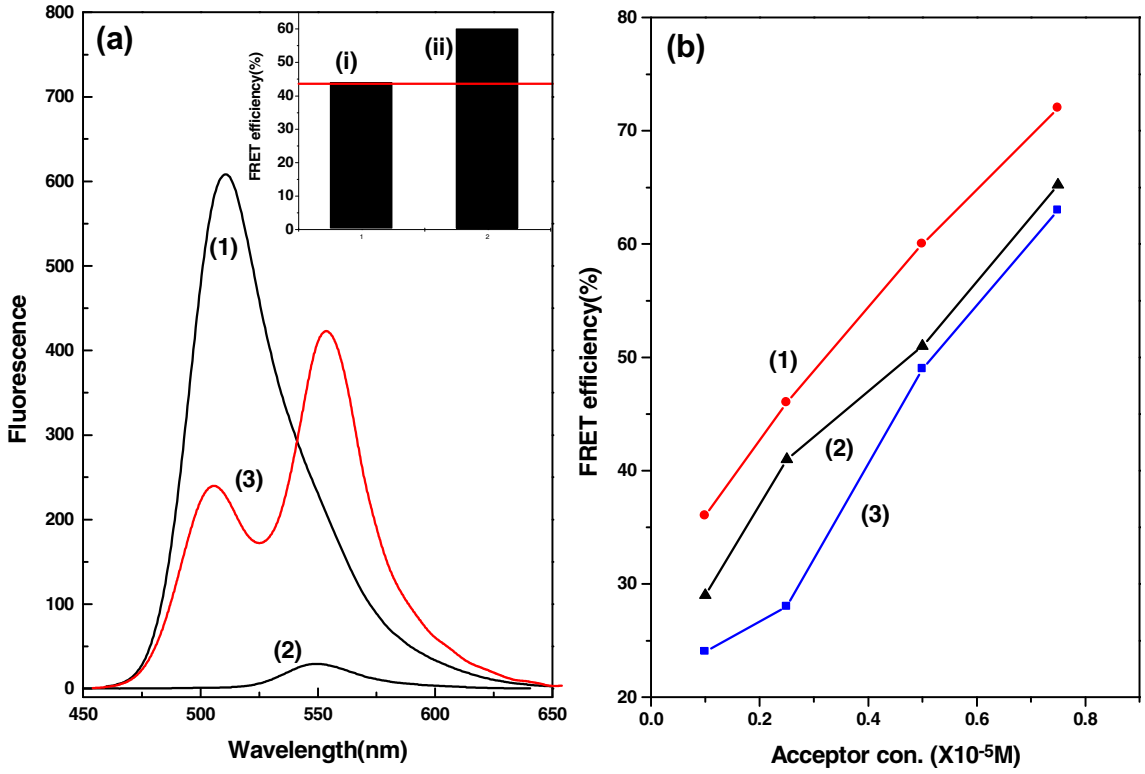

Fig. 2. (a) Fluorescence spectra of pure Flu (curve 1), pure R6G (curve 2) and Flu + R6G (1:1 volume ratio) in clay dispersion (curve 3). Inset showing the comparison of FRET efficiency between Flu and R6G without (i) and with (ii) clay. (b) Variation of FRET efficiency between Flu and R6G for different acceptor (R6G) concentration in aqueous – clay dispersion concentration of Flu was fixed at 10^{-6} M. Clay concentrations were 0.5 (curve 3), 1 (curve 1) and 2 ppm (curve 2).

Table 2
Values of spectral overlap integral ($J(\lambda)$), Förster radius (R_0), donor–acceptor distance (r) and energy transfer efficiency (E%) for FRET between Flu and R6G with different acceptor (R6G) concentration in aqueous – clay dispersion. Clay concentrations were 0.5, 1 and 2 ppm. The donor (Flu) concentration was fixed at 10^{-6} M for all the cases (these are calculated from the spectral characteristics of Figs. S2–S4 of Supporting information).

