Fluorescence quantum yield evaluation of strongly absorbing dye solutions as a function of the excitation wavelength

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Abstract
A simple method to evaluate fluorescence quantum yields based on corrected fluorescence emission spectra of dyes in dilute and strongly absorbing solutions using different excitation wavelengths is presented. The method is supported by a detailed knowledge of the apparatus geometry and energy profile of excitation.

Several recommended quantum counters were used (9,10-diphenylanthracene, rhodamines 101 and B, cresyl violet, oxazine 1 and 1-ethyl-4-(4-(p-dimethylaminophenyl)-1,3 butadienyl)-quinolinium perchlorate, (LDS 798) to cover the emission range from the UV to the visible and near-IR. A curve of correction factor f vs. the optical density of the samples was obtained enabling an accurate determination to be made of the fluorescence quantum yield as a function of concentration and excitation wavelength.

A case study of two squaraines (bis[4-(dimethylamino)phenyl]squaraine (HSQ) and bis [4-(dimethylamino)-2-methylphenyl]squaraine (MeSQ)) is presented. The quantum yields of these squaraines were obtained in saturated solutions in dichloromethane and in dilute samples at room temperature. The quantum yields determined are the same for high and low concentrations of the compounds indicating that no aggregation effects occur.

1. Introduction

Determination of the quantum yield of fluorescence is essential in studies involving concentration or excitation wavelength effects. Several methods for determining fluorescence quantum yields have been reported and a detailed discussion has been presented in refs. 1 and 2. Some techniques correct the fluorescence spectra for the self-absorption effect [3–5], which alters the shape of the emission in the high energy part and causes a shift of the maximum to the red, whereas other methods correct the fluorescence quantum yield for reabsorption effects [1, 6, 7].

Three common optical geometries are used in fluorescence emission or excitation measurements: front surface or reflection geometry, 90° geometry and transmission geometry. All of these geometries give the same fluorescence spectrum, quantum efficiency and lifetime for very dilute solutions (approximately 10⁻⁶ M) [1].

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An increase in concentration reduces the intensity $I_F^{\text{OBS}}$ because of self-absorption and this effect is at a maximum in the transmission geometry and is also important in the $90^\circ$ geometry.

In both cases the fluorescence light must cross significant amounts of unexcited solution, the optical path being determined by the geometry of the system, and an inner filter effect occurs depending on the analysis wavelength, reducing the amount of light reaching the detector. Whenever this effect is present, the evaluation of the quantum yields by integration of the corrected emission spectra causes obvious errors in the values of $\phi_F^{\text{OBS}}$, which are dependent on the concentration of the sample (optical density) and the geometry of the apparatus.

Reflection geometry (front face) is used for fluorescence studies of more concentrated solutions where the self-absorption effect can be minimized by excitation at the maximum absorption, thereby decreasing the penetration depth $\delta$ of the exciting radiation. A decrease in the optical density of the sample implies that the penetration depth $\delta$ of the exciting radiation will increase and the self-absorption correction will become increasingly important.

In this paper we describe a method which has been developed to correct the intensities observed at different excitation wavelengths; the intensities are related to the different optical densities of the sample and also depend on the geometry of the system. The method is based on a detailed study of the dependence of the fluorescence intensity on the optical density of the sample using recommended quantum counters in the UV, visible and near-IR regions, in order to evaluate fluorescence quantum yields of strongly absorbing samples.

2. Instrumentation and techniques

2.1. Experimental details

Rhodamine 101, oxazine 1, oxazine 170 and LDS 798 were purchased from Exciton (dye laser grade); rhodamine B and cresyl violet were obtained from Aldrich (dye laser grade); 9,10-diphenylanthracene was purchased from Nuclear Enterprises Ltd. (scintillation grade). All of these compounds were used without further purification. Purity was confirmed by thin layer chromatography (TLC) (one spot on Kieselgel 60 F254, Merck). Ethanol was of spectroscopic grade (Romil Chemicals).

The experimental arrangement for obtaining the fluorescence emission spectra (front face geometry) is shown schematically in Fig. 1.

