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A Guide to Recording Fluorescence Quantum Yields

Introduction:

When a fluorophore absorbs a photon of light, an energetically excited state is formed. The fate of this species is varied, depending upon the exact nature of the fluorophore and its surroundings, but the end result is deactivation (loss of energy) and return to the ground state. The main deactivation processes which occur are fluorescence (loss of energy by emission of a photon), internal conversion and vibrational relaxation (non-radiative loss of energy as heat to the surroundings), and intersystem crossing to the triplet manifold and subsequent non-radiative deactivation.

The fluorescence quantum yield (ΦF) is the ratio of photons absorbed to photons emitted through fluorescence. In other words the quantum yield gives the probability of the excited state being deactivated by fluorescence rather than by another, non-radiative mechanism. The most reliable method for recording ΦF is the comparative method of Williams et al.,1 which involves the use of well characterised standard samples with known ΦF values. Essentially, solutions of the standard and test samples with identical absorbance at the same excitation wavelength can be assumed to be absorbing the same number of photons. Hence, a simple ratio of the integrated fluorescence intensities of the two solutions (recorded under identical conditions) will yield the ratio of the quantum yield values. Since ΦF for the standard sample is known, it is trivial to calculate the ΦF for the test sample.

In practice, the measurement is slightly more complicated than this because it must take into account a number of considerations. For example:

- The presence of concentration effects, e.g. self-quenching;
- The use of different solvents for standard and test samples;
- The validity in using the standard sample and its ΦF value.

These considerations are answered by

- Working within a carefully chosen concentration range and acquiring data at a number of different absorbances (i.e. concentrations) and ensuring linearity across the concentration range;
- Including the solvent refractive indices within the ratio calculation;
- Cross-calibrating the standard sample with another, to ensure both are behaving as expected and allowing their ΦF values to be used with confidence.

The measurement of ΦF values is challenging if the values are to be trusted. Incorrect quantum yields are all too easy to obtain!

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General Experimental Considerations:

Standard samples. The standard samples should be chosen to ensure they absorb at the excitation wavelength of choice for the test sample, and, if possible, emit in a similar region to the test sample. The standard samples must be well characterised and suitable for such use. A number of lists of good fluorescent standard samples are available, including:

• J. R. Lakowicz, Principles of Fluorescence Spectroscopy, Kluwer Academic/Plenum Press, New York, 1999, Second Edition.
• J. C. Scaiano (Ed.), Handbook of Organic Photochemistry, CRC Press, 1989.

A list of standard samples, with their literature quantum yields, is given at the end of this document.

Cuvettes. Standard 10 mm path length fluorescence cuvettes are sufficient for running the fluorescence measurements. In order to minimise errors in calculating the absorbance of each solution, it is advisable to use absorption cuvettes with extended path lengths (for example, 20 mm, 50 mm).

Concentration range. In order to minimise re-absorption effects (Dhami et al.2) absorbances in the 10 mm fluorescence cuvette should never exceed 0.1 at and above the excitation wavelength. Above this level, non-linear effects may be observed due to inner filter effects, and the resulting quantum yield values may be perturbed. Remember that this maximum allowable value of the recorded absorbance must be adjusted depending upon the path length of the absorption cuvette being used (for example, 10 mm = 0.1 maximum, 20 mm = 0.2 maximum etc).

Sample preparation. It is vital that all glassware is kept scrupulously clean, and solvents must be of spectroscopic grade and checked for background fluorescence.

Procedure:

1. Record the UV-vis absorbance spectrum of the solvent background for the chosen sample. Note down the absorbance at the excitation wavelength to be used.

2. Record the fluorescence spectrum of the same solution in the 10 mm fluorescence cuvette. Calculate and note down the integrated fluorescence intensity (that is, the area of the fluorescence spectrum) from the fully corrected fluorescence spectrum.

3. Repeat steps 1. and 2. for five solutions with increasing concentrations of the chosen sample. (There will be six solutions in all, corresponding to absorbances at the excitation wavelength of ~0/solvent blank, 0.02, 0.04, 0.06, 0.08 and 0.10.)

4. Plot a graph of integrated fluorescence intensity vs absorbance. The result should be a straight line with gradient m, and intercept = 0.