Acceptor (R6G) concentration (in M)	For clay = 0.5 ppm				For clay = 1 ppm				For clay = 2 ppm			
	$J(\lambda) \times 10^{15}$ m^{-1} cm^{-1} nm^4	R_0 nm	r nm	E (%)	$J(\lambda) \times 10^{15}$ m^{-1} cm^{-1} nm^4	R_0 nm	r nm	E (%)	$J(\lambda) \times 10^{15}$ m^{-1} cm^{-1} nm^4	R_0 nm	r nm	E (%)
10^{-6}	4.125	6.4	7.75	24.02	4.522	6.5	7.15	36.00	4.422	6.48	7.52	29.01
0.25×10^{-5}	4.321	6.45	7.54	28.13	4.915	6.59	6.76	46.10	4.753	6.56	6.97	41.00
0.5×10^{-5}	5.021	6.62	6.66	49.21	5.137	6.64	6.20	60.12	5.025	6.62	6.57	51.17
0.75×10^{-5}	5.109	6.64	6.07	63.04	5.523	6.72	5.97	72.00	5.23	6.66	6.00	65.23

Flu–R6G decreases with increase in R6G concentration, where as the value of spectral overlap integral increases, resulting an increase in FRET efficiency. Maximum energy transfer efficiency was found to be 63% for acceptor concentration of 0.75×10^{-5} M (Table 1). There are several reports where increase in FRET efficiency has been observed with increasing acceptor concentration [25].

Study of FRET in laponite clay dispersion

Synthetic clay minerals are most abundant natural nano materials with cation exchange capacity, layer structure, intercalation and swelling properties [7,26]. Under certain condition Synthetic clay minerals can also have anion exchange capacity [26]. Accordingly synthetic clay minerals are considered as the ideal host material to incorporate ionic, as well as neutral molecules through ion exchange reaction and intercalation [26]. It has been observed that spectral properties of dye molecules change when they are incorporated onto clay matrix [6,7]. Also the molecules come close to each other when they are adsorbed onto clay surface or intercalated in between the interlayer space of synthetic clay minerals. Accordingly the FRET efficiency is largely affected when dye molecules are incorporated onto synthetic clay matrix [6,7]. Therefore, it is very interesting to study the energy transfer between Flu and R6G in presence of synthetic clay minerals.

In order to investigate the effect of laponite on FRET, we have measured the fluorescence spectrum of Flu–R6G mixture (1:1) in laponite clay dispersion. Fig. 2a shows the fluorescence spectra of pure Flu, R6G and their mixture (1:1 volume ratio) in presence of laponite dispersion (laponite concentration was 1 ppm). The most interesting observation in laponite dispersion was that the Flu fluorescence intensity decreases further in favor of R6G fluorescence intensity (Fig. 2a, curve 3), resulting an increase in FRET efficiency. It has been observed that in certain cases in presence of laponite the energy transfer efficiency increases to 60.12% which was 44.15% in absence of clay (Tables 1 and 2, the acceptor concentration was 0.5×10^{-5} M).

It is worthwhile to mention in this context that laponite particles are negatively charged and have layered structure with an ion exchange capacity [6,27]. The dyes Flu and R6G under

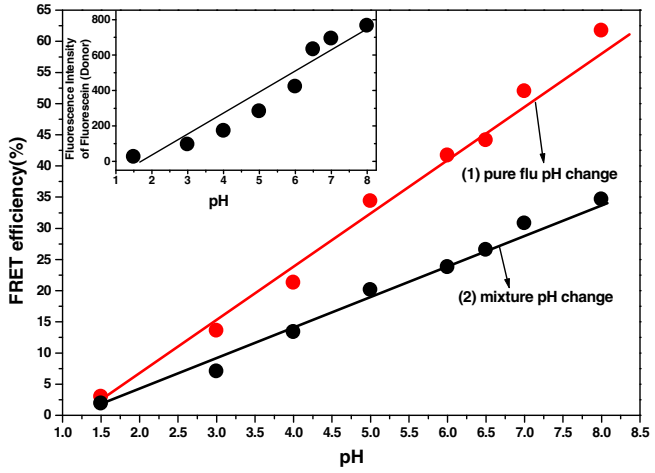

Fig. 3. Variation of FRET efficiency between Flu and R6G at different pH of pure Flu in aqueous solution and at different pH of Flu–R6G mixture in aqueous solution (1:1 volume ratio). Dye concentration was 10^{-6} M (Flu) and 0.5×10^{-5} M (R6G), respectively. Curve 1: pH of the pure Flu solution was changed and then mixed with R6G solution at ambient pH (6.5). Curve 2: pH of the Flu – R6G solution was changed. Inset shows the variation of fluorescence intensity of pure Flu at different pH of pure Flu in aqueous solution.

