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Measurement of Fluorescence Quantum Yields

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Key Words

- Fluorescence Quantum Yield
- Relative Fluorescence Quantum Yield

Introduction

The fluorescence quantum yield is an intrinsic property of a fluorophore and is important for the characterization of novel fluorescent probes. The fluorescence quantum yield is the ratio of photons absorbed to photons emitted through fluorescence.

$$Q = \frac{\text{photons}_{em}}{\text{photons}_{abs}}$$

The quantum yield Q can also be described by the relative rates of the radiative k_r and non-radiative k_{nr} relaxation pathways, which deactivate the excited state.

$$Q = \frac{k_r}{k_r + \Sigma\, k_{nr}}$$

While measurements of the absolute quantum yield require sophisticated instrumentation, it is easier to determine the relative quantum yield of a fluorophore by comparison to a reference fluorophore with a well-known quantum yield.

There are two methods for relative quantum yield measurements: a single-point and a comparative method.[1] Using the single-point method the quantum yield is calculated using the integrated emission intensities from a single sample and reference pair at identical concentration. While this method is fast and easy, it is not always reliable due to the inaccurate measurement of the fluorophore's absorbance. The second is the comparative method of Williams *et al.*, which involves the use of multiple well characterized references with known fluorescence quantum yields.[2] It is more time consuming but provides much higher accuracy by calculating the slope of the line generated by plotting the integrated fluorescence intensity against the absorption for multiple concentrations of fluorophore.

In this technical note, the comparative method was applied to determine the fluorescence quantum yield of Rhodamine B by comparison to Rhodamine 6G which has fluorescence quantum yield of 0.95.[3] Results from both the comparative and the single-point methods are compared and evaluated against the well-known value of the Rhodamine B.

Rhodamine and rhodamine derivative dyes are commonly used as fluorescence probes for biological assays. Nucleic acids, proteins and some cell features can be labeled and monitored using these fluorescence probes. Many rhodamine and rhodamine derivative dyes are commercially available with different attachment chemistries.

Reagent and Apparatus

1. Rhodamine B, Rhodamine 6G: Generally used as a reagent for detection of the DNA, RNA and Protein on fluorescence analysis

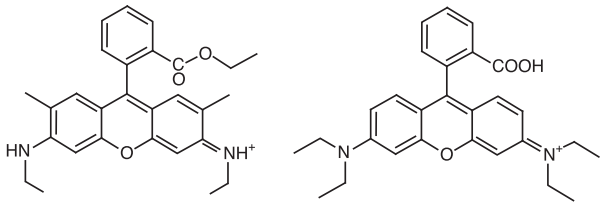

Figure 1: Molecular structure of Rhodamine 6G (left) and Rhodamine B (right)

2. Ethanol
3. Thermo Scientific Evolution Array UV-Visible spectrophotometer controlled by Thermo Scientific VISION*collect* software
4. Thermo Scientific Lumina fluorescence spectrometer controlled by Luminous software
5. Fluorescence cuvette (beam pathlength is 10 mm)

Procedure

1. Prepare 4 ~ 5 samples each of Rhodamine B and Rhodamine 6G having different absorbances between 0.01-0.1 at the excitation wavelength 535 nm
2. Measure the fluorescence spectrum from 500 to 700 nm with the prepared samples using an excitation wavelength of 535 nm
3. Calculate the integrated fluorescence intensity from the spectrum
4. Plot the magnitude of the integrated fluorescence intensity against the absorbance of the solution absorbance
5. Calculate the fluorescence quantum yield

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Instrument Parameter

Absorbance spectra were acquired using the Wavelength Monitoring mode of the VISION*collect*™ software. The following parameters were used for the acquisition of fluorescence data using the Lumina™ fluorescence spectrometer.