5. Repeat steps 1. to 4. for the remaining samples.

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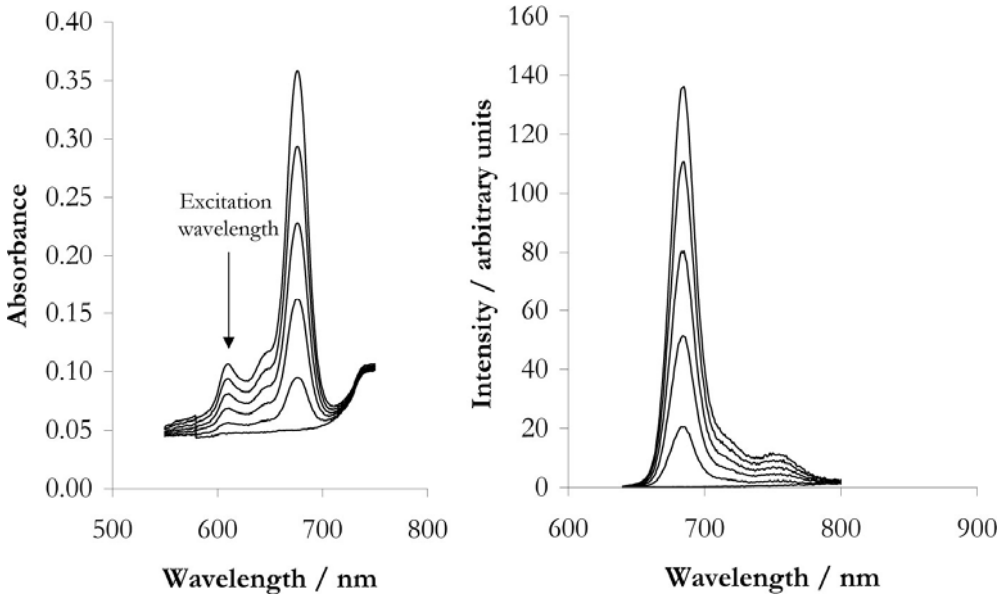

Figure 1: Example of an absorption and emission data set obtained for five different concentrations of a sample. Note that the absorption measurements have been recorded with a 50 mm path length cuvette, and have not been background corrected – the solvent blank absorption is shown in the figure.

Note that all the fluorescence spectra must be recorded with constant slit widths. Changing this parameter between samples will invalidate the quantum yield measurement.

Ideally the band pass of the excitation monochromator should be set to the same value as the UV-vis absorbance spectrometer used for the absorbance measurements, although so long as the difference between the two is not excessive the success of the measurement will not be compromised. Another approach to this, when using the Jobin Yvon spectrofluorometers (FluoroLog and FluoroMax), is the in situ measurement of optical transmission through the sample whilst in the fluorometer sample chamber, by use of the transmission accessory. However, this does remove the opportunity of using long path length absorbance cells for increased accuracy, and whilst speeding up the acquisition process, may not be suitable for more difficult measurements.

Although the above methodology does require the use of a single excitation wavelength throughout the quantum yield measurements, when using the Jobin Yvon FluoroLog and FluoroMax systems the use of different excitation wavelengths for specific samples is possible. In this case, the data must be corrected for the changes in excitation intensity, by dividing the spectrally corrected emission data by the spectrally corrected excitation intensity, corresponding to Sc/Rc. However, the practice of maintaining just one excitation wavelength for all samples will reduce the chance for errors in the inherently difficult quantum yield measurement.

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Calculation of Fluorescence Quantum Yields from Acquired Data:

The gradients of the graphs obtained in 4. above are proportional to the quantum yield of the different samples. Absolute values are calculated using the standard samples which have a fixed and known fluorescence quantum yield value, according to the following equation:

$$\Phi_X = \Phi_{ST}\left(\frac{Grad_X}{Grad_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Where the subscripts ST and X denote standard and test respectively, Φ is the fluorescence quantum yield, Grad the gradient from the plot of integrated fluorescence intensity vs absorbance, and η the refractive index of the solvent.

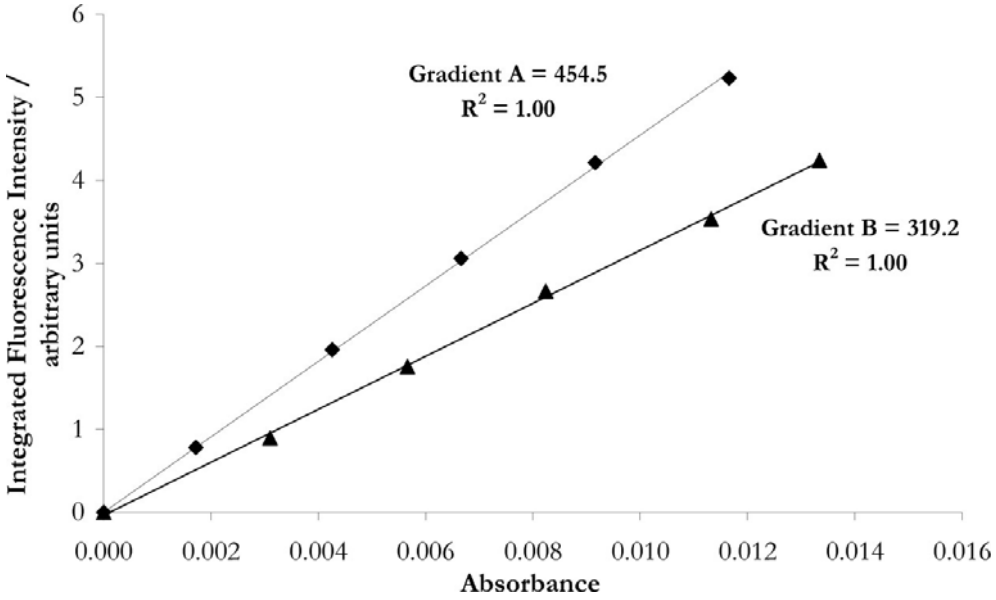

Figure 2: linear plots for two standard samples. The gradient for each sample is proportional to that sample's fluorescence quantum yield. Conversion into an absolute quantum yield is achieved through the equation given in the text.