A xenon lamp (450 W) was used for excitation (Muller Electronick Optic, model SVX 1450 + LAX 1450) together with an excitation monochromator (Hilger and Watts, model D2330). For analysis in the visible region, a Jobin Yvon monochromator (model H20) and a Hamamatsu photomultiplier (PM) (R928) (as detector) were used. This detection system was connected to an amplifier (Par model 134) and the data acquisition and control of the system were performed with a microprocessor equipped with a 12 bit, analogue and digital input/output (I/O) board (Data Translation, model DT 2811). Scanning was performed using step motors and data were acquired every 0.5 or 1.0 nm depending on the run, and stored on the hard disc of the microcomputer.

A second detection system was also installed, and was routinely used for studies in the UV region. It consisted of a double grating monochromator blazed in the UV (Bausch and Lomb) with an EMI 9635QB PM tube connected to a Par 134 amplifier.

This detection system was used to monitor the intensity of radiation $I_0(\lambda_{exc})$ which split the excitation beam into a main excitation beam and a reference beam incident
on a suitable quantum counter. The apparatus could be used in a double-beam mode, using the beam splitter, or converted into a single-beam apparatus using a totally reflecting aluminium-coated mirror instead of the beam splitter (see Fig. 1).

Front face geometry enabled us to obtain emission from samples with high or low optical density at the exciting wavelength. Figure 2 shows the optical paths of excitation and emission radiation in a rectangular cell (1 cm) and defines the angles relevant to the present study.

2.2. System energy profile for excitation

The intensity of the excitation light varies with the selected wavelength; this dependence originates from the lamp profile, but is also affected by the efficiency of the excitation monochromator and the geometry of the system.

An evaluation of the system energy profile is required to determine accurate quantum yields at different excitation wavelengths and to obtain corrected excitation spectra. Some fluorescent dyes have recently been recommended for use as quantum
counters to extend the correction range from the visible to the near-IR region \[8, 9\]; some of these compounds were used here, namely oxazine 1, cresyl violet and LDS 798. In the visible region rhodamines 101 and B were used and 9,10-diphenylanthracene (DPA) was employed in the UV. Ethanol was used as solvent in all cases, except with DPA where cyclohexane provided better solubility. The limiting factor for low concentrations results from the minimum absorption of the compound. The recommended minimum optical densities should not be below ten to obtain a good energy profile [8] (preferably higher than 20 for a 5% error in the energy profile as will be discussed in Section 3.1). Owing to this, the solubility of the dye is an important limiting factor in its use as a quantum counter; it should also possess a quantum yield which is independent of the excitation wavelength.

At high dye concentrations, the excitation spectra reflect the energy profile of the excitation system, and are independent of the extinction coefficient of the dye \((I_F \propto I_0 \phi_F)\). However, at low optical densities (lower than 0.02) there is a linear dependence of the fluorescence intensity on the optical density \((I_F \propto I_0 \phi F2.303s(\lambda_{exc})Cl)\). Thus, in the latter case, the excitation spectra reflect the normal ground state absorption spectra provided that \(\phi_F\) is independent of \(\lambda_{exc}\).

The system energy profile was obtained by setting the emission monochromator in the low energy part of the fluorescence emission spectra of the dye, and never at the fluorescence emission maximum, since in the latter self-absorption distorts the energy profile. The excitation monochromator was then scanned in the calibration range, usually from 250 nm until the end of absorption of the dye.

Some typical results are shown in Fig. 3. Curve (a) shows the calibration of the excitation system in the UV region and curves (b)–(e) extend the calibration to the visible and near-IR regions.

Care must be taken when choosing a suitable energy profile since, for some of the compounds tested here, deviations were found in these curves, usually corresponding to
Fig. 3. Energy profiles of the excitation system obtained with different quantum counters: (a) 9,10-diphenylanthracene in cyclohexane (5×10^{-2} M); (b) rhodamine B in ethanol (1.0×10^{-2} M); (c) rhodamine 101 in ethanol (8.5×10^{-3} M); (d) oxazine 1 in ethanol (6.0×10^{-3} M); (e) LDS 798 in ethanol (5.0×10^{-3} M).

to the region where the extinction coefficients of the dyes were at a minimum. However, the good agreement between the curves (a)–(e) in Fig. 3 (except in the region of minimum molar absorptivity of the dyes: 450–530 nm for oxazine 1; 380–450 nm for LDS 798) shows that these dyes can be used as quantum counters as expected [8, 9]. We can determine the true energy profile of the apparatus by normalizing curves (b)–(e) to the maximum intensity (λ≈470 nm) and neglecting the part of the curve obtained with the dye in the region of the minimum optical density. The resulting energy profile is presented in Fig. 4.

