Studying The Fluorescence Resonance Energy Transfer between Fluorescein and Rhodamine B in an aqueous solution

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Abstract

The energy transfer of fluorescence resonance in aqueous solution was studied between two organic dyes, Fluorescein and Rhodamine B. Both dyes were found in solution as monomers, according to spectroscopic studies. Energy transfer was observed in fluorescence resonance imaging solutions containing fluorescein and rhodamine B. with different concentrations of the acceptor RhB dye in the range of (1.5×10^{-5} M to 3.5×10^{-5} M). Studies using Both UV–vis absorption and fluorescence spectroscopy demonstrated that the two dyes, when dissolved in solution, appear largely as monomers.

Keywords: FRET, Fluorescein, Rhodamine B, HPCL Water

1. Introduction

The fluorescence resonance energy transfer (FRET) is a physical mechanism that transfers nonradiatively excited energy from one excited molecular fluorophore (the donor) to another fluorophore through intermolecular long-range dipole–dipole coupling (the acceptor)[1]. In the fields of biology, physiology, medicine, and pharmacology, fluorescent sensors are utilized quite frequently[2]. When it comes to scientific investigation, they have drawn the interest of a great number of chemists and biologists[3]. Utilizing detection methods that are based on fluorescence sensors comes with a number of advantages, such as ease of use, low cost, high sensitivity, quick and simple adaptation to automated analysis, the ability to support spatially resolved images, and a number of different signal output modes[4, 5]. In general, fluorescent sensors offer a one-of-a-kind method for detecting analytes that are relevant from a physiological or ecological perspective[6]. Theodor Forster, who created an equation in 1948 to determine the efficiency of electronic excitation transfer from a donor to an acceptor, is remembered today thanks to the acronym FRET (which stands for his name)[7]. It is possible that FRET is an accurate method for measuring molecule closeness, and it is particularly useful at angstrom distances (10–100)[8]. if the donor and acceptor are within the Forster radius, the donor's excitation energy will be transferred to the acceptor[9]. This distance is typically between 3 and 6

nanometers[10]. FRET efficiency can be affected by a variety of distinct aspects, such as the fluorescence quantum yield of the donor in the absence of an acceptor, the refractive index of the solution, the dipole angular orientation of each molecule, and the spectrum overlap integral of the donor emission and the acceptor absorption, amongst others[11]. If any of these characteristics change as a result of the presence of an outside agent, then the efficiency of energy transfer will change as a result[12]. Because of this, the FRET process has the potential to advance sensor technology[13]. It is essential to discover new FRET pairs since fluorescence spectroscopy has developed into a potent technique for detecting transition and heavy metal ions by investigating and quantifying their FRET mechanism. This study presents the outcomes of our tests on FRET between two dyes, namely acriflavine and rhodamine B [14]. Metal ions are one of those that are mentioned in Metal ions have a substantial impact on both the fluorescence and absorption spectra[15]. It's possible that this will have some kind of effect on the FRET mechanism that occurs between Flu and other dye molecules[11]. As a consequence of this, it is of the utmost importance to carry out research on the FRET between Flu and other dyes under a number of different conditions[16]. The findings indicate that, given a constant concentration of donors, the efficiency of energy transfer improves with decreasing donor concentrations

2. Experimental

2.1 Materials and Methods

Donor and acceptor laser dyes were Flu and RhB, both of which were purchased in Lancashire, United Kingdom. Flu can be prepared in the form of. It is available as a dark orange/red powder. It possesses the chemical formula of $C_{20}H_{12}O_5$ and a molecular weight of 332.311 gm/mole, in addition to RhB the chemical formula $C_{28}H_{31}ClN_2O_3$ and a molecular weight of 479.02 gm/mole. As a solvent, Milli-Q water with a molecular weight of 18.02 u was utilized.
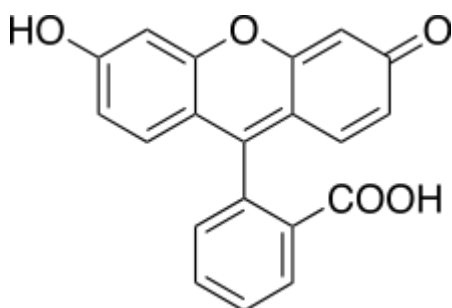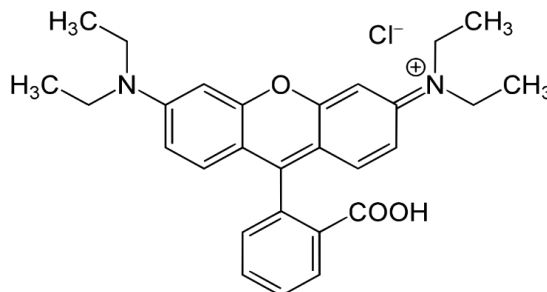
Fluorescein   Rhodamine

Fig.1 Formulations of curcumin made with chemicals in the Flu and RhB.

