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ORIGINAL PAPER

Solvent Dependency in the Quantum Efficiency of 4-[(4-Aminophenyl)-(4-imino-1-cyclohexa-2, 5- dienylidene) methyl] Aniline Hydrochloride

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Abstract In the present work dual beam thermal lens technique is used for studying the solvent dependency on the quantum efficiency of a novel dye used for biomedical applications. The role of solvent in the absolute fluorescence quantum yield of 4-[(4-Aminophenyl)-(4-imino-1-cyclohexa-2, 5-dienylidene) methyl] aniline hydrochloride is studied using thermal lens technique. It is observed that the variation in solvents and its concentration results considerable variations in the fluorescence quantum yield. These variations are due to the non-radiative relaxation of the absorbed energy and because of the different solvent properties. The highest quantum yield of the dye is observed in the polar protic solvent-water.

Keywords Basic fuchsin · Solvent dependency · Quantum efficiency · Absolute fluorescence quantum yield · Thermal lens

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Introduction

The absolute fluorescence quantum yield (FQY) value is a very important parameter since it characterizes the lasing ability of dyes. The FQY is defined as the ratio of the number of photons emitted to the number of photons absorbed. Among the various methods for determining the FQY [1–7], the popular one is the comparative method in which a standard material of known quantum yield is used to determine the quantum yield of an unknown sample. However due to the lack of suitable standard materials, photothermal techniques such as thermal lens spectroscopic techniques are used for FQY calculations instead of comparative method [6–8]. The thermal lens technique (TL) is a versatile, viable and sensitive technique which can be used for the determination of FQY [9, 10] and thermal diffusivity [11]. One of the advantages of this method is that low absorptive samples can also be studied using this technique. The FQY is strongly dependent on environmental conditions such as solvent-solute interactions, temperature, pH, viscosity, concentration of dye etc. [12–15]. In the present experiment three solvents are used to study the variations of FQY with different dye concentrations using TL technique. In TL technique a Gaussian laser beam is used as pump beam and the non-radiative relaxation of the excited molecules is detected using a probe beam. The variations in the thermo-optical parameters of the sample are indicated by the intensity variations of the detected beam [16]. TL technique can also be used for calorimetric trace analysis [17], absorption spectroscopy of liquids [17, 18], multiphoton absorption studies [18, 19] investigation of the photochemical rate and absolute quantum yield [20] etc.

4-[(4-Aminophenyl)-(4-imino-1-cyclohexa-2, 5-dienylidene) methyl] aniline hydrochloride, known as basic fuchsin is a dye from the triaminotriphenylmethane family with molecular formula $C_{20}H_{20}ClN_3$ (Fig. 1) and is a mixture

of three dyes: Pararosaniline, Rosaniline, and Magenta II. The dye with molecular weight of 337.85 g/mol is purchased from Sigma Aldrich. The molecular structure is shown in Fig. 1. It is a dark colored powder in solid form and gives a magenta color when dissolved in solvents. This dye possesses anesthetic, bactericidal, and fungicidal properties and is inflammable in nature. Apart from its application as a coloring agent for textile and leather materials, this dye is also used for biomedical applications.

Experimental

The experimental set-up used for the present study is given in Fig. 2. A diode pumped solid state (DPSS) laser (100 mW, 532 nm) is used as the pumping source and a low power Helium-Neon laser (5 mW, 632 nm) is used as the probe beam. The intensity modulated pump beam and the probe beam is made collinear and passed through a quartz cuvette containing sample solution through an assembly of dichroic mirror and convex lens (focal length 20 cm). Since the absorption coefficient of the dye at 632 nm is very narrow as compared to that of the pump beam, the perturbation in refractive index due to probe beam can be neglected. The thermal lens signal generated is filtered to allow the passage of signal at 632 nm only. Optical density filters were used in between the pumping source and sample to vary the laser intensity. A DPSS laser with output power of 80 mW is used in the present for heating the sample. The thermal lens signal is then filtered and then collected by the tip of an optical fiber which in turn is connected to a monochromator-PMT-Digital Storage Oscilloscope assembly.

The stock solution of molarity $3*10^{-2}$ mol/L at the quenching concentration was prepared by an accurately weighed amount of dye and dissolving it in ethanol, water and acetone. From the stock solution samples of various concentrations from 10^{-2} mol/L to 10^{-5} mol/L are prepared.

