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Thermal lens technique to evaluate the fluorescence quantum yield of a schiff base

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ABSTRACT The fluorescence spectrum of the schiff base obtained from salicylaldehyde and 2-aminophenol is studied using an argon-ion laser as the excitation source and its fluorescence quantum yield (Q_f) is determined using a thermal lens method. This is a nondestructive technique that gives the absolute value of Q_f without the need for a fluorescence standard. The quantum-yield values are calculated for various concentrations of the solution in chloroform and also for various excitation wavelengths. The value of Q_f is relatively high, and is concentration dependent. The maximum value of Q_f obtained is nearly 0.78. The high value of the fluorescence quantum yield will render the schiff base useful as a fluorescent marker for biological applications. Photostability and gain studies will assess its suitability as a laser dye.

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1 Introduction

Fluorescence quantum yield (Q_f) is one of the most important properties of fluorescent materials. It is a measure of the rate of nonradiative transitions that compete with the emission of light. From both theoretical and practical points of view, fluorescence quantum yield values are important. For example, they provide information on radiationless processes in molecules and in the assignment of electronic transitions. It is also of use in fluorimetric determination of materials, for determining their purity, and for judging their suitability as wavelength shifters and laser media. Its significance is well recognised in the studies of organic laser dyes because the knowledge of Q_f of such dyes and its concentration dependence are essential for selecting efficient laser media.

It is well known that the conventional measurements of Q_f require the use of accurate luminescence standard samples and comparison of the given sample with a standard for which the fluorescence yield is known [1]. However, the reliability of such relative determinations is limited both by the accuracy of the standard yield value and by the confidence that can be placed on the comparison technique. Even after making various corrections for system geometry, re-absorption, polarisation, etc., the accuracy of the quantum-yield values obtained from photometric measurements is rather poor. In order to evaluate absolute quantum efficiency, we have to consider both the radiative and nonradiative processes taking place in the medium. As the contribution from nonradiative processes is not directly measurable using the traditional optical detection methods, thermo-optic techniques such as photoacoustic [2, 3] and thermal lens methods have been adopted for this purpose. Measurements based on photothermal effects are capable of giving fluorescence yields of highly fluorescent solutions as well as solids with high accuracy and reproducibility.

It was Hu and Whinnery [4] who pointed out that when combined with conventional transmission data, a thermal blooming measurement permits calculation of luminescence quantum yield. Brannon and Magde [5] presented a detailed theory for the calculation of the luminescence quantum yield and reported successful results with experiments on fluorescein. However, these were all essentially single-beam thermal lens methods, where an auxiliary lens of suitable focal length is used to create a beam waist in the laser beam. Usually the sample cell is centred one confocal distance past the lens focus, where the laser-beam size is $\omega_1 = \sqrt{2}\omega_0$, where ω_0 is the minimum beam-waist radius. At this point the fractional change in the radius of curvature of the laser beam is largest for a given lens. 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 [6].

In the case of experiments where we have to change the excitation wavelength, the detector should be carefully chosen and corrected taking into account its wavelength response. The above problem can be overcome by the dual-beam technique [7–11]. The advantage is that the detection optics and the detectors can be optimised for a single convenient probe wavelength. This is particularly useful in recording thermal lens absorption spectra. The dual-beam thermal lens method has been used by various researchers for determination of fluorescence quantum yield, to detect small absorption, to record the thermal lens spectra, and for the determination of various thermo-optic parameters like thermal diffusivity, temperature coefficient of refractive index, etc. [12, 13]. In this paper, we report the determination of fluorescence quantum

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yield of a schiff base, exploiting the merits of the dual-beam technique.

2 Principle of the measurement

The theory is developed based on the fundamental and simple concept of energy conservation [5, 14, 15]. 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}:

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

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

$$T = \frac{P_t}{P_L}\,. \tag{2a}$$

If fractional absorption is defined as

$$A = 1 - T, \tag{2b}$$

then we may write

$$P_f = AP_L - P_{th}\,. \tag{2c}$$

The emission quantum yield, by definition, is given by

$$Q_f = \frac{P_f/\langle\nu_f\rangle}{(P_L - P_t)/\nu_L}\,; \tag{3}$$

here, ν_L is the laser frequency and $\langle\nu_f\rangle$ is the mean fluorescence emission frequency, evaluated as

$$\langle\nu_f\rangle = \frac{\int \nu_f \mathrm{d}N(\nu_f)}{\int \mathrm{d}n(\nu_f)}\,, \tag{4}$$

where the quantity $\mathrm{d}N(\nu_f)$ is the number of photons emitted per second in an incremental bandwidth centred at ν_f. Equation (3) may be rewritten in the form

$$Q_f = \frac{\nu_L}{\langle\nu_f\rangle}\left[1 - \frac{P_{th}}{AP_L}\right], \tag{5}$$