According to the following equation, and in order to create the two dyes, a concentration of 1×10^{-3} M was used Eq.1. [17] (1)

$$m = C\ V\ Mw \qquad (1)$$

Where: m is the weight of the dye in grams that is required to obtain the desired concentration, C is the concentration that must be prepared, V is the volume of solvent in liters that must be added to the dye, and Mw is the molecular weight of the dye that is being used in g/mol.

The produced dye solutions were diluted in accordance with the equation that is presented below (2)

$$C_1\ V_1 = C_2\ V_2 \qquad (2)$$

Eq.2.[18] Where: C1 and C2 refer to the primary and secondary concentrations (M), respectively. Both V_1 and V_2 refer to the volume (in liters) of the solution before and after it have been diluted. Figure 2 shows Flu and RhB dyes in different concentrations 10^{-3}, 10^{-4}, 10^{-5} M .The mixing ratio used for donor and acceptor in this research is (3ml: 3ml).

(a)   **(b)**

Fig.2 After being diluted with water, the dye rhodamine B (a) and the dye Fluorescein (b)

2.2 The measurement of UV–Vis absorption as well as fluorescence spectra

The Ultraviolet Spectrophotometer (T70/T80 series UV/Vis Spectrometer) was utilized in order to take readings for the spectra of UV–Vis absorption as well as steady-state fluorescence. Fluorescence emission was measured using a Spectrofluorophotometer (RF-5301pc Shimadzu). The fluorescence light was collected from the sample surface at an angle of ninety degrees, and the excitation wavelength was 420 nanometers while the sample was at room temperature.

2.3. Theoretical considerations

Perrin[19] proposed the use of dipole–dipole interactions as a method by which molecules might interact without colliding at distances greater than their molecular diameters This was done in order to take into consideration the fact that molecules can interact with one another at distances greater than their molecular diameters without really coming into touch with one another[18]. Forster developed an elegant theory that, by using his famous phrase, offered a quantitative explanation for non-radiative energy transfer[21]. This theory was made possible by Perrin's notion, which Forster relied on[21]. This theory was presented in the context of Forster's presentation.

$$K_T(\text{r}) = \left(\frac{R_0}{r}\right)^6 \frac{1}{\tau_D} \qquad (3)$$

Where $k_T(r)$ is the known rate of energy transfer from donor to acceptor, τ_D is the donor lifetime in the absence of acceptor [18]. r is the distance between donor and acceptor and R_o is The Förster or critical transfer distance at which the energy transfer rate is equal to the decay rate. The value of R_o can be defined by the following expression:
[11] [22].

$$R_0^6 = \frac{2.07}{128\,\pi^5 N_A} \frac{k^2 Q_D}{n^4} \int_0^\alpha F(\lambda)\varepsilon_A(\lambda)\lambda^4\,d\lambda \qquad (4)$$

where F_D is the normalized fluorescence intensity of the donor; $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor (in $M^{-1}\,cm^{-1}$); λ is the wavelength (in nm); ϕ_D is the fluorescence quantum yield of the donor when there is no acceptor present [9, 11]; n is the refractive index of the medium; k^2 is the

orientation factor of the transition dipole moment between the donor (D) and the acceptor (A)[11]; The spectral overlap integral $J(\lambda)$ is the name given to the integral that is a part of Equation 2, and its value may be found by using the formula[23]:

$$(\lambda) = \int_0^\alpha F(\lambda)\varepsilon_A(\lambda)\lambda^4 \, d\lambda \qquad (5)$$