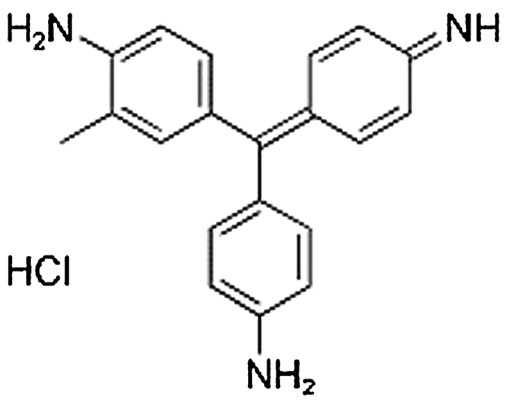

Fig. 1 Molecular structure of dye

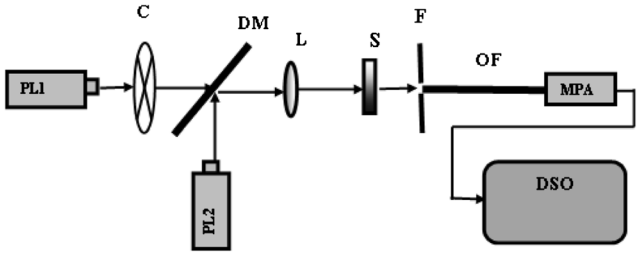

Fig. 2 Schematic representation of the experimental set-up. *PL1* Pump Laser (DPSS, 532 nm), *C* Chopper, *L* Lens, *DM* Dichroic Mirror, *S* Sample cell, *OF* Optical Fiber, *PL2* Probe Laser (He-Ne, 632 nm), *MPA* Monochromator and PMT Assembly, *DSO* Digital Storage Oscilloscope

Theoretical Background

The fluorescence quantum yield Q_f is the ratio of the number of photons emitted to the number of photons absorbed through fluorescence. The method is based on the principle of energy conservation. Let P_0 be the power of the incident beam, P_t be the power of the transmitted beam, P_e be the emission power and P_{th} be the thermal power degraded to heat. In the absence of any photochemical reaction the absorbed power can be written as a sum of transmitted power, thermal power degraded to heat and the emission power as given by Eq. (1).

$$P_0 = P_t + P_{th} + P_e \tag{1}$$

Considering reflection and scattering losses are negligibly small, the transmittance can be written as

$$T = \frac{P_t}{P_0} \tag{2}$$

having absorbance, A as

$$A = 1 - T \tag{3}$$

Hence the absorbed power, AP_0 is

$$AP_0 = P_{th} + P_e \tag{4}$$

Re-arranging Eq. (4)

$$P_e = AP_0 - P_{th} \tag{5}$$

In case of completely fluorescence quenched sample the quantum efficiency can be calculated by [18]

$$Q_f = \left(\frac{\lambda_f}{\lambda}\right)\left(\frac{P_e}{AP_0}\right) = \left(\frac{\lambda_f}{\lambda}\right)\left(1 - \frac{\eta}{\eta_\alpha}\right) \tag{6}$$

where $P_\alpha = AP_0$

The ratio of the fluorescence wavelength λ_f to the excitation wavelength λ corresponds to the Stokes shift. The thermal power degraded to heat, P_{th} is directly proportional to η, the thermal lens signal measured for each sample and P_α is proportional to thermal lens signal η_α corresponding to the concentration at which the fluorescence intensity is quenched

completely [21]. The thermal lens signal η has been measured as the variation of intensity at the center of the probe beam at a far field as a result of the TL effect in the medium [22].