investigation were anionic and cationic, respectively at ambient condition. R6G molecules were adsorbed onto the laponite surface through cation exchange reaction as well as electrostatic attraction [7]. On the other hand, anionic Flu may adsorb onto laponite through anion exchange reaction as well as intercalation [26]. It is relevant to mention in this context that in certain condition (pH < 11.5) the structural OH groups at the edges and also on the basal surface of the laponite are exchanged with anions [26]. It has also been observed that the dye Flu can be intercalated in the Layered Double Hydroxide (LDH) matrix which is similar to anionic synthetic clay [26]. Confinement of the dyes Flu and R6G on the laponite layer causes a decrease in the distance between the donor–acceptor pair. This in turn enhances the FRET efficiency. Therefore, laponite particles play an important role in concentrating the dyes to make a better favorable condition for close interaction to occur between energy donor and acceptor in contrast to the aqueous solution.

Analysis of fluorescence spectra (Fig. 2a) reveal that in certain cases the spectral overlapping integral $J(\lambda)$ between the fluorescence spectrum of Flu and absorption spectra of R6G increases from $4.852 \times 10^{15}\ M^{-1}\ cm^{-1}\ nm^4$ to $5.137 \times 10^{15}\ M^{-1}\ cm^{-1}\ nm^4$ due to incorporation of laponite sheets. Also the intermolecular distance between Flu and R6G decreases from 7.13 nm to 6.20 nm. Therefore laponite particles play a vital role in concentrating the dyes and thus reducing the intermolecular distance providing a favorable condition for efficient energy transfer. Consequently the energy transfer efficiency increases up to a maximum value of 72% in presence of laponite platelets (Tables 1 and 2, the acceptor concentration was 0.75×10^{-5} M).

There are several reports where synthetic clay minerals play vital role for the enhancement of FRET efficiency [28–30]. Probably the first record on efficient energy transfer in clay mineral system was based on the interaction between two different dyes Cyanine and Rhodamine simultaneously adsorbed onto clay mineral surfaces [28]. Czimorova et al. reported prominent energy transfer among laser dyes in saponite dispersion [29]. Bujdak et al. studied FRET between two rhodamines Rh123 (donor) and Rh610 (acceptor) in both solution and in presence of nanoclay saponite [30]. Our research group has also shown that the energy transfer efficiency increased to a large extent in presence of nanoclay laponite [6,7]. The synthetic clay mineral layers work as templates for concentrating the dyes, accordingly the intermolecular separation between them decreases. Also the orientation of dye molecules changes when they are incorporated onto the restricted geometry of synthetic clay mineral layers. As a result the energy transfer efficiency affected to a large extent in presence of synthetic clay mineral.

Fig. 2b represents the variation of FRET efficiencies between Flu and R6G in presence of laponite for three different concentrations (0.5 ppm, 1 ppm and 2 ppm) of laponite with varying acceptor concentration (corresponding fluorescence spectra are shown in Figs. S2–S4 of Supporting information). The FRET parameters as calculated from the fluorescence spectra are listed in Table 2. It is observed that the FRET efficiency increases with increasing acceptor concentration for all the laponite concentration. However, FRET efficiencies are higher in case of 1 ppm of laponite concentration compared to 0.5 ppm. But the FRET efficiencies for 2 ppm laponite concentration are lower than that in case of 1 ppm laponite concentration. Increasing the concentration of laponite (from 0.5 ppm to 1 ppm) will increase the number of laponite layers in the dispersion. As a result maximum number of dyes will be adsorbed onto the laponite surfaces. This in turn results an increase in FRET efficiency. But for the laponite concentration of 2 ppm there could be a possibility of aggregation of the dye molecules onto the laponite surface which results in a slight decrease in FRET efficiency compared to 1 ppm laponite concentration [31].