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,

$$Q_f = \frac{P_f}{AP_L}\frac{\lambda_f}{\lambda} = \left(1 - \frac{P_{th}}{P_\alpha}\right)\frac{\lambda_f}{\lambda}\,, \tag{6}$$

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 thermal lens (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

$$Q_f = \frac{\lambda_f}{\lambda}\left(1 - \frac{\eta}{\eta_\alpha}\right). \tag{7}$$

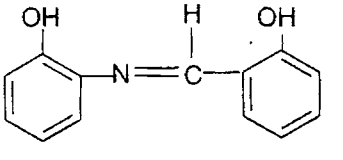
3 Experimental

The schiff base 2-hydroxyphenyl imino methyl phenol was synthesised from aminophenol and salicylaldehyde. Salicylaldehyde (1 mM) in methanol was refluxed with o-amino phenol (1 mM) for 2 h. The reaction mixture was cooled, filtered, collected, and recrystallised from methanol. It has a structure as shown in Fig. 1. It was dissolved in chloroform and filtered carefully to get the stock solution.

There are schiff bases having important applications in medicine and biotechnology. For example, a thiosemicarbazone possessing antitumour activity against L1210 leukaemia in mice was reported [16]. In addition, 2-acetylpyridine thiosemicarbazone is a class of compounds that has shown a broad spectrum of chemotherapeutic properties, including antimalarial and antitumour activity as well as antibacterial, antitrypanosomal, and antiviral activities. In our experiments, we are unable to explore the therapeutic application of the compounds; instead, we are more interested in the photonic-based applications.

The sample for the TL and fluorescence studies was prepared by diluting the stock solution to the required concentration. The absorption spectrum (Fig. 2) was taken using a Jasco V570 UV-VIS spectrophotometer. The most important characteristic of the sample is that it is extremely stable in its optical and physical properties. There is not much degradation in its fluorescence intensity or change in the spectrum with time. In addition, it is highly fluorescing. Its suitability as a laser dye has to be explored in future by carrying out photostability and gain studies.

The dual-beam thermal lens setup has been reported previously [10]. The thermal lens was produced by the excitation of the sample with a continuous-wave (cw) argon-ion



2-{(E)-[(2-hydroxyphenyl)imino]methyl}phenol

FIGURE 1 Structure of the schiff base 2-hydroxyphenyl imino methyl phenol

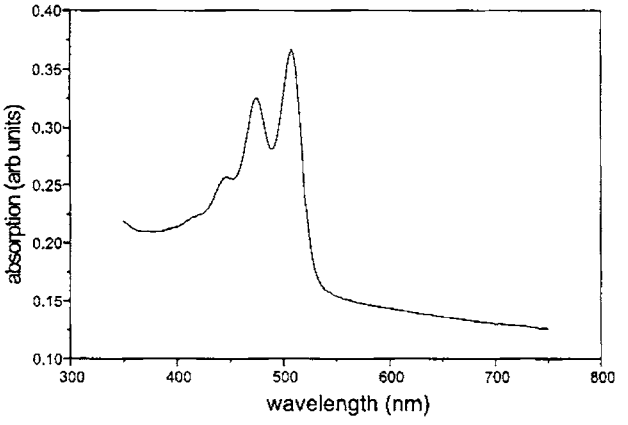


FIGURE 2 Typical absorption spectrum of the sample

laser (Spectra Physics model 171 with 270 exciter) that generates the pump field at wavelengths 457 nm, 476 nm, 488 nm, 496 nm, and 514 nm. The power was attenuated to 5 mW for all these wavelengths 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 pump beam is intensity modulated using a chopper operating at a low frequency of 8 Hz (model SR 540). This frequency was selected because we found that the maximum thermal lens signal will be obtained when the chopping frequency is between 7 Hz and 9 Hz. The formation of the thermal lens was probed with an intensity-stabilised helium–neon cw laser (JDS Uniphase model 1507-0) 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. Both the pump and the probe were focused using the same lens of focal length 35 cm, forming a mode-matched configuration, and are made collinear by a dichroic mirror.