Therefore the above definition of R_o in Eq. (4) can be rewritten in terms of (λ) with units $M^{-1}cm^1nm^4$ as

$$R_o = 0.2108[k^2 n^{-4}(\lambda)]^{\frac{1}{6}} \qquad (6)$$

where R_O is expressed in units of A^o. E The steady state is a method that can be used to determine the FRET's effectiveness[24]. measurements and is expressed as

$$E = 1 - \frac{F_{DA}}{F_D} \qquad (7)$$

where F_{DA} and F_D refer to the donor fluorescence intensity with and without an acceptor, respectively[25]. F_{DA} stands for donor fluorescence intensity with and F_D stands for donor fluorescence intensity without[6]. The exact distance, r, that separates the donor and the acceptor can then be calculated using

$$r = R_o \left[\left(\frac{1}{E} - 1\right)\right]^{\frac{1}{6}} \qquad (8)$$

In this case, Egs were used to calculate the values of (λ), R_o, E, and r. (4) – (8).

When the donor's fluorescence quantum yield (D) was estimated in the absence of an acceptor, the value of 0.91 was discovered to be associated with pure fluorine when it was dissolved in water[26]. The number that was determined to be quite near to the value that was specified for fluorine was found. In addition to the angles created by these two dipole moments and the vector joining their centers, the angle between the transition dipole moments of D and A molecules has an effect on the orientation factor k^2, which in principle can take on any value between 0 and 4[27]. When the dipoles are oriented in such a way that they are perpendicular to one another, k^2 equals zero, k^2 equals four, k^2 equals two-thirds (when both dyes are spinning freely), and k^2 equals 0.47 (when the dipoles are collinear) (in the case of solid films in which the dipole moments of individual molecules are orientational but do not rotate independently).

$k^2 = 2/3$ (in the case of the dipole moments, individual molecules are orientational and spin by themselves). The value of the medium's refractive index (n) was also utilized based on the references. A water solution has a pH value of 4/3[11, 25].

Results and discussion

Figure 3 demonstrates. the normalized UV–Vis absorption and steady-state fluorescence spectra of Pure Flu and RhB in aqueous systems. Monomer features can be seen in both the absorption and the fluorescence spectra[28]. The excitation wavelength of 420 nm was used for the purpose of searching for donor and acceptor energy transfer measurements. Also, this length was the best wavelength to excite the fluorescence spectra.This particular wavelength was decided upon because, at this particular frequency, the amount of RhB that may be absorbed is practically nonexistent. We note from Figure 4 that the concentration of Flu 10^{-5} M, 10^{-4} M and RhB the concentration of is 10^{-5} M, 10^{-4} M.

The wavelength of absorption was chosen in order to come as close as possible to simulating direct flu molecule excitation while simultaneously avoiding or minimizing direct flu molecule excitation. The RhB molecules are made active. Figure 5a illustrates the fluorescence spectra of pure Flu and RhB, as well as their mixtures in aqueous solution (50:50 volume ratios). the concentration of Flu 10^{-5} M is fixed and RhB the best concentration of energy transfer is $3.5 * 10^{-5}$ M. For the purpose of the energy transfer mechanism, because this concentration was found to be one of the most effective concentrations when mixed with varying concentrations of the acceptor RhB dye in the range of 1.5×10^{-5}M to 3.5×10^{-5}M Figure 5b demonstrates that Flu has its own unique fluorescence band. The RhB fluorescence band, on the other hand, is not very noticeable in dye solution. The more concentration the RhB, the lower the fluorescence. It is essential to take into account the fact that the intensity of the RhB fluorescence increases while the intensity of the Flu fluorescence drops when the concentration is held constant at 10^{-5} M. As a consequence of this, it achieves its highest value as energy transfer at a concentration for acceptor of 3.5×10^{-5}M with a concentration of the donor that remains constant.

