ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Fluorescence Resonance Energy Transfer between acridinedione and rhodamine – 6G.

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ABSTRACT

Fluorescence Resonance Energy Transfer between two acridinedione and Rhodamine 6G was investigated in an alcoholic solution. Spectroscopic studies suggest that both the dyes were present mainly as monomer in solution. Fluorescence Resonance Energy Transfer occurred from acridinedione to Rhodamine 6G in an alcoholic solution. Energy transfer efficiency was measured during the spectroscopic analysis.
Keywords: acridinedione, rhodamine-6G,FRET, efficiency

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ISSN: 0975-8585

INTRODUCTION

The Fluorescence Resonance Energy Transfer (FRET) between two molecules is an important physical phenomenon with considerable interest for the understanding of some biological systems and with potential applications in optoelectronic and thin film device development [1, 2]. FRET is also finding a widespread application today more and more in biomedical research and drug delivery. It is established as an effective tool for measuring distance parameters between the component molecules. Due to its sensitivity to distance, FRET has been used to investigate molecular level interactions. Recent advances in the technique have led to qualitative and quantitative improvements, including increased spatial resolution, distance range and sensitivity. Through the Förster mechanism, fluorescence energy transfer occurs by means of a radiationless, coulombic, dipole-dipole interaction and remains active over the 10 to 100Å range[3]. This makes the FRET technique suitable to probe nanoscale processes. The coulombic, dipole-dipole interaction needs spectral overlap between an acceptor's absorption and the donor emission, along with the appropriate orientation of their transition dipoles.

A pair of molecules that interact in such a manner that FRET occurs is often referred to as a donor-acceptor pair. The phenomenon of FRET is not mediated by photon emission. An Introduction to Fluorescence Resonance Energy Transfer (FRET) even does not require that the acceptor chromophore to be fluorescent. The process of FRET seems to be more efficient when there is an appreciable overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor [4]. The efficiency of energy transfer by FRET mechanism is defined as "the fraction of photons absorbed by the donor that is transferred to the acceptor." This parameter can be experimentally determined by comparing the fluorescence intensities of the donor (D) in the presence and absence of acceptors (A) as given by Eq. (1).

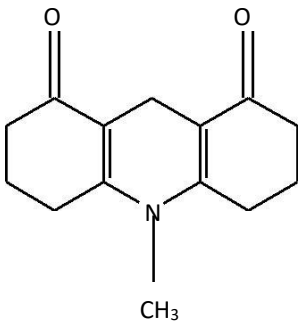
$$E = 1 - I_{DA} / I_D$$

where I_{DA} represents the intensity of emission of donor in presence of acceptor and I_D represents the intensity of emission of donor in absence of acceptor. Alternatively, energy transfer efficiency can also be calculated as:

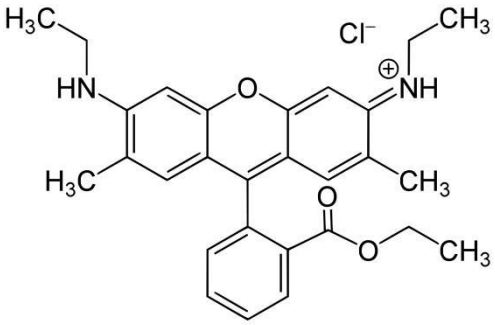
$$E = 1 / 1 + (r/R^{\circ})6$$

where '**r**' is the distance between D and A chromophores, 'R°' is the so-called Forster distance, at which the energy transfer efficiency is 50%. Thus R° is the critical distance, at which energy transfer and spontaneous decay of the excited donor are equally probable. [5].

The FRET pair under study in this paper is acridinedione (ADR) as donor and rhodamine-6G (Rh-6G). Both being fluorescent molecules, an effective FRET analysis is targeted here. The structures of the donor (D) and acceptor (A) are depicted as shown:



ADR (donor)



Rh-6G (acceptor)

ISSN: 0975-8585

EXPERIMENTAL METHODS

Chemicals used:

Acridinedione (ADR) used was prepared in the laboratory following reported procedures [6]. Rhodamine-6G was obtained from Qualigens India Ltd. and used as received. Methanol used is of HPLC grade obtained from SRL.