The sample was placed in a quartz cuvette of path length 1 cm 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 fibre. The signal was processed using an SR 850 DSP lock-in amplifier and thus the values of η and η_α are obtained.

For fluorescence studies, the same sample cell and excitation source were used. The front-surface emission was collected and focused by a convex lens of focal length 10 cm to the same detection system. The fluorescence emission was recorded by wavelength scanning in the region from 520 nm to 570 nm. The spectrum needs no correction since the monochromator has a flat response in the wavelength range from 200 nm to 600 nm.

We have carried out thermal blooming and fluorescence studies of the material in the concentration range from 17.8 to 319 μM and have calculated the quantum yield using (7).

4 Results and discussion

A typical fluorescence spectrum of the material is shown in Fig. 3. It is recorded with the 514-nm excitation. The spectrum is invariant with respect to excitation wavelength, with the exception that the relative intensities reflect the wavelength dependence of the absorption coefficient. The excitation spectrum clearly explains this observation. Under the same conditions, the fluorescence-emission spectrum is independent of the excitation wavelength, due to the partial dissipation of excitation energy during the excited-state lifetime. The emission intensity is proportional to the strength of the absorption at the excitation wavelength. When an aromatic chromophore in solution, such as a dye, is excited by visible light, the transition will be to various vibronic levels of the first excited singlet state S_1 [17]. Molecules in the higher vibronic levels of S_1 will decay through nonradiative relaxation to lower vibronic levels of S_1. This means for every excited state of energy $E(S_v')$, that fraction of the energy corresponding to $E(S_v') - E(S_v'')/E(S_v')$ will immediately appear as heat coming from the sample. Radiative relaxation from ${S_1}^{v''}$ to one of the vibronic levels of S_0 will result in fluorescence emission. Fluorescence emission is affected by a variety of environmental factors, like interactions between the fluorophore and surrounding solvent molecules (dictated by solvent polarity), other dissolved inorganic and organic compounds, temperature, pH, and the localised concentration of the fluorescent species. There is an important parameter in the fluorescence studies, namely the Stokes shift, which represents the energy lost while the molecule was in the excited state. It is important for many reasons but, from a practical point of view, it allows the emitted fluorescence photons to be easily distinguished from the excitation photons, leading to the possibility of very low backgrounds in fluorescence studies. A high value of polarity of the solvent molecules will increase the Stokes shift, usually by several tens of nanometres. The relative polarity of chloroform, which has been used as a solvent in our case, is only 0.259,

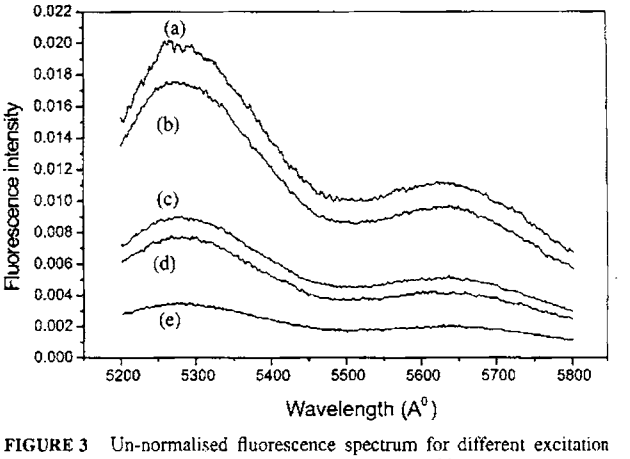


FIGURE 3 Un-normalised fluorescence spectrum for different excitation wavelengths (a) 514 nm, (b) 496 nm, (c) 476 nm, (d) 488 nm, (e) 457 nm

Concentration (μmol/L)	514 nm	496 nm	488 nm	479 nm	457 nm
17.8	528.25	528.25	528.25	528.15	528.15
49.7	532.26	532.26	532.27	532.79	532.54
70.9	532	532.2	532	532	532
128	534.51	534.51	534.8	534.5	534.5
159.5	535.99	536.1	535.9	535.5	535.6
212.6	538.34	538.23	538.04	538.41	538.04
319	539.3	539.4	539.25	539.2	539.2

TABLE 1 Variation of peak fluorescence wavelengths for various excitation wavelengths

which is rather too low to have any appreciable effect on this shift.