Optical densities higher than 20 are important to avoid geometrical errors. As an example, it was observed that 1,1',3,3,3',3'-hexamethylindotricarbocyanine (HITC) iodide in ethanol did not give a good energy profile and could not be used in the near-IR region, although HITC perchlorate in methanol has recently been reported to be a reasonably good quantum counter [9]. Oxazine 1 (perchlorate) in a saturated solution in ethanol at room temperature (approximately 6×10^{-3} M) showed some irregularities in the energy profile connected with an apparent decrease in φɛ (about 20% at approximately 485 nm). Recently reported data [10] have shown an excellent spectral flatness (better than 1%) of oxazine chloride (Basic Blue 3) in methanol. It can easily be shown that, for a saturated solution of oxazine 1 in ethanol at room temperature, the optical density is low (approximately 8.5 at 485 nm).

If corrections are needed for only small parts of the spectral range, the individual curves in Fig. 3 may be used, but great care must be taken in choosing the quantum counters to avoid the geometrical errors that may occur.
The profile in Fig. 4 is routinely used to obtain corrected excitation spectra in our laboratory. It is also used to check the detection system or to evaluate if a new dye can be used as a quantum counter in a large or restricted range of wavelengths.

2.3. **Correction of the fluorescence emission spectra**

The energy profile of Fig. 4 can also be used to correct fluorescence intensities, obtaining data independent of the intensity of excitation $I_0(\lambda_{exc})$.

**Fully corrected fluorescence emission spectra** are necessary so that the data are independent of the apparatus used. The corrected emission spectra are used to make accurate measurements of the quantum yields of the compounds, aggregation, etc.

A detailed description of the methods used to obtain the appropriate fluorescence emission correction curves can be found in the literature [8, 9, 11], as well as a discussion of the use of different fluorescent dyes as quantum counters or emission standards [12–14].

Basically, the methods consist of determining the ratio between a synchronously scanned spectrum (the emission and excitation monochromators are simultaneously scanned) for a standard reflector and an energy profile obtained with a good quantum counter. This ratio provides a correction curve which eliminates the influence of wavelength-dependent instrument parameters that affect the observed emission spectra of every sample.

An alternative simple approach for correcting the spectra can be obtained by using a correction curve which is the product of the relative efficiency of the analysing grating monochromator as a function of the wavelength and the relative quantum efficiency of the photomultiplier, which is also dependent on the wavelength, provided that both curves are accurately known.

Both methods can be carried out routinely if a microprocessor is available to acquire and store the data and also to perform the calculations rapidly. Correction curves obtained using quantum counters and those obtained by the simple method described above show reasonable agreement, and the major differences exist in regions
where the extinction coefficient of the chosen quantum counter is small. In this work the use of the simpler method was found to be perfectly adequate.

Recommended standards for fluorescence emission in the visible and near-IR regions [12-14] are difficult to use since, as a rule, emission spectra are often greatly distorted by self-absorption and therefore many spectra reported in the literature are probably incorrect. Aggregation effects and acid-base equilibria of dyes may also be an important source of error.

2.4. Quantum yield determination

The fluorescence quantum yield of an unknown sample can be evaluated using different equations depending on the optical density of the sample. In the case of optically dense measurements [2]

\[
\phi_u = \frac{A_u n_u^2 I_0^u(\lambda_{exc})}{A_s n_s^2 I_0^s(\lambda_{exc})}
\]

where the subscripts \(u\) and \(s\) refer to the unknown and standard samples, \(A\) is the integrated area under the corrected emission spectrum, \(I_0^u(\lambda_{exc})/I_0^s(\lambda_{exc})\) is the relative intensity of the exciting light at the excitation wavelength for each sample and \(n\) is the refractive index of the solution.

Several assumptions are made in eqn. (1), i.e. the optical paths for the unknown and standard samples are the same, the fluorescence intensity is proportional to the intensity of the light absorbed, the excitation beams are monochromatic and the reflection losses are the same for the unknown and standard samples.

