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Thermal lens technique to study the effect of pH on electronic energy transfer in organic dye mixtures

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Abstract

The effect of pH on the fluorescence efficiency of fluorescein is evaluated using thermal lens technique. Fluorescence efficiency increases as the sample becomes more and more alkaline. But when fluorescein is mixed with rhodamine B fluorescence quenching of fluorescein takes place with the excitation of rhodamine B. The electronic energy transfer in this mixture is investigated using Optical Parametric Oscillator as the excitation source. The effect of pH on the efficiency of energy transfer in fluorescein–rhodamine B mixture is presented.

Keywords: Thermal lens; Energy transfer; Organic dyes

1. Introduction

Transfer of excitation energy between molecules is an important controlling factor in various fields, ranging from radiation physics to biology. The energy transfer study has become a powerful spectroscopic tool for obtaining information about molecular excited states not obtainable by conventional spectroscopic methods. Its significance is well recognized in the studies of organic laser dyes for judging their suitability as wavelength shifters [1–3]. The effect of pH on energy transfer was essential for selecting efficient laser media.

When a molecule is excited to higher energy levels, transfer of energy from one molecule to another is one of the different ways to give off its excess energy. Transfer of energy between alike molecules in liquid solutions results in the self-quenching of the fluorescence of such solutions. Between unlike molecules, the transfer process results in the quenching of the fluorescence of one species and the sensitization of the fluorescence of the other. The main mechanisms involved in the electronic energy transfer in molecular systems are of (a) radiative and (b) nonradiative. In radiative transfer, the emission of a quantum of light by one molecule (donor) is followed by the absorption of the emitted photon by a second molecule (acceptor). Nonradiative transfer involves the simultaneous de-excitation of the donor and the excitation of the acceptor, a one step process and it is mainly due to coulombic or exchange interaction. Coulombic resonance energy transfer does not require physical contact of the interacting partners and it is dependent on the inverse sixth power of the intermolecular separation [4]. The probability of such transfer is large if the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor partially. The exchange interaction is a collisional transfer, that requires close approach of the donor and acceptor and the distance between them is of the order of collisional diameters (5–10 Å) [5,6].

Fluorescence energy transfer is a technique now widely applied to probe biological and other complex systems [7]. In this technique donor molecules are excited in the presence of acceptor molecules and the luminescence yield of donor and or acceptor are measured as a function of concentration. Hence this method cannot be applied to nonfluorescent samples. However, photothermal methods measure the photon energy, which has been converted into heat and hence it can be competently used for energy transfer studies. As the contribution from nonradiative processes is not directly measurable using the traditional optical detection methods, thermo-optic techniques have been adopted for this purpose. Among the various photothermal techniques thermal lensing is a versatile and viable technique [8], which

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can be used to study the energy transfer mechanisms in organic dye mixtures. One of the important features of this technique is that it is a nondestructive method that provides high sensitivity for measuring the absorbance of light in a large number of samples. This method has been used by various researchers for determination of fluorescence quantum yield, to record the thermal lens spectra, for the study of the multiphoton processes, to realize optical logic gates and for the determination of various thermo-optic parameters like thermal diffusivity, temperature coefficient of refractive index, etc. [9–14].

When a medium is illuminated with a Gaussian laser beam, some of the energy absorbed by the molecules in the ground state is excited to higher energy states. The nonradiative decay process causes the heating of the sample, creates a refractive index gradient in the medium, and the medium acts like a lens called the thermal lens (TL). The formation of a thermal lens will expand the beam radius, which can be detected at the far field as a corresponding reduction in the detected power. The thermal lens signal is given by the fractional change in the detected power at the far field or in terms of the change in the beam area [15].

2. Principle of the measurement

In order to study the pH dependence on efficiency of energy transfer between fluorescein (donor) and rhodamine B (acceptor) first of all we have studied the effect of pH on fluorescence quantum efficiency of fluorescein alone. Then fluorescein is mixed with rhodamine B and determined the energy transfer rate between these molecules at different pH values. First part of the principle of measurement gives the theory to find the fluorescence quantum efficiency of fluorescein and the second part deals with the theory of rate of transfer of energy between unlike molecules.