We have also observed a concentration quenching of fluorescence. Our results also show the usual concentration-dependent red shift in fluorescence emission (Table 1). This effect can be explained based on solvent relaxation and Stokes shift. For all the excitation wavelengths that we have considered, the change in Stokes shift is almost 11 nm in the concentration ranges in which the study was conducted. This loss of energy of the emitted photons is due to several dynamic processes which include energy losses arising from dissipation of vibrational energy, redistribution of electrons in the surrounding solvent molecules induced by the altered dipole moment of the excited fluorophore, re-orientation of the solvent around the excited-state dipole, and specific interactions between the fluorophore and the solvent, like dipole–dipole interaction.

The effects of the environmental parameters on fluorescence vary widely from one fluorophore to another, but the absorption and emission spectra, as well as the quantum yields, can be heavily influenced by environmental variables. In fact, the high degree of sensitivity in fluorescence is primarily due to interactions that occur in the local environment during the excited-state lifetime. A fraction, $1 - Q_f$, of all the energy absorbed by $S_1 \leftarrow S_0$ transitions will appear as heat. The photothermal lensing signal will be proportional to the total heat emitted by the sample excited to the S_1 state. The dependence of the thermal lens signal on concentration and on excitation

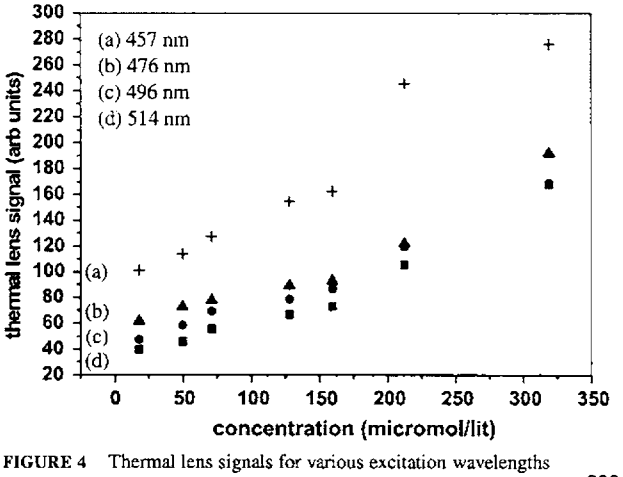


FIGURE 4 Thermal lens signals for various excitation wavelengths

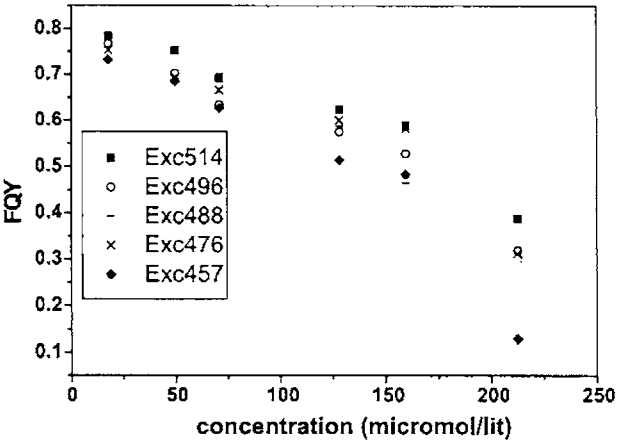


FIGURE 5 Calculated value of fluorescence quantum yield

wavelength is as shown in Fig. 4. The graph shows a large signal for the highest frequency of excitation, which is complemented by a smaller fluorescence signal at this frequency. This can take place when the excited molecule undergoes a radiationless transition to the various lower vibrational levels of S_1 or if there is energy transfer to a nearby chromophore (energy transfer – ET) or intersystem crossing (ISC) to a triplet state.

The calculated quantum yield is highest for a concentration of 17.8 μM, and varies slightly with excitation wavelength. It is seen that at higher concentrations the yield is significantly lower for 457-nm excitation. One reason is the increased probability of nonradiative de-excitation, which results in a higher thermal lens signal as seen from Fig. 4. The complementary nature of TL and fluorescence signals is also clear from Fig. 4 and Fig. 5, in which there are clear enhancements in the TL signal above 200 μM concentration corresponding to strong fluorescence quenching. To determine the cross section for other nonradiative decay processes like resonance energy transfer to other fluorophores, triplet to triplet excitation, etc., more detailed structural and chemical analysis is necessary. It is assumed that the aggregation of the compound in solution is negligible [18].

5 Conclusion

We have calculated the fluorescence quantum yield of the synthesised schiff base using a dual-beam thermal lens method. The moderately high value of the fluorescence and the extremely high stability of the material might render it useful as a fluorescent marker for biological applications. Gain studies and photobleaching studies will assess the suitability of the material as a laser dye.

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