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

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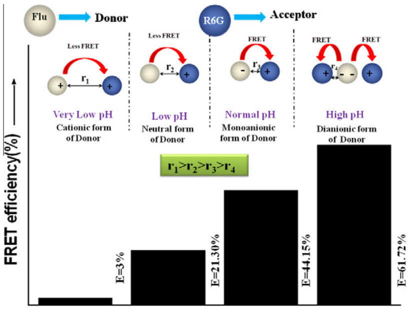
HIGHLIGHTS

- 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].

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

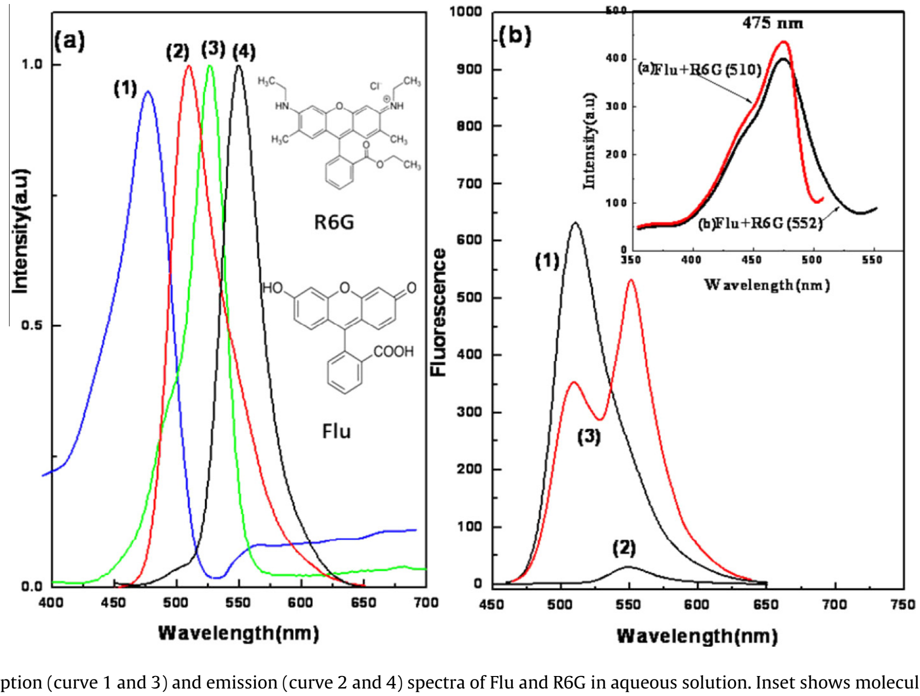

Fig. 1. (a) Normalized absorption (curve 1 and 3) and emission (curve 2 and 4) spectra of Flu and R6G in aqueous solution. Inset shows molecular structure of Flu and R6G (b) fluorescence spectra of pure Flu (curve 1), pure R6G (curve 2) and Flu–R6G (1:1 volume ratio) mixture in aqueous solution (curve 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).