Techniques used:

Absorption spectra were recorded in an Agilent 8453 diode array spectrophotometer. Fluorescence spectra were recorded using a Perkin–Elmer LS5B luminescence spectrophotometer and Horiba Jobin Yvon Fluoromax 4P spectrophotometer [7].

RESULTS AND DISCUSSION

In the present work, the FRET between acridinedione donor and rhodamine-6G acceptor combination is studied in an alcoholic medium. For spectroscopic measurements, a 1x 10^{-4} M stock solution of ADR in 0.6% in methanol was prepared and used. The acceptor stock solution was of concentration 1 x 10^{-3} M for rhodamine -6G in methanol. Suitable dilutions were prepared for the analysis with methanol.

The absorption and emission spectra of ADR in methanol shows a characteristic maximum around 388 and 470 nm. This band has been assigned to an intra molecular charge transfer from the ring nitrogen to the ring carbonyl oxygen rich centre within the ADR molecule [8]. The choice of rh-6G is based on the fact that the acceptor absorption in the region of absorption of the donor (388 nm) is practically negligible and there occurs an appreciable spectral overlap between the donor emission and the acceptor absorption spectra. The overlap between the normalized emission spectrum of the donor and the normalized absorption spectrum of the acceptor are shown in **Fig. 1**.

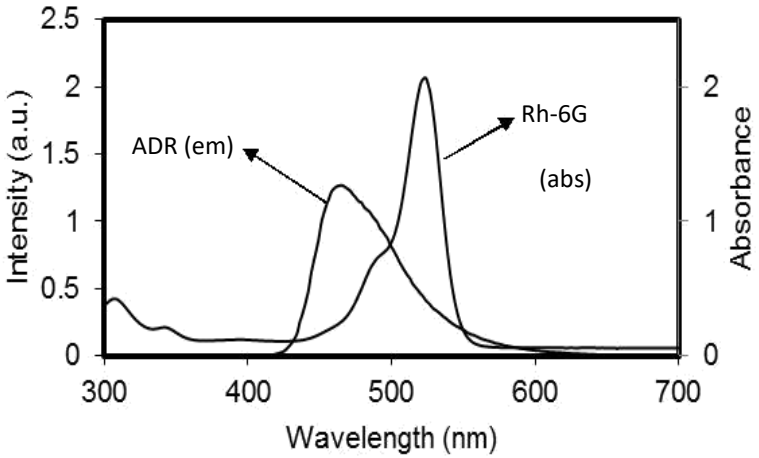


Fig1: Spectral overlay of emission of Acridnedione (ADR) and absorption of Rhodamine-6G (Rh-6G) in methanol.

Fig. 2 shows the absorption spectra of 1.1x 10^{-5} M ADR in presence of varying concentrations (2x10^{-5} M, 4x10^{-5} M M, 6x10^{-5} M, 8x10^{-5} M) of Rh-6G in methanol. It was revealed that there was no appreciable change in the

ISSN: 0975-8585

absorbance of the donor at the λ_{max} (388 nm for free ADR in methanol) with increasing concentrations of acceptor. This indicates that there occurs no dimer or higher aggregate formation for the concentrations used here. Hence steady state studies were carried out by exciting at this wavelength (388 nm) . **Fig. 3** shows the steady – state emission spectrum of ADR with Rh-6G acceptor in methanol. The concentrations of the donor and acceptor were maintained the same as with the absorption measurements. It was found that the excitation wavelength of 388 nm was absorbed dominantly by the ADR donor and showed a characteristic emission at 480 nm. With successive additions of acceptor concentration, donor emission intensity decreased with a blue shift (4-5 nm) and acceptor emission increased with a slight red shift (8-10 nm). Such an observation is often considered as the evidence for energy transfer from any donor to acceptor. The blue shift in donor fluorescence is attributed to the radiative transfer that occurs from donors to acceptors and the red shift in acceptor fluorescence is due to the radiative migration that occurs due to self absorption [5].