A geometrical correction may be necessary to compensate for the different penetration depth of the exciting light into the sample which changes as the optical density varies. These corrections may be performed as in ref. 3, although in most apparatuses, including ours, this influence is reduced since the depth of penetration \(\delta\) is usually much smaller than the distance from the face of the cell to the aperture of the detector (see Fig. 2). However, we must remember that the fluorescence emission intensity depends on the absorbed light and this absorption occurs according to Beer's law. At the same time the fluorescence intensity also depends on the geometry of the detection system. All of these factors must be considered when an accurate evaluation of the fluorescence emission intensities is required, as will be shown in Section 2.5 (“factor \(f\) correction method”).

If the solvent used in the standard solution and in the unknown sample is the same, no refractive index correction is needed in eqn. (1). However, when different solvents are used the \(n_u^2/n_s^2\) correction must be included [1, 12].

The \(n_u^2/n_s^2\) term in eqn. (1) is only strictly valid for normal viewing and a more accurate correction can be made according to ref. 1. Again, since in our geometry \(\beta' = 68^\circ\) (see Fig. 2), the deviations from the \(n_u^2/n_s^2\) correction term are generally negligible.

2.5. The factor \(f\) correction method

The intensity of the exciting radiation absorbed by the dye at the excitation wavelength \(\lambda_{exc}\), in a layer \(dx\) at a distance \(x\) from the face of the cell (see Fig. 2), is given by

\[
-dI^{ABS}(\lambda_{exc}) = I_0(\lambda_{exc}) \mu(\lambda_{exc}) C e^{-\mu(\lambda_{exc})Cx} \ dx
\]

where \(I_0(\lambda_{exc})\) is the intensity of the exciting radiation at \(\lambda_{exc}\), \(C\) is the concentration of the absorbing species and \(\mu(\lambda_{exc})\) is related to the extinction coefficient by \(\mu(\lambda_{exc}) = 2.303\epsilon(\lambda_{exc})\). The emitted fluorescence is then
\[ dI_F(\lambda_{\text{exc}}, x, x + dx) = S \phi_F \ dI_{\text{ABS}}(\lambda_{\text{exc}}) = I_0(\lambda_{\text{exc}}) S \mu(\lambda_{\text{exc}}) C \phi_F \ e^{-\mu(\lambda_{\text{exc}})Cx} \ dx \]

where \( S \) is a geometrical factor. In eqn. (3), the fluorescence self-absorption is not included in order to simplify the analysis. The observed emitted fluorescence is then

\[ I_F^{\text{OBS}}(\lambda_{\text{exc}}) = \int_0^{\delta-x_{\text{max}}} dI_F = I_0(\lambda_{\text{exc}}) S \phi_F \{1 - e^{\mu(\lambda_{\text{exc}})C\delta}\} \]

where \( \delta = z/\sin \alpha \) is the maximum depth of penetration of the exciting radiation (\( \delta \) is the maximum value for \( x \) as can be seen in Fig. 2).

When exciting at a chosen wavelength \( \lambda_{\text{exc}} \) where the dye exhibits a high extinction coefficient and by using high concentrations of the dye, we have

\[ \varepsilon(\lambda_{\text{exc}})C \delta = 1 \]

and then eqn. (4) becomes

\[ I_F^{\text{OBS}}(\lambda_{\text{exc}}) = I_0 \phi_F S \]

We can now define a correction factor \( f \), which is the ratio of the observed intensity of fluorescence in the general case \( I_F^{\text{OBS}}(\lambda_{\text{exc}}) \) to the maximum value for the intensity obtained at very high optical densities

\[ f = \frac{I_F^{\text{OBS}}(\lambda_{\text{exc}})}{I_F^{\text{OBS,MAX}}(\lambda_{\text{exc}})} = 1 - e^{-2.303 \varepsilon(\lambda_{\text{exc}})C\delta} \]

This equation is valid in the absence of aggregation or concentration quenching effects. Self-absorption is also not included in eqn. (7). An \textit{a posteriori} introduction of all of these effects may be carried out in the data treatment.

The \( f \) factor affects the fluorescence emission intensity and derives from a small or large value of penetration depth of the exciting radiation. \( f \) varies with the geometry of each apparatus by the term \( \delta = z/\sin \alpha \), with the solvent since \( n = \sin(90 - \alpha)/\sin(90 - \alpha') \) and with the value of \( \varepsilon(\lambda_{\text{exc}})C \).

Equation (1) may now be written as

\[ \phi_u = \phi_s \frac{A_u}{A_s} \frac{n_u^2}{n_s^2} \frac{I_0(\lambda_{\text{exc}})^2}{I_0(\lambda_{\text{exc}})} \frac{1}{f} \]

An accurate experimental determination of the \( f \) factor for a given apparatus means that it can be used for samples with high or low optical density (front face geometry).