2.1. Quantum yield of the donor

The theory is based on the fundamental and simple concept of energy conservation [15–17]. The laser power incident on any sample (P_L) must be equal to the sum of the power transmitted, P_t, and the power emitted as luminescence, P_f, plus the power degraded to heat, P_{th}:

$$P_L = P_t + P_f + P_{th} \tag{1a}$$

where it is assumed that the reflection and scattering losses are negligibly small. The transmission is defined as:

$$T = \frac{P_t}{P_L} \tag{1b}$$

If fractional absorption is defined as:

$$A = 1 - T \tag{1c}$$

then we may write:

$$P_f = AP_L - P_{th} \tag{1d}$$

The emission quantum yield, by definition, is given by:

$$Q_f = \frac{P_f/\langle\nu_f\rangle}{(P_L - P_t)/\nu_L} \tag{2}$$

here, ν_L is the laser frequency and ν_f is the mean fluorescence emission frequency, evaluated as:

$$\langle\nu_f\rangle = \frac{\int \nu_f \, dN(\nu_f)}{\int dn(\nu_f)} \tag{3}$$

where the quantity $dN(\nu_f)$ is the number of photons emitted per second in an incremental bandwidth centered at ν_f. Eq. (2) may be rewritten in the form:

$$Q_f = \frac{\nu_L}{\langle\nu_f\rangle}\left[1 - \frac{P_{th}}{AP_L}\right] \tag{4}$$

where the ratio $\nu_L/\langle\nu_f\rangle$ takes account of the Stokes shift. This entails some amount of heat deposition in the sample even for 100% Q_f. Here the absorption can be measured by a spectrophotometer and P_{th} can be measured by the thermal lensing technique. If we can use a nonfluorescing sample in the same solvent as a reference, we can write $AP_L = P_{th}$ (for the reference sample). It is adequate if the reference sample is less than 0.5% fluorescing. This can also be the same sample under study because mostly it will exhibit a concentration quenching of fluorescence. The concentration at which fluorescence quenches should be found and in this case we can consider the entire excitation energy to be converted into nonradiative relaxation process and hence:

$$Q_f = \frac{P_f}{AP_L}\frac{\lambda_f}{\lambda} = \left(1 - \frac{P_{th}}{P_\alpha}\right)\frac{\lambda_f}{\lambda} \tag{5}$$

where $P_\alpha = AP_L$ and λ_f and λ are the peak fluorescence wavelength and the excitation wavelength, respectively. P_{th} is directly proportional to the TL signal (η) and P_α is directly proportional to the TL signal (η_α) corresponding to the concentration at which the fluorescence intensity is quenched to a value less than 0.5%. This concentration is determined by recording the fluorescence spectrum for a large number of concentrations and finding the concentration at which it quenches to nearly zero. Thus, the quantum efficiency can be calculated by:

$$Q_f = \left(1 - \frac{\eta}{\eta_\alpha}\right)\frac{\lambda_f}{\lambda} \tag{6}$$

2.2. Electronic energy transfer rate

Stern–Volmer developed a quantitative expression for the rate of electronic energy transfer due to long-range dipole–dipole interaction is given as [18]:

$$\frac{\phi_f^0}{\phi_f^A} = 1 + K_f\tau[A] \tag{7}$$

where ϕ_f^0 and ϕ_f^A are the quantum yield of fluorescence emission in the absence and presence of acceptor respectively, K_f the resonance transfer rate due to long range dipole–dipole interaction, $[A]$ the acceptor concentration and τ is the lifetime of the excited donor in the absence of acceptor. Thermal lens method

measure the photon energy which has been converted into heat while fluorescence observes the re-emitted photons and hence both thermal and fluorescence spectroscopy are complementary to each other. Hence, Eq. (7) can be modified as [19]:

$$\frac{\eta_L^0}{\eta_L^A} = 1 - K_L \tau [A] \quad (8)$$

where the thermal lens signal η_L^0 and η_L^A are measured by the fractional change in the detected power for donor alone and with the acceptor, respectively.

3. Experimental

Fluorescein is an important xanthene dye with large variety of technical applications. Due to its large absorption in the visible range and the availability of a variety of chemistries that can be used to conjugate it to functional biomolecules, fluorescein is widely used as fluorephore in biosciences [20]. It is also used to label primers in automated DNA sequencing [21] and fluorescein angiography turn out to be a very useful procedure for assessing retinal disease. Together with rhodamine it can be used in energy transfer measurements to determine distances within and between molecules [22].