Results and Discussions

TL studies were carried out to study the effect of solvent in the FQY of dye. The three different solvents used in the present study are water, ethanol and acetone. Acetone is a polar aprotic solvent which is having large dielectric constants and large dipole moments, but they do not participate in hydrogen bonding. Ethanol and water are polar protic solvents which participate in hydrogen bonding. These also have high dielectric constants and high dipole moments and possess O-H or N-H bonds. The solvent properties are given in Table 1. Number of researchers has studied the effect of solvent on the optical parameters of various dyes [23–27]. The absorption spectrum and fluorescence spectrum of the dye for a concentration 10^{-4} mol /L on the three solvents is given in Figs. 3 and 4 respectively. The absorption spectrum was taken using a Jasco U-570 UV/VIS/NIR spectrophotometer and fluorescence spectrum was taken using a Varian Cary Eclipse fluorescence spectrophotometer. It is observed that the absorption peak resides around 550 nm in each of the solvents. It is clear from figure that the absorption spectrum is independent on the nature of solvent used but there is a shift in the fluorescence spectrum. The peak fluorescence wavelength and FQY variations with varying concentration for the three solvents are shown in Figs. 5 and 6 respectively. The FQY and fluorescence spectra are dependent on solvent polarity, viscosity, rate of solvent relaxation, probe conformational changes, rigidity of the local environment, internal charge transfer, proton transfer and excited state reactions, probe–probe interactions, changes in radiative and non-radiative decay rates etc. [14].

Effects of Solvent Polarity and Dipole Moment

Emissions from fluorophores occur at longer wavelengths compared to the wavelength at which absorption occurs. This energy loss is due to various processes because of the light absorption. The fluorophore is excited to the first singlet state (S_1), usually to an excited vibrational level within S_1. The

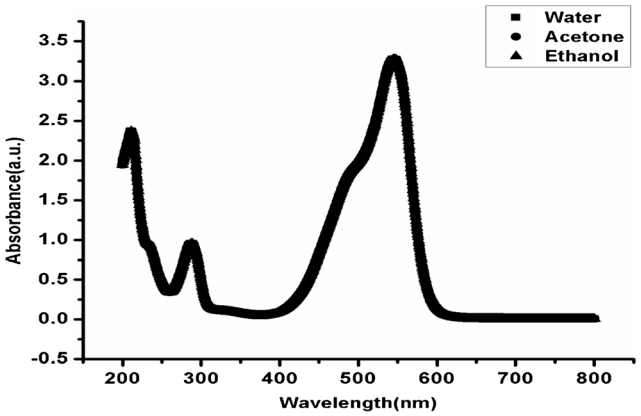

Fig. 3 Absorbtion spectrum of dye in the three solvents for a molarity of 10^{-4} g/L

excess vibrational energy is rapidly lost to the solvent. If the fluorophore is excited to the second singlet state (S_2), it rapidly decays to the S_1 state in 10^{-12} s due to internal conversion. Solvent effects shift the emission to lower energy due to stabilization of the excited state by the polar solvent molecules [28]. Generally the fluorophore has a larger dipole moment in the excited state (μ_E) compared to the ground state (μ_G). Because of the solvent relaxation or reorientation around the solvent dipoles, the energy of the excited state is decreased. As a result the emission is shifted to longer wavelengths as the solvent polarity is increased. Only fluorophores that are polar display a large sensitivity to solvent polarity.

Fluorescence lifetimes (1–10 ns) are usually much longer than the time required for solvent relaxation. For fluid solvents at room temperature, solvent relaxation occurs in 10–100 ps. For this reason, the emission spectra of fluorophore are representative of the solvent relaxed state. Absorption spectra are less sensitive to solvent polarity compared to emission spectra. Absorption of light occurs in about 10^{-15} s, a time too short for motion of the fluorophore or solvent. Absorption spectra are less sensitive to solvent polarity because the molecule is exposed to the same local environment in the ground and excited states [29]. In contrast, the emitting fluorophore is exposed to the relaxed environment, which contains solvent molecules oriented around the dipole moment of the excited state. Solvent dependent emission spectra can be described using Lippert equation (Eq. 7) which relates Stokes shift with refractive index (n) and dielectric constant (ε). These general solvent effects occur when a fluorophore is dissolved in any

Table 1 Properties of the three solvents

Solvent	Property				
	Dielectric constant	Refractive index	Polarity	Dipole moment	Viscosity (at 0 °C)
Water	78.3	1.33	9	1.85D	1.787 mPa·s
Ethanol	24.3	1.35	5.2	1.69D	1.720 mPa·s
Acetone	20.7	1.36	5.1	2.88D	0.4013 mPa·s