Fig. 3. Normalized UV–Vis absorption and fluorescence spectra of Flu
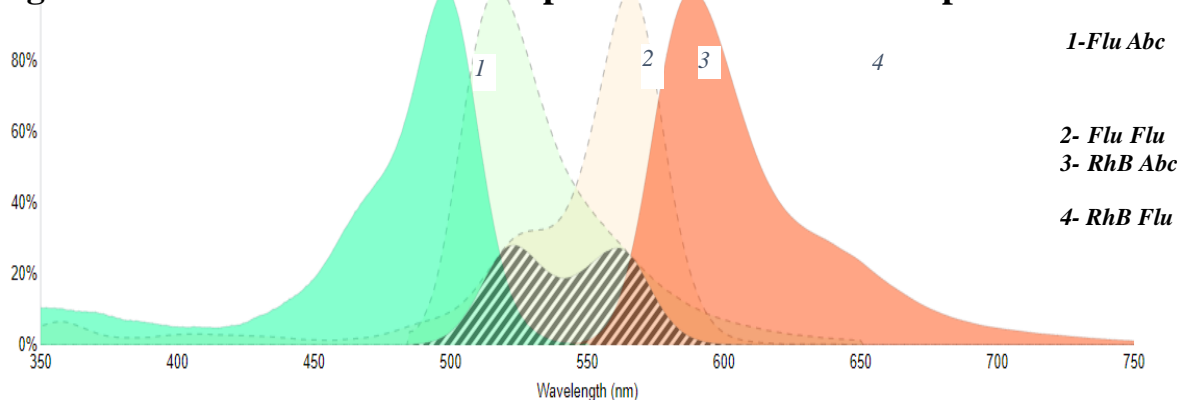
and RhB in aqueous solution. The overlap between Flu fluorescence (2) and RhB absorption (3) spectra is shown by shaded region.
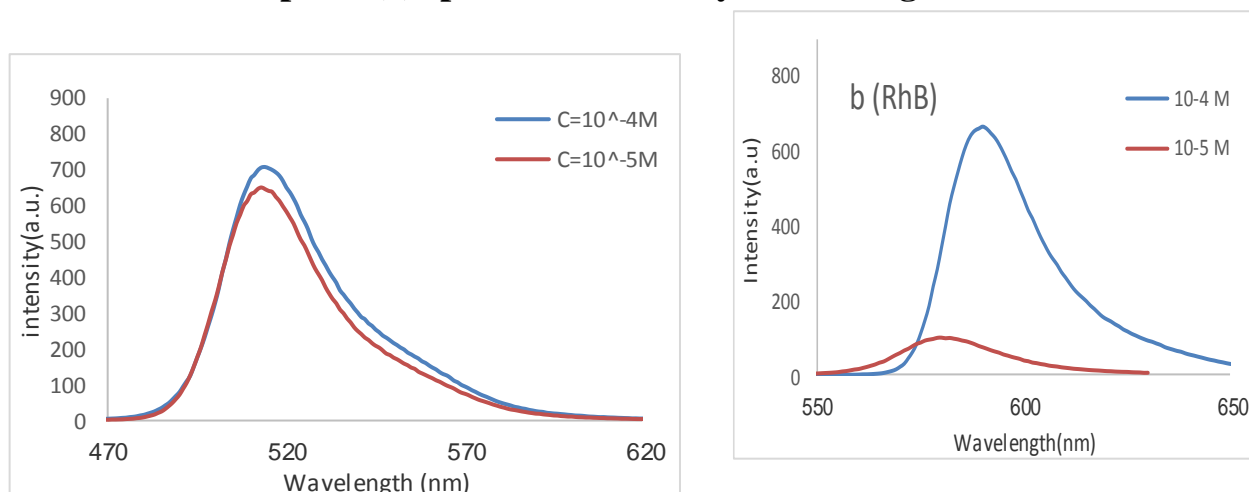