It is relevant to mention that with increase in synthetic mineral concentration, normally dye molecules form aggregate. Martínez et al. reported the formation of aggregates of R6G molecules with increasing clay concentration [32]. With increase in loading, dye molecules tend to form self aggregate and dimmers. Cyanine dyes were also found to form aggregate with increase in laponite concentration [31].

Table 3
Values of spectral overlap integral ($J(\lambda)$), Förster radius (R_0), donor–acceptor distance (r) and energy transfer efficiency (E%) for FRET between Flu and R6G at different pH of Flu. The dye concentration was 10^{-6} M and 0.5×10^{-5} M for Flu and R6G, respectively (these are calculated from the spectral characteristics of Figs. S5 and S6 of Supporting information).

pH of the (pure Flu) solution	$J(\lambda) \times 10^{15}\ m^{-1}\ cm^{-1}\ nm^4$	R_0 nm	r nm	FRET efficiency (%)
1.5	1.054	5.104	9.110	3.00
3.0	2.136	5.741	8.551	8.39
4.0	3.691	6.289	7.819	21.30
5.0	4.152	6.413	7.144	34.35
6.0	4.753	6.552	6.926	41.70
Ambient pH (6.5)	4.850	6.582	6.844	44.15
7.0	5.021	6.620	6.532	52.00
8.0	5.12	6.642	6.133	61.72

Effect of pH on FRET in aqueous solution

The dye Flu used in the present study is pH sensitive [15]. Flu is highly fluorescent under basic or neutral conditions, whereas the fluorescence intensity decreases under acidic conditions. Under basic or neutral conditions they exist mainly in ring opened form whereas, they are in spirocyclic form under acidic conditions. At pH values < 2 Flu become cationic in aqueous solution. Flu exists in its neutral species within the range of pH from 2 to 4. As the pH value increases from 4 to 6.5, the mono-anionic form is generated. The dianionic form is generated at pH above 6.5. The fluorescence intensity of Flu increases with increasing pH. The change in fluorescence intensity of Flu with varying pH (from pH = 1.5 to pH = 8) is shown in the inset of Fig. 3 (corresponding fluorescence spectra are shown at Fig. S5 of the Supporting information). This change in fluorescence intensity of Flu with pH may in turn cause

a change in spectral overlapping of the donor fluorescence and acceptor absorbance which results a change in FRET efficiency. Therefore, it is very interesting to study the FRET phenomenon between Flu and R6G at varying pH range. Consequently, we have measured the fluorescence spectra of Flu–R6G mixed solution at different pH following two processes.

(i) Flu solutions prepared at different pH were mixed with R6G aqueous solution prepared at normal pH.
(ii) Solution of the Flu–R6G mixture was prepared at different pH.

The FRET parameters calculated from the spectra measured at different pH are listed in Tables 3 and 4, respectively. The plot of FRET efficiency vs pH for both the cases is shown in Fig. 3. The corresponding fluorescence spectra have been shown in Figs. S6 and S7 in the Supporting information.

From Fig. 3, it has been observed that for both the cases the FRET efficiency increase almost linearly with increase in pH within pH range from 1.5 to 8.0. pH dependent ionic interaction occurred between Flu and R6G in aqueous solution. This is because the ionic nature of Flu is largely dependent on the pH of the solution [15]. At normal pH, Flu remains in monoanionic form in aqueous solution. At acidic medium Flu becomes either neutral (pH range from 2 to 4) or cationic (pH < 2) [15]. At higher pH (>6.4) Flu remains in dianionic form in aqueous solution. On the other hand R6G is cationic in nature. As a result with increasing pH the electrostatic interaction between Flu and R6G increases in aqueous solution and they come closer to each other. This results in an increase in FRET efficiency. Again pH dependent enhancement in Flu fluorescence intensity causes an increase in $J(\lambda)$ values (Tables 3 and 4) with increase in pH values. These also contribute to the FRET enhancement at higher pH.