The experimental setup of the dual beam thermal lens technique employed in the present investigation is shown in Fig. 1. In the dual beam configuration separate lasers are used for pump and probe beams. This technique is more advantageous since only a single wavelength (probe) is always detected and are needed no correction for the spectral response of the optical elements and detector. The excitation radiation employed in the present investigation is 470 nm radiation from an Optical Parametric Oscillator (Quantaray mopo-700). The power was attenuated to 5 mW so as to avoid aberration in the signal due to full-wave shifts. In addition, at these power levels we expect no multiphoton processes or nonlinear effects to take place in the medium. The formation of the thermal lens was probed with an intensity-stabilised helium–neon cw laser (JDS Uniphase model 1507) operating at 632.8 nm and with the power attenuated to 1 mW. The absorption of the molecule at the probe wavelength is negligible compared to that at the pump wavelengths. The probe beam is made to pass collinearly through the sample using a dichroic mirror. The sample was placed in a quartz cuvette of path length 1 mm and positioned at one confocal distance past the beam waist of the probe beam. This position is determined by a Z-scan method. The formation of the thermal lens causes the probe beam to expand and is detectable at the far field. This is detected as a change in the output voltage of a photomultiplier tube (PMT). The monochromator associated with the PMT (McPherson 275 with a model 789A controller) is set to operate at 632.8 nm so that it also serves as a filter. The beam is carefully positioned using mirrors and coupled to the entrance slit of the monochromator–PMT assembly using an optical fiber. The signal output from PMT is processed using a digital storage oscilloscope (Tektronix, TDS 220) and thus thermal lens signals are obtained. The present work is done at a temperature of 26 °C.

The absorption spectrum of donor and acceptor in ethanol are recorded with a UV–VIS–IR spectrophotometer (Jasco V-570). For the fluorescence study, the front surface emission is collected and focused by a lens to the entrance slit of a 1 m Spex monochromator, which is coupled to a PMT having an S20 cathode. The PMT output is fed to a lock-in amplifier (SR 850). The emission wavelength is scanned in the specified region (400–650 nm). The spectrum needs no correction since the monochromator has a flat response in the wavelength range from 200 to 800 nm.

First, the pH of the solvent is adjusted to 2 using concentrated HCl and then solvents having different pH ranging from 4 to 9 are prepared by adding appropriate amount of NaOH solution. Ethanol is used as the solvent since it has no absorption in the specified wavelength region. An accurately weighed amount of fluorescein and rhodamine B are dissolved in pH-adjusted ethanol separately to make the concentration of each to be 0.24 mmol l^{-1}.

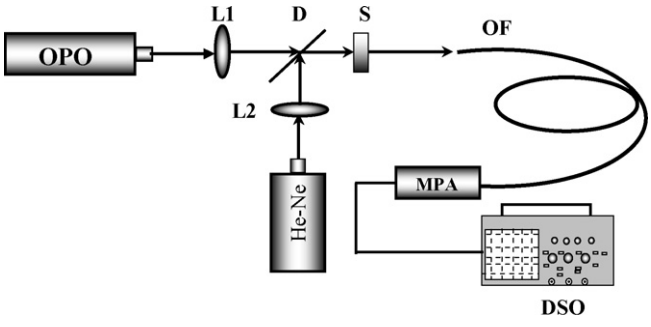

Fig. 1. Schematic diagram of the experimental set up. L_1, L_2, lens; DM, dichoric mirror; S, sample; OF, optic fibre, MPA, monochromator-PMT assembly; DSO, digital storage oscilloscope.

4. Results and discussion

Fig. 2 depicts the absorption spectrum of fluorescein at different pH values. It has maximum absorption at 454 and 482 nm since fluorescein exists in different structure forms. In