First, the two standard compounds are cross-calibrated using this equation. This is achieved by calculating the quantum yield of each standard sample relative to the other. For example, if the two standard samples are labelled A and B, initially A is treated as the standard (ST) and B as the test sample (X), and the known ΦF for A is used. Following this, the process is reversed, such that B is now treated as the standard (ST) and A becomes the test sample (X). In this manner, the quantum yields of A and B are calculated relative to B and A respectively.

The values of ΦF(A) and ΦF(B) obtained by experiment in this way should match the literature values. The match is generally considered valid and acceptable if the data obtained is of good quality (i.e., good linearity with a zero intercept) and the experimental quantum yields match their literature counterparts within ±10%. If the values show a larger error than this, fresh standard solutions should be prepared, and the process repeated. Possible reasons for a poor match include contamination or degradation of the samples or inaccurate spectral correction of the spectrofluorimeter.

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Once the standard samples have been cross-calibrated and an acceptable match obtained, the quantum yield values for the test samples can then be calculated, using the same equation above. For each test sample, two ΦF values will be obtained, one relative to standard A, the other to standard B. The simple average of these two values represents the quantum yield of the test sample. The error in the reported value must be considered based upon the observed errors in the gradients; a value of ±10% is normal.

Planning:

As can be seen above, the measurement of fluorescence quantum yields is laborious and time consuming. Nonetheless, with careful planning the process can be made as efficient as possible. For example, there is no point in performing all measurements until the standard samples have been cross-calibrated (a long day's work recording data for two standard samples and six test samples may come to nothing if, at the end of the day, analysis of the two standard samples shows an unacceptable match). Take measurements for the standard samples only, and check their cross-calibration is acceptable before moving on to record data for the remaining test samples.

Conclusions:

The measurement of fluorescence quantum yields can often be difficult and troublesome, and the need for absolute care during every step cannot be over emphasised. However, it is possible to make such measurements routinely, and following these guidelines should make this an achievable goal.

Acknowledgements:

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References:

1) A. T. R. Williams, S. A. Winfield and J. N. Miller, Relative fluorescence quantum yields using a computer controlled luminescence spectrometer, Analyst, 1983, 108, 1067.

2) S. Dhami, A. J. de Mello, G. Rumbles, S. M. Bishop, D. Phillips and A. Beeby, Phthalocyanine fluorescence at high concentration: dimers or reabsorption effect? Photochem. Photobiol., 1995, 61, 341.

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A Table of Standard Materials and Their Literature Quantum Yield Values

Compound	Solvent	Literature Quantum yield	Emission range / nm	Reference
Cresyl violet	Methanol	0.54	600-650	J. Phys. Chem., 1979, 83, 696
Rhodamine 101	Ethanol + 0.01% HCl	1.00	600-650	J. Phys. Chem., 1980, 84, 1871
Quinine sulfate	0.1M H2SO4	0.54	400-600	J. Phys. Chem., 1961, 65, 229
Fluorescein	0.1M NaOH	0.79	500-600	J. Am. Chem. Soc., 1945, 1099
Norharmane	0.1M H2SO4	0.58	400-550	J. Lumin., 1992, 51, 269-74
Harmane	0.1M H2SO4	0.83	400-550	J. Lumin., 1992, 51, 269-74
Harmine	0.1M H2SO4	0.45	400-550	J. Lumin., 1992, 51, 269-74
2-methylharmane	0.1M H2SO4	0.45	400-550	J. Lumin., 1992, 51, 269-74
Chlorophyll A	Ether	0.32	600-750	Trans. Faraday Soc., 1957, 53, 646-55
Zinc phthalocyanine	1% pyridine in toluene	0.30	660-750	J. Chem. Phys., 1971, 55, 4131
Benzene	Cyclohexane	0.05	270-300	J. Phys. Chem., 1968, 72, 325
Tryptophan	Water, pH 7.2, 25C	0.14	300-380	J. Phys. Chem., 1970, 74, 4480
2-Aminopyridine	0.1M H2SO4	0.60	315-480	J. Phys. Chem., 1968, 72, 2680
Anthracene	Ethanol	0.27	360-480	J. Phys. Chem., 1961, 65, 229
9,10-diphenyl anthracene	Cyclohexane	0.90	400-500	J. Phys. Chem., 1983, 87, 83