A close look to the Fig. 3 shows that the extent of increase in FRET efficiency is higher when the pH of the Flu solution was changed followed by mixing of R6G at normal pH. In case of first method, the FRET efficiency changes from 3.00% to 61.72% for a change in pH from 1.5 to 8.0 in the mixture, whereas, the FRET efficiency changes from 1.91% to 34.65% for a change in pH of Flu–R6G mixture from 1.5 to 8.0 for the second method.

The difference in the increase in FRET efficiency may be explained in terms of the change of ionic nature of Flu in aqueous solution with pH change. In the first method, the pH of the individual Flu solution was first changed following the addition of R6G solution. In the second process the pH of the Flu–R6G mixture was changed. Eventually, there may be a possibility of change in ionic form of Flu (cationic, neutral, monoanionic, dianionic) both in rate as well as in number with increasing pH for the first method. Therefore the observed linear change in FRET efficiency with increasing pH may be used for the sensing of pH. This situation has been explained through a schematic diagram given in Fig. 4.

Table 4
Values of spectral overlap integral ($J(\lambda)$), Förster radius (R_0), donor–acceptor distance (r) and energy transfer efficiency (E%) for FRET between Flu and R6G at different pH of Flu –R6G mixture. The dye concentration was 10^{-6} M and 0.5×10^{-5} M for Flu and R6G, respectively (these are calculated from the spectral characteristics of Figs. S5 and S7 of Supporting information).

pH of the mixed (Flu–R6G) solution	$J(\lambda) \times 10^{15}$ m^{-1} cm^{-1} nm^4	R_0 nm	r nm	FRET efficiency (%)
1.5	1.129	5.163	9.954	1.91
3.0	2.082	5.717	8.780	7.05
4.0	2.781	5.999	8.153	13.38
5.0	3.642	6.275	7.896	20.12
6.0	3.762	6.309	7.660	23.78
Ambient pH (6.5)	3.881	6.342	7.513	26.56
7.0	4.023	6.380	7.301	30.80
8.0	4.190	6.424	7.140	34.65

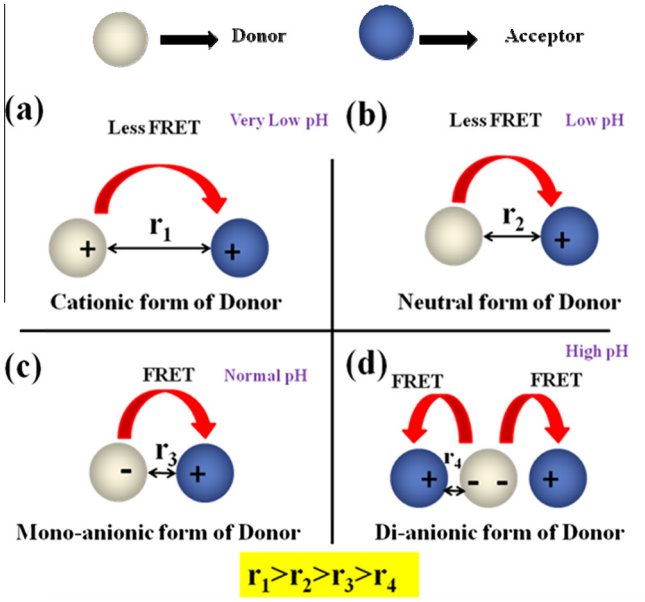

Fig. 4. Schematic representations showing the change in ionic nature of donor (Flu) with change in pH and FRET between Flu and R6G at different pH.