Fig.4. (a) The fluorescence spectra of Flu at concentrations of $10^{-4}M$ (1) and $10^{-5}M$ (2). (b) The concentrations of RhB at $10^{-4}M$ (1) and 10^{-5} M (2). Excitation wavelength of 420 nm was used for the measurement of each spectrum.
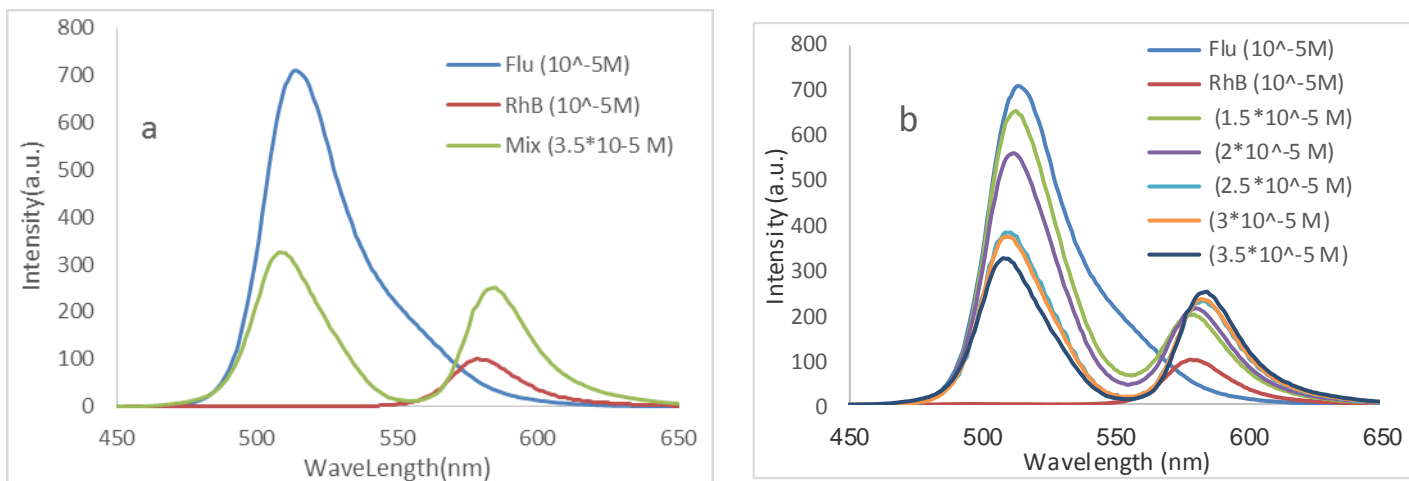
Fig. 5. (a) Fluorescence spectra of RhB (3), Flu (1), and Flu+ RhB (50:50) mixture (2) in aqueous solution. RhB concentration was 10^{-5} M; Flu concentration was 10^{-5}M. (b) Fluorescence spectra of a mixture of Flu and RhB with varying acceptor concentrations for a constant amount of donor. The effectiveness of the FRET process is shown below as a function of the acceptor concentration. Excitation wavelength of 420 nm was used for all of the spectra's measurements.

Table 1 presents the values of the spectral overlap integral $J(\lambda)$, the Forster radius (Ro), the donor–acceptor distance (r), and the energy transfer efficiency (E %) for FRET between Flu and RB at various acceptor concentrations in aqueous solution. The concentration of the donor was maintained at 10^{-5} M throughout the experiment (these values were derived from the spectral properties shown in Fig.3) (Supporting information).

Acceptor (RB) concentration (in M)	$J(\lambda) \times 10^{16} \ M^{-1}cm^{-1}nm^4$	E (%)	Ro (nm)	r(nm)
$1.5*10^{-5}$	1.61	0.078	6.36	9.43
$2*10^{-5}$	1.73	0.21	6.43	7.9
$2.5*10^{-5}$	1.89	0.45	6.52	6.7
$3*10^{-5}$	1.92	0.47	6.54	6.65
$3.5*10^{-5}$	2.08	0.54	6.62	6.44

4. Conclusion

The term "FRET" refers to the process through which energy is transferred between two fluorescent dyes. Research on Fluorescein and Rhodamine B in solution has been carried out to a satisfactory level. Experiments with UV-Vis absorption and fluorescence spectroscopy show that, and that the Flu and RhB absorption spectra overlap sufficiently to allow FRET from Flu to RhB. Additionally. There was a transfer of energy, The efficiency of the solution was greater with the mixed dye system, which consisted of 50% Flu and 50% RhB. The observed energy transfer was at the concentration $3.5 * 10^{-5}$ M, which is considered to be the best concentration and which had the transfer efficiency (0.54%). Another observation we can make based on the results is that the shapes of the pigments change according to the concentrations before and after the energy transfer.

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دراسة نقل طاقة الرنين الفلوري بين الفلورسين والرودامين ب في محلول مائي

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مستخلص البحث:

تمت دراسة نقل الطاقة لرنين التألق في محلول مائي بين صبغتين عضويتين ، الفلوريسين والرودامين ب. تم العثور على كلا الصبغين في محلول كمونومرات ، وفقًا للدراسات الطيفية. لوحظ نقل الطاقة في محاليل التصوير بالرنين الفلوري المحتوية على الفلورسين والرودامين ب. بتركيزات مختلفة من الصبغة المستقبلة RhB في نطاق   (1.5×10^{-5} م إلى 3.5 م. $\times 10^{-5}$ م). أظهرت الدراسات التي أجريت باستخدام كل من امتصاص الأشعة فوق البنفسجية والتحليل الطيفي الفلوري أن الصبغتين ، عند ذوبانهما في المحلول ، تظهران إلى حد كبير على هيئة مونومرات .