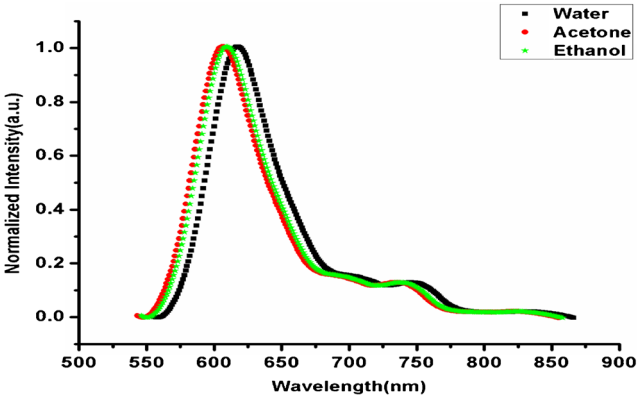

Fig. 4 Normalized fluorescence spectrum of dye in three solvents for a molarity of 10^{-4} g/L

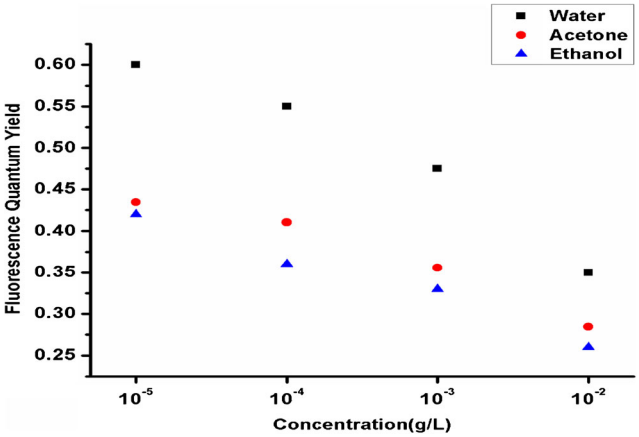

Fig. 6 Fluorescence quantum yield variations of the dye with varying concentrations

solvent, and are independent of the chemical properties of the fluorophore and the solvent.

According to Lippert equation,

$$\overline{\nu}_A - \overline{\nu}_F = \frac{2}{hc}\left(\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2-1}\right)\frac{(\mu_E-\mu_G)^2}{a^3} + constant \qquad (7)$$

The terms *h,* a and c *in* Eq. 7 corresponds to Plank's constant (=6.6256×10 $^{-27}$ ergs), radius of the cavity where the fluorophore resides and speed of light (=2.9979×10^{10} cm/s). $\overline{\nu}_A$ and $\overline{\nu}_F$ are the wavenumbers (cm^{-1}) of the absorption and emission respectively. The first term (ε– 1) / (2ε+1) describes the spectral shifts due to the reorientation of the solvent dipoles and to the redistribution of the electrons in the solvent molecules. The second term (n^2 – 1)/(2n^2+1) accounts for the redistribution of electrons. The difference of these two terms accounts for the spectral shifts due to reorientation of the solvent molecules and is defined as orientation polarizability (Δ*f*). An increase in *n* will decrease the energy loss and an increase in ε results in a larger difference between $\overline{\nu}_A$ and $\overline{\nu}_F$. The refractive index (*n*) is an instantaneous high-frequency response that occurs by light absorption and depends on the motion of electrons within the solvent molecules whereas the dielectric constant (ε) is a static property, which depends on both electronic and molecular motions. An increase in n (described by electronic polarizability) allows both the ground and excited states to be instantaneously stabilized by movements of electrons within the solvent molecules. This electron redistribution results in a decrease in the energy difference between the ground and excited states. An increase in ε (described by molecular polarizability) will also result in stabilization of the ground and excited states. However, the energy decrease of the excited state due to the dielectric constant occurs only after reorientation of the solvent dipoles. As a result, stabilization of the ground and excited states of the fluorophore depends on ε and is time dependent. The rate of solvent relaxation also depends on the temperature and viscosity of the solvent [15]. The excited state shifts to lower energy on a timescale comparable to the solvent reorientation time. The ground state and excited state are instantaneously stabilized by the redistribution of electrons and hence the refractive index and electronic redistribution has a comparatively minor effect on the Stokes shift. Most fluorophore have nonzero dipole moments in the ground and excited states. If the solvent is polar then the Stokes shift is increased because of the larger orientation polarizability which is a consequence of the increased dipole moment [28].