There are few reports where energy transfer phenomenons have already been used for pH measurement [33–38]. Chan et al. used semi conducting polymer dots as a platform and designed ratiometric pH nanoprobes by using the concept of FRET within the range of pH from 5 to 8 [33]. By introducing polymer doped with either Congo red (pH range 3–5) or methyl red (pH range from 5 to 7) Egami et al. has reported a fiber optic pH sensor [34]. Jiangli Fan et al. observed FRET by using 1,8-naphthalimide as donor and rhodamine as acceptor [35]. Xianfeng Zhou et al. has designed a ratiometric chemosensor by using two pH sensitive dyes coumarine (donor) and amino-naphthalimide derivative (acceptor) [36]. Georgiev et al. has reported a pH sensitive and selective ratiometric PAMAM wavelength-shifting bichromophoric system where the systems surface is labelled with yellow-green emitting 4-(N-piperazinyl)-1,8-naphthalimide as donor and Rhodamine 6G as acceptor [37]. Dennis et al. described a nanoparticle-based ratiometric pH sensor by using semiconductor quantum dot (QD) and pH sensitive fluorescent proteins (FPs). It has been observed that Forster Resonance Energy Transfer (FRET) between QD and multiple FPs modulates the FP/QD ratio exhibiting a >12-fold change between pH 6 and 8 [38]. In the present system pH dependant change in FRET parameters can be used to design a pH sensor that can measure pH over a wide range from 1.5 to 8. This is one advantage of our system with respect to previous systems and has proven the significance of this work.

Conclusion

A new FRET pair Flu and R6G has been identified. FRET between these two dyes were successfully investigated in solution both in presence and absence of synthetic clay particle laponite. UV–Vis absorption and fluorescence spectroscopy studies reveal that both the dyes present mainly as monomer in solution and there exist sufficient overlap between the fluorescence spectrum of Flu and absorption spectrum of R6G which is a prerequisite condition for FRET to occur from Flu to R6G. Energy transfer occurred from Flu to R6G in solution in presence and absence of laponite. FRET

efficiency increases with increasing acceptor concentration. The energy transfer efficiency increases in presence of laponite. The maximum efficiency was found to be 72.00% for the mixed dye system (50% RhB + 50% Acf) in laponite dispersion. Flu is pH sensitive and it was observed that the overlap between Flu fluorescence and R6G absorption spectrum changes with change in pH. Consequently energy transfer efficiency was found to be pH sensitive. The energy transfer efficiency varies from 3.00% to 61.72% for a change in pH of Flu solution from 1.5 to 8.0. However, the energy transfer efficiency varies from 1.91% to 34.65% when the pH of the Flu–R6G mixture solution was changed from 1.5 to 8.0. With proper calibration it is possible to use the present system under investigation to sense pH over a wide range of pH from 1.5 to 8.0.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2015.04.027.