Fluorescence intensities may only be compared when emissions are free from all geometrical effects introduced by different depths of penetration of the exciting radiation into the sample, according to Beer's law, and by different viewing angles of emission detection, which are characteristic of each apparatus. The experimental determination of \( f \) as a function of the optical density enables the emission spectra to be corrected to a situation where \( \delta \approx 0 \) (see eqn. (5)), thus isolating possible aggregation or concentration quenching effects.

2.6. \textit{The self-absorption effect}

Correction for self-absorption effects in the fluorescence emission spectrum of a certain compound can be performed using equations similar to eqns. (2), (3) and (4),
by introducing the term $e^{-\mu(A_{\text{exc}})\delta C}$, which corrects the blue part of the emission spectrum (this is affected by self-absorption effects for each $A_{\text{em}}$). Equation (4) then becomes

$$I_F^{\text{OBS}}(\lambda_{\text{exc}}, \lambda_{\text{em}}) = I_0 S \phi_F \frac{\mu(\lambda_{\text{exc}})}{\mu(\lambda_{\text{exc}}) + \mu(\lambda_{\text{em}}) \sin \alpha / \sin \beta} \{1 - e^{-(\mu(\lambda_{\text{exc}}) + \mu(\lambda_{\text{em}})(\sin \alpha / \sin \beta)\delta C)}\}$$

(9)

and the self-absorption correction may be included by the use of [3-5]

$$I_F(\lambda_{\text{exc}}, \lambda_{\text{em}}) = I_F^{\text{OBS}} \frac{\mu(\lambda_{\text{exc}}) + \mu(\lambda_{\text{em}}) \sin \alpha / \sin \beta}{\mu(\lambda_{\text{exc}})} \times \frac{1 - e^{-\mu(\lambda_{\text{exc}})\delta C}}{1 - e^{-(\mu(\lambda_{\text{exc}}) + \mu(\lambda_{\text{em}}) \sin \alpha / \sin \beta)\delta C}}$$

(10)

Equation (10) shows that the self-absorption correction depends on the geometry of the apparatus through the values of $\delta$, $\sin \alpha / \sin \beta$ and $\lambda_{\text{exc}}$ which affects $\varepsilon(\lambda_{\text{exc}})$. It also depends on the overlap between the spectral emission and absorption through $\varepsilon(\lambda_{\text{em}})$. For a very high concentration of the dye and for high values of the extinction coefficient where

$$\mu(\lambda_{\text{exc}}) \gg \mu(\lambda_{\text{em}}) \frac{\sin \alpha}{\sin \beta}$$

(11)

then

$$I_F(\lambda_{\text{exc}}, \lambda_{\text{em}}) = I_F^{\text{OBS}} \ (f = 1)$$

(12)

and, for these conditions, the fluorescence emission spectrum is the same as that obtained for very dilute samples where self-absorption effects are unimportant, provided that no aggregation occurs.

Results and discussion

3.1. Determination of the $f$ factor

Several experiments were performed in order to verify the validity of eqn. (7) and to determine a curve for $f$ as a function of the optical density of the sample. Different compounds should provide the same $f$ curve. Quantum yields can easily be determined as a function of concentration or excitation wavelength using eqn. (5), provided that accurate values of $f$ and accurate energy profiles (to determine $I_0(\lambda_{\text{exc}})/I_0^0(\lambda_{\text{exc}})$) are known.

Several recommended quantum counters were used: DPA for emission in the UV range (approximately 400–500 nm), rhodamines B and 101 for the visible region (approximately 550–700 nm) and cresyl violet, oxazine 1 and LDS 798 for the visible and near-IR regions. Some reasons for choosing these compounds are as follows: 9,10-diphenylanthracene does not form excimers [1]; cresyl violet shows no concentration dependence up to approximately $10^{-3} \text{ M}$ [14] and oxazine 1 does not form molecular aggregates [3]; the fluorescence emission maximum of LDS 798 is not dependent on concentration and is not shifted on dilution [9], indicating that self-absorption is unimportant; rhodamine 101 has a quantum efficiency of almost unity, independent of solvent and temperature [1]. Although some problems occur with rhodamine B in ethanol due to the presence of two forms of the dye (determined by the acid–base equilibrium [13]), this compound was also employed since it has been the most popular and widely used quantum counter up to the present time.
The $f$ values were determined from emission spectra obtained for different concentrations of the compounds and different excitation wavelengths in order to cover a wide range of optical densities. Special care was taken to avoid self-absorption errors by choosing, in each case, an analysis wavelength in the red part of the spectrum. The fluorescence emission intensities were normalized to unity at the highest optical density used.