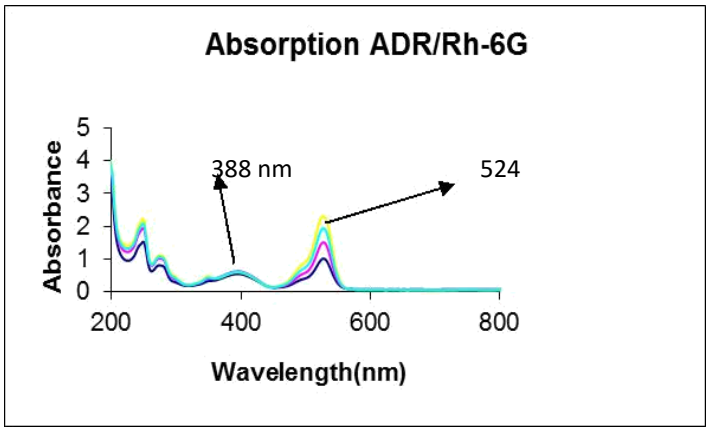


Fig 2: Absorption spectrum of ADR/rhodamine-6G system in methanol. [ADR]: 1.1x 10^{-5} M, [rhodamine-6G]: (a) 2x10^{-5} M, (b) 4x10^{-5} M M, (c) 6x10^{-5} M, (d) 8x10^{-5} M.

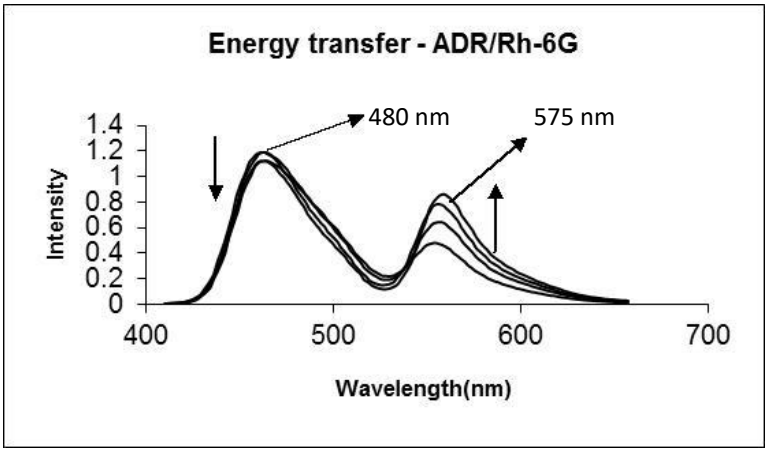


Fig3: Steady-state emission of ADR/rhodamine-6G system in methanol. [ADR]:1.1x 10^{-5} M, [rhodamine-6G]: (a) 2x10^{-5} M, (b) 4x10^{-5} M M, (c) 6x10^{-5} M,(d) 8x10^{-5} M. λ_{max} = 388 nm.

Here, in our case, for the pair of ADR & Rh-6G, it was confirmed that there is no significant contribution of the acceptor fluorescence at 575 nm on direct excitation at 388 nm by performing blank experiments. As shown in fig 3, with increase in concentration of Rh-6G, the emission at acceptor wavelength (575 nm) is found to increase which is evidencing the fluorescence resonance energy transfer happening between ADR and Rh-6G viz., energy is being transferred from donor ADR to acceptor Rh-6G. From the intensity values of the

donor in the absence and presence of different concentration of acceptors, efficiency of energy transfer (Z) etc., were calculated. As indicated by the equation 2, the efficiency of energy transfer was calculated as 62%.

CONCLUSION

A FRET analysis was carried out between the ADR and Rh-6G molecules. From the overlay spectrum, it was inferred that the choice of donor-acceptor combination is a promising one. From the absorption and emission of ADR in presence of varying concentrations of Rh-6G, an effective non-radiative energy transfer happening was identified. From the intensity values of donor in the presence and absence of acceptor, the FRET efficiency was found to be a most favorable one.

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