References

[1] T. Yanqing, F. Emily, K. Summer, L. Fred, S. Fengyu, Z. Liqiang, C. Shih-hui, R.M. Deirdre, Sens. Actuators, B 188 (2013) 1–10.
[2] G. Ghini, C. Tronoa, A. Giannetti, G.L. Puleob, L. Luconi, J. Amadouc, G. Giambastiani, F. Baldini, Sens. Actuators, B 179 (2013) 163–169.
[3] R.E. Campbell, Anal. Chem. 81 (2009) 5972–5979.
[4] J.A. Thomas, R.N. Buchsbaum, A. Zimniak, E. Racker, Biochemistry 18 (1979) 2210–2218.
[5] Q.A. Best, N. Sattenapally, D.J. Dyer, C.N. Scott, M.E. Mccarroll, J. Am. Chem. Soc. 135 (2013) 13365–13370.
[6] D. Dey, D. Bhattacharjee, S. Chakraborty, S.A. Hussain, Sens. Actuators, B 184 (2013) 268–273.
[7] D. Dey, J. Saha, A. Datta Roy, D. Bhattacharjee, S.A. Hussain, Sens. Actuators, B 195 (2014) 382–388.
[8] M. Chao, Z. Fang, H. Lifang, W. Shuizhu, J. Phys. Chem. B 115 (2011) 874–882.
[9] T.H. Förster, Z. Naturforsch. 4A (1949) 321.
[10] N.L. Rosi, C.A. Mirkin, Chem. Rev. 105 (2005) 1547–1562.
[11] E.M. Nolan, S.J. Lippard, Chem. Rev. 108 (2008) 3443–3480.
[12] S.C. Dodani, Q. He, C.J. Chang, J. Am. Chem. Soc. 131 (2009) 18020–18021.
[13] X.L. Zhang, Y. Xiao, X.H. Qian, Angew. Chem. 47 (2008) 8025–8029.
[14] H. Ueyama, M. Takagi, S. Takenaka, J. Am. Chem. Soc. 124 (2002) 14286–14287.
[15] J. Panchompoo, L. Aldous, M. Baker, M.I. Wallace, R.G. Compton, Analyst 137 (2012) 2054–2062.
[16] T. Förster, Action of Light and Organic Molecules. Modern Quantum Chemistry. Part 2, Academic Press, New York, 1965.
[17] S. De, A. Girigoswami, J. Colloid Interface Sci. 271 (2004) 485–495.
[18] A.T.R. Williams, S.A. Winfield, J.N. Miller, Analyst 108 (1983) 1067–1071.
[19] M. Adamczyk, J. Grote, J.A. Moore, Bioconjugate Chem. 10 (1999) 544–547.
[20] G. Blagoi, N. Rosenzweig, Z. Rosenzweig, Anal. Chem. 77 (2005) 393–399.
[21] Q. Yang, T. Qiu, W. Wu, C. Zhu, L. Liu, J. Ying, S. Wang, ACS Appl. Mater. Interfaces 3 (2011) 4539–4545.
[22] M. Fischer, J. Georges, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 53 (1997) 1419–1430.
[23] Z. Grauer, D. Avnir, S. Yariv, Can. J. Chem. 62 (1984) 1889–1894.
[24] P.D. Sahare, V.K. Sharma, D. Mohan, A.A. Rupasov, Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 69 (2008) 1257–1264.
[25] T. Guangchao, D. Liping, S. Xingguang, Food Chem. 141 (2013) 4060–4065.
[26] F. Bergaya, B.K.G. Theng, G. Lagaly, Handbook of Clay Science Chapter 5, vol. 1, Elsevier Ltd, 2006, pp. 141–245.
[27] R.H.A. Ras, B.V. Duffel, M.V. Auweraer, F.C.D. Schryver, R.A. Schoonheydt, Molecular and particulate organisation in dye-clay films prepared by the Langmuir–Blodgett method, in: Proceedings of the 12th International Clay Conference, Bahia Blanca, Argentina 2001, pp. 473–480.
[28] R.A. Schoonheydt, Clays Clay Miner. 50 (2002) 411–420.
[29] A. Czímerováa, J. Bujdák, N. Iyi, J. Photochem. Photobiol., A 187 (2007) 160–166.
[30] k.J. Bujdá, D. Chorvát, N. Iyi, J. Phys. Chem. C 114 (2010) 1246–1252.
[31] A. Dixon, Ç. Duncan, H. Samha, Am. J. Undergraduate Res. 3 (2005) 29–34.
[32] V.M. Martınez, F.L. Arbeloa, J.B. Prieto, I.L. Arbeloa, J. Phys. Chem. B 109 (2005) 7443–7450.
[33] Y.H. Chan, C. Wu, F. Ye, Y. Jin, P.B. Smith, D.T. Chiu, Anal. Chem. 83 (2011) 1448–1455.
[34] C. Egami, Y. Suzuki, O. Sugihora, H. Fujimura, N. Okamoto, Jpn. J. Appl. Phys. 36 (1997) 2902–2905.
[35] F. Jiangli, L. Chunying, L. Honglin, Z. Peng, W. Jingyun, C. Shuang, H. Mingming, C. Guanghui, P. Xiaojun, Dyes Pigm. 99 (2013) 620–626.
[36] Z. Xianfeng, S. Fengyu, L. Hongguang, P. Senechal-Willis, T. Yanqing, R.H. Johnson, D.R. Meldrum, Biomaterials 33 (2012) 171–180.
[37] N.I. Georgiev, A.M. Asiri, A.H. Qusti, K.A. Alamry, B.B. Vladimir, Dyes Pigm. 102 (2014) 35–45.
[38] A.M. Dennis, W.J. Rhee, D. Sotto, S.N. Dublin, G. Bao, ACS Nano 6 (2012) 2917–2924.