Figures 5–10 show the results obtained for the experimentally determined $f$ values superimposed on a unique curve calculated using eqn. (7). The consistency of the $f$ values is demonstrated in Figures 5–10.
results for compounds emitting in the UV, visible and near-IR regions is very good and strongly supports the use of this equation.

In the equation, the penetration depth $\delta$ of the exciting radiation increases when the optical density decreases, and may be calculated from the $f$ data by

$$
\delta = \frac{-\ln\{1 - f(\lambda_{\text{exc}})\}}{2.303 \epsilon(\lambda_{\text{exc}})C}
$$

(13)
Fig. 9. \( f \) values as a function of the optical density for rhodamine B in ethanol. \( \lambda_{\text{anal}} = 610 \) nm (\( \cdots \)). Calculated curve (eqn. (7)) (---).

Fig. 10. \( f \) values as a function of the optical density for 9,10-diphenylanthracene in cyclohexane. \( \lambda_{\text{anal}} = 440 \) nm (\( \cdots \)). Calculated curve (eqn. (7)) (---).

It is interesting to note that the penetration depth \( \delta \) is 0.03 cm for \( \epsilon C = 100 \), 0.09 cm for \( \epsilon C = 10 \), 0.25 cm for \( \epsilon C = 1 \) and 0.70 cm for \( \epsilon C = 0.1 \). These results clearly show that the use of 1 mm cells to avoid geometrical problems is incorrect, since \( f \) changes by about 20% from a very high optical density down to \( \epsilon C \approx 8 \), where \( \delta \) is approximately 1 mm.

Another important conclusion from the \( f \) curves is that at \( \epsilon C = 20 \), \( f = 0.95 \). This means that for optical densities above this value, the error will be less than 5% in the energy profile determinations. If quantum counters with optical densities lower
than 20 at minimum absorption are used, $f$ corrections are needed. The omission of this correction causes obvious errors in energy profiles or correction curves for fluorescence emission spectra.

3.2. Fluorescence quantum yield determination of some squaraines

Quantum yield determinations of squaraines have recently been reported [15, 16] using very low concentrations (approximately $10^{-7}$ M) of the dyes in dichloromethane; sulphorhodamine 101 in ethanol was used as reference ($\phi_F = 1.0$). In order to investigate the concentration or $\lambda_{\text{exc}}$ effects on the fluorescence of the squaraines, we carried out a detailed study over a large range of concentration (from saturated solutions down to approximately $10^{-6}$ M; scanning the excitation wavelength) using the squaraines bis[4-(dimethylamino)-2-methylphenyl]squaraine (MeSQ) and bis[4-(dimethylamino)phenyl]squaraine (HSQ) with dichloromethane as solvent.

As reference we chose oxazine 1 and oxazine 170 which emit in a very similar region to the squaraines (Fig. 11). The oxazine emission obtained at $\lambda_{\text{exc}} = 600$ nm in ethanol (using saturated solutions at room temperature) is the same as that obtained for very dilute samples (approximately $10^{-6}$ M), although at intermediate concentrations severe self-absorption effects are detected in both cases, as reported for oxazine 1 in ref. 8. This behaviour is expected according to eqns. (10)–(12).

Some results obtained for the squaraines studied are shown in Figs. 12 and 13. The optical densities used at each wavelength, the corresponding $f$ factors and the quantum yield values are collected in Table 1.

At intermediate optical densities a self-absorption effect is present, consistent with the "apparent" red shift of the spectral maxima, which is not observed in more dilute solutions. The decrease in quantum yield observed in these samples can be assigned to this effect.