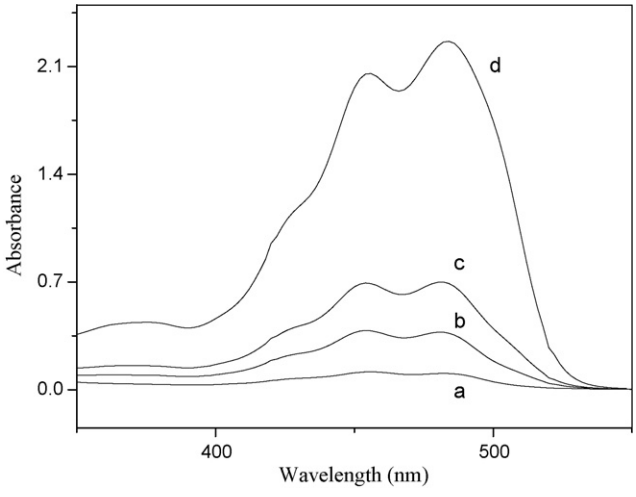

Fig. 2. Absorption spectra of fluorescein, for different pH values: (a) pH 6.5, (b) pH 7, (c) pH 7.7, and (d) pH 8.4.

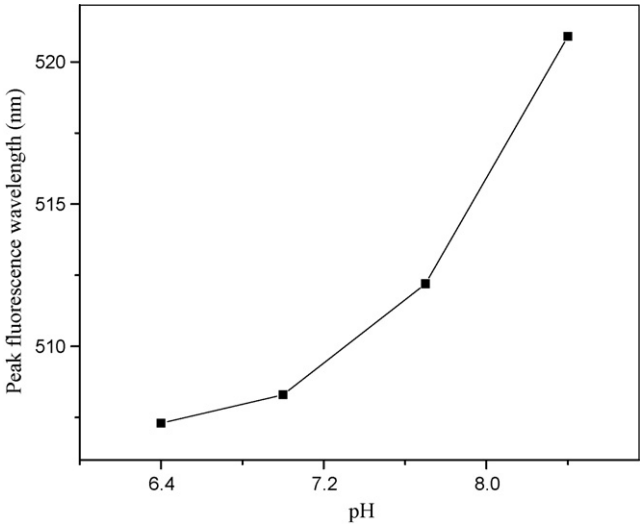

Fig. 3. The pH dependence of peak fluorescence wavelength of fluorescein.

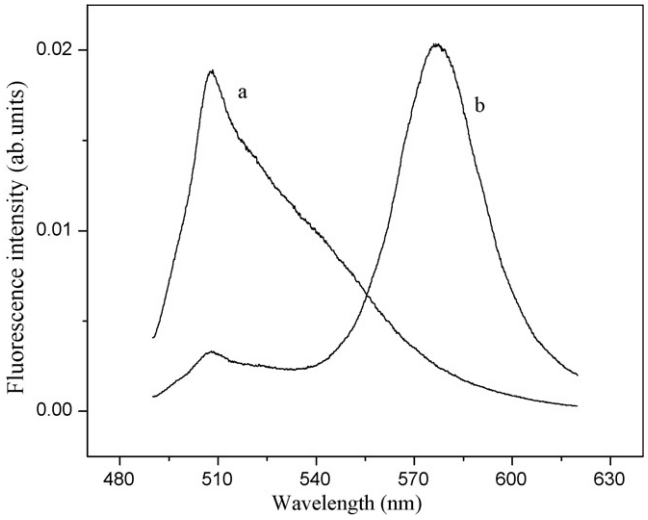

Fig. 5. Variation of fluorescence intensities: (a) fluorescein alone and (b) fluorescein–rhodamine B system.

the molecule of fluorescein the carboxyl phenyl is almost perpendicular to the xanthene ring and therefore is not conjugated to the xanthene group. Thus the ionization and alkylization of the carboxyl is not expected to influence the absorption spectrum in the visible region. Fig. 3 represents the peak fluorescence wavelength as a function of pH. The auxochrome OH attached to the chromophore shifts the peak fluorescence wavelength to longer wavelength.

The variation of fluorescence quantum yield with pH is shown in Fig. 4. Fluorescein exists in solution in a number of ionic and neutral forms such as: (a) cationic, (b) neutral, (c) monoanionic, and (d) the dianionic forms depending upon pH [23]. The low fluorescence yield of fluorescein in neutral and slightly acidic solutions is due to the formation of lactonic form of the dye from the excited state of the quinonoid form. In alkaline medium the anionic form attains the rigidity introduced by O^- bridge makes the molecule highly fluorescent. Martin and Lindqvist have reported that hydrogen bonding plays a central role in the nonradiative deactivation of the dye and that equilibrium is established in the excited state between the H-bonded dyes and nearly nonfluorescent free dyes [24].