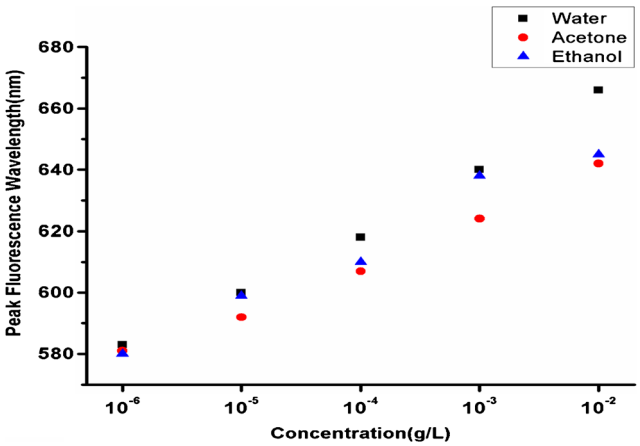

Fig. 5 Variations in the peak fluorescence wavelength with concentrations for the dye in the three solvents

Specific interactions, produced by the neighboring molecules can be determined by the specific chemical properties of both the fluorophore and solvent. These effects can be due to hydrogen bonding, acid–base chemistry or charge-transfer interactions etc. The solvent–fluorophore interactions occur either in the ground state or in the excited state which can be identified by examining emission spectra in a variety of solvents. If the interaction is occurred in the excited state, then the polar additive would not affect the absorption spectra. If the interaction occurs in the ground state, then some change in the absorption spectrum is expected. In the present case

absence of changes in the absorption spectra indicates that no ground-state interaction occurs. If the fluorophore and polar solvent are associated in the ground state, then an immediate spectral shift upon excitation is expected. If the fluorophore and polar solvent only associate in the excited state, then the appearance of the specific solvent effect will depend on the rates of diffusion of the fluorophore and polar solvent.

In addition to specific solvent–fluorophore interactions, many fluorophores can form an internal charge transfer (ICT) state, or a twisted internal charge transfer (TICT) state. If the fluorophore contains both an electron-donating and an electron-accepting group, there can be an increase in charge separation within the fluorophore upon excitation. If the solvent is polar, then a species with charge separation (the ICT state) may become the lowest energy state whereas if the solvent is non-polar, the species without charge separation, the so-called locally excited (LE) state, may have the lowest energy. Hence, the role of solvent polarity is not only to lower the energy of the excited state due to general solvent effects, but also to govern which state has the lowest energy. The quantum yield can be changed due to change in the rate of nonradiative decay (k_{nr}) or due to a conformational change in the fluorophore [30].

Effects of Temperature

Like solvent polarity, the temperature also plays a major role in Stokes shift [31, 32]. The effects of high temperature are similar to those of high polarity solvents. At low temperatures the solvent is more viscous and hence the time required for solvent reorientation is higher. Assume that upon excitation the fluorophore is initially in the Franck-Condon state (F). If the solvent relaxation rate is slower than the decay rate, then the emission spectrum is expected to be from the unrelaxed F state. If the solvent relaxation rate is higher, the emission spectrum is assumed to be from the relaxed state (R). At intermediate temperatures, emission and relaxation occurs simultaneously resulting in an intermediate spectrum.

Effects of Viscosity

Viscosity is also having a dramatic effect on the emission of fluorophores and hence on FQY. In a highly viscous environment, the decay is radiative since the molecule is not able to display internal rotation as needed for charge transfer whereas in case of less viscous environment the molecule displays internal rotation and charge transfer resulting in radiationless decay [33].

Conclusion

A quantitative description of the effects of environment on fluorescence emission spectra is perhaps the most challenging topic in fluorescence spectroscopy. No single theory or type of interactions can be used in all circumstances. The solvent dependency on the FQY of a novel dye of triaminotriphenylmethane family is studied. Effects of solvent polarity, dipole moment, changes in viscosity and temperature are discussed with respect to three solvents. The variation in FQY is expected to because of the variations in the properties of the solvents. Moreover the reduction in FQY at high concentrations is due to the higher aggregate formation.

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