At lower optical densities, the self-absorption effect is unimportant and the quantum yield does not vary with the excitation wavelength. Indeed, the introduction of the correction factor $f$ gives the same quantum yield at all wavelengths studied, and

![Fig. 11. Corrected fluorescence emission spectra of the squaraines in dichloromethane (saturated solutions at room temperature): 1, HSQ (1.1 $\times 10^{-3}$ M); 2, MeSQ (3.2 $\times 10^{-3}$ M). The reference compounds are oxazine 1 (3) and oxazine 170 (4) in saturated ethanol solutions, with $\phi_F$ values of 0.11 and 0.60 respectively ($\lambda_{\text{exc}} = 600$ nm in both cases).](image-url)
Fig. 12. Corrected fluorescence emission spectra of a saturated MeSQ solution in dichloromethane ($C_{MeSQ} = 1.1 \times 10^{-3}$ M): (1) $\lambda_{exc}=625$ nm; (2) $\lambda_{exc}=600$ nm; (3) $\lambda_{exc}=575$ nm; (4) $\lambda_{exc}=550$ nm; (5) $\lambda_{exc}=525$ nm.

Fig. 13. Corrected fluorescence emission spectra of a saturated HSQ solution in dichloromethane ($C_{HSQ} = 3.2 \times 10^{-3}$ M): (1) $\lambda_{exc}=625$ nm; (2) $\lambda_{exc}=600$ nm; (3) $\lambda_{exc}=575$ nm; (4) $\lambda_{exc}=550$ nm; (5) $\lambda_{exc}=525$ nm; (6) $\lambda_{exc}=475$ nm.

identical, within experimental error, with that obtained with the larger optical density (greater than 20), for which the $f$ factor approaches unity.

Our fluorescence emission data disagree with the report in the literature [16] of a multiple emission in these compounds and no evidence for a red-shifted band is detected. However, the quantum yields $\phi_e$ in concentrated and dilute samples (Table 1) are in excellent agreement with the published values of 0.023 (MeSQ) and 0.65 (HSQ) [16], and also show the absence of aggregation or concentration quenching effects.
| λ<sub>ex</sub> (nm) | MeSQ in CH<sub>2</sub>Cl<sub>2</sub> (M) | | HSO in CH<sub>2</sub>Cl<sub>2</sub> (M) | |
|----------------|---------------------------------|---------------------------------|
|               | 1.1×10⁻³<sup>a</sup>            | 1.1×10⁻⁵                          | 1.1×10⁻⁶                          | 3.2×10⁻⁵<sup>a</sup>             | 7.0×10⁻⁶                          |
|               | f  | OD  | φ<sub>F</sub> | f  | OD  | φ<sub>F</sub> | f  | OD  | φ<sub>F</sub> | f  | OD  | φ<sub>F</sub> |
| 625           | 1.00 | 140 | 0.016 | 0.49 | 1.3 | 0.021 | 0.21 | 0.2 | 0.024 | 0.87 | 9.6 | 0.67 | 0.57 | 2.1 | 0.69 |
| 600           | 0.99 | 55  | 0.011 | 0.27 | 0.4 | 0.023 | 0.21 | 0.2 | 0.024 | 0.62 | 2.7 | 0.58 | 0.36 | 0.6 | 0.64 |
| 575           | 0.91 | 14  | 0.008 | 0.17 | 0.1 | 0.025 | 0.37 | 0.7 | 0.59 | 0.18 | 0.2 | 0.62 |
| 550           | 0.67 | 3.5 | 0.007 | 0.17 | 0.1 | 0.025 | 0.26 | 0.3 | 0.49 | 0.37 | 0.7 | 0.59 | 0.18 | 0.2 | 0.62 |

<sup>a</sup>Saturated dichloromethane solution at room temperature.
4. Conclusions

An accurate knowledge of the energy profile of excitation and the geometry of our fluorometer has enabled us to establish a method for obtaining fluorescence quantum yields of samples with high or low optical density, using a front face geometry. The method can be easily applied to any other apparatus provided that a microprocessor is available to acquire and treat the data.

The method is based on the usual equation for determining $\phi_F$ employing a standard compound with a known $\phi_F$ value, but an additional $f$ correction term is included which depends on the geometry of the system and the optical density of the sample.

The accuracy of the quantum yield determination depends on the $f$ vs. optical density curve, experimentally obtained using appropriate quantum counters. The depth of penetration $\delta$ of the exciting radiation into the sample can also be calculated from the $f$ values.

The method has been applied to some squaraines at high concentrations in a saturated dichloromethane solution. The fluorescence quantum yield obtained for saturated and very dilute samples is the same, showing no aggregation effects in these compounds.

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