When fluorescein is excited with blue light (470 nm), it will normally give off green light. However, if fluorescein is in close proximity to rhodamine B, it will transfer its total energy to the rhodamine B in a radiationless transfer. Now rhodamine B will give off red fluorescence as can be seen from Fig. 5. It is clear from Fig. 5 that the contribution to acceptor fluorescence due to direct excitation is very small.

To study the effect of pH on energy transfer between fluorescein and rhodamine B thermal lens signal corresponding to the pH-adjusted donor alone is evaluated. Now rhodamine B is added to fluorescein and thermal lens signal of the mixture is measured. In order to calculate the energy transfer rate constant in the mixture, the ratio of the TL signal of donor alone to the TL signal of the mixture are investigated. By considering the lifetime τ of the excited donor in the absence of acceptor as $\sim$3.7 ns and the concentration of the acceptor, the value of K_L is determined using Eq. (8). This is repeated for different values

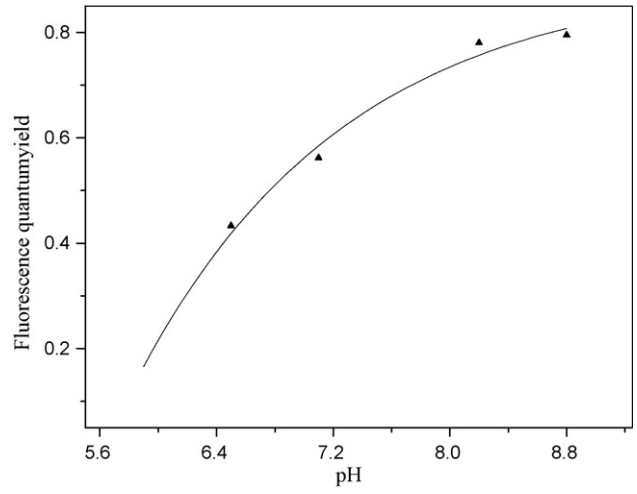

Fig. 4. Variation of quantum yield of fluorescein with pH.

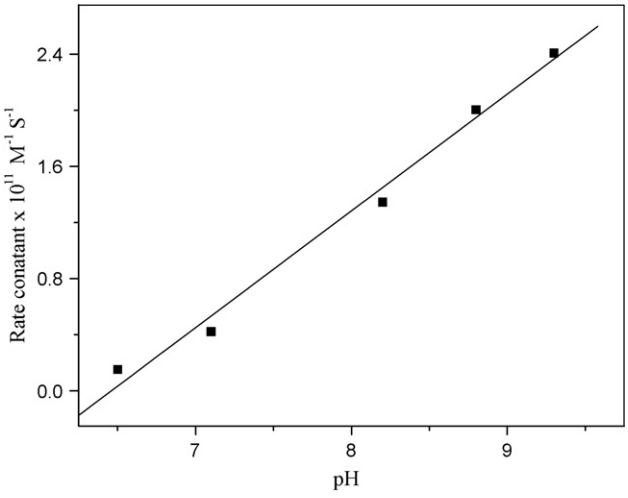

Fig. 6. Variation of energy transfer rate with pH.

of pH of the mixture and the variation of K_L with pH is shown in Fig. 6, which shows a linear dependence in pH. The non-radiative energy transfer is found to be increasing for a highly basic solution since absorption of fluorescein increases with pH. The relaxation from S1 to one of the vibronic levels of S0 will result in fluorescence. When fluorescein is in close proximity of rhodamine B fluorescence quenching of fluorescein and the excitation of rhodamine B takes place nonradiatively. The TL signal will be proportional to the total heat emitted by the sample excited to the S1 state.

5. Conclusion

The thermal lens technique has been successfully employed for the determination of the energy transfer rate between the donor and acceptor at different pH environment. The fluorescence quantum yield of fluorescein is evaluated at different pH values. The high value of the fluorescence quantum yield will render fluorescein as a fluorescent marker for biological applications.

Since the experiment is conducted at low concentration donor–donor transport and translational diffusion may influence energy transfer. The energy transfer by exchange interaction has been neglected because (a) the concentration of donor and acceptor was taken less than 2×10^{-3} mol l^{-1} and hence collisional encounter due to short range would be very rare (b) no new fluorescence peaks were detected in the mixture to indicate any fluorescence exciplex formation.

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