Scan Mode: Emission
Data Mode: Fluorescence
Repeat Number: 1
Repeat Interval: 1
PMT Voltage: 700 V
Scan Speed: 300 nm/min
Integration Time: 20 ms
Response Time: 0.1 s

EX Slit: 2.5 nm
EM Slit: 2.5 nm
EX Filter: Air
EX Filter: Air
EX Wave: 535 nm
EM Start: 500 nm
EM End: 700 nm

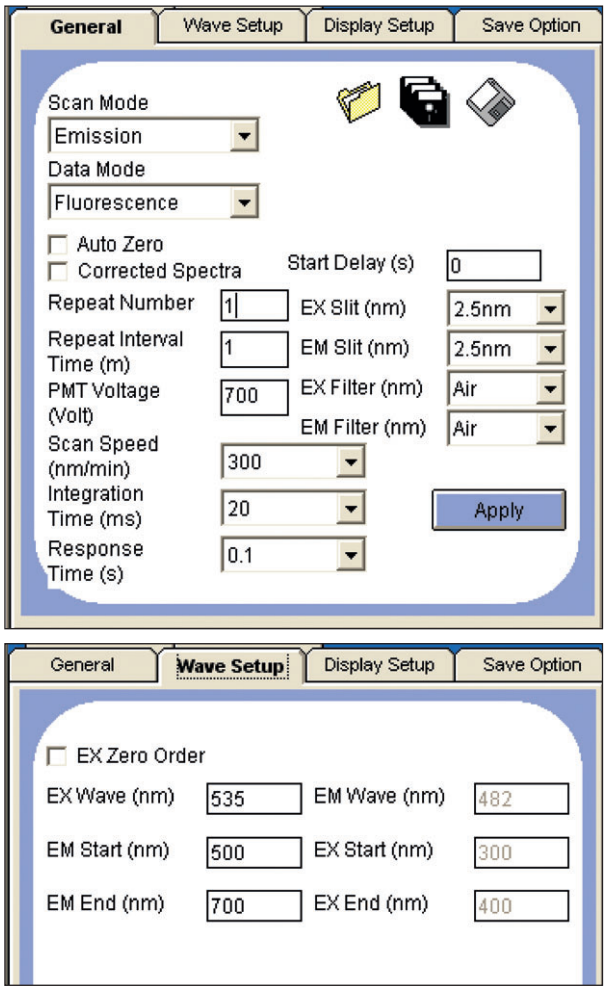

Figure 2: Parameters set up for measuring fluorescence

Result

Figure 3 shows the absorbance and fluorescence spectra of Rhodamine 6G and Rhodamine B. The area of the fluorescence spectrum, calculated using the Area calculation feature of Luminous software, is shown in Figure 4. The area for each sample is given in Table 1.

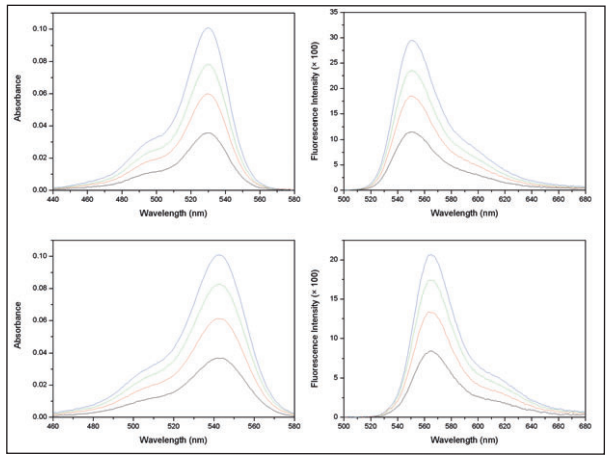

Figure 3: Absorbance (left) and Fluorescence (right) spectra of Rhodamine 6G (top) and Rhodamine B (bottom)

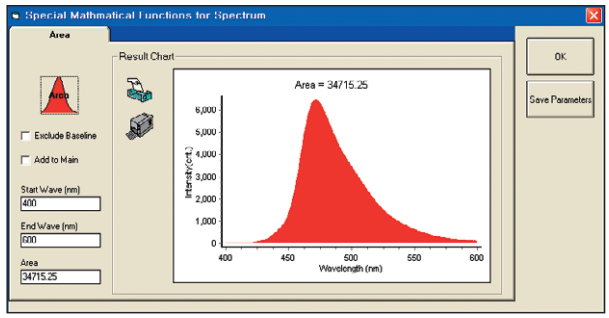

Figure 4: The area of the fluorescence spectrum

Rhodamine 6G		Rhodamine B	
Absorbance	**Area of Fluorescence**	**Absorbance**	**Area of Fluorescence**
0.032	58903	0.032	41162
0.053	93579	0.053	66010
0.07	119226	0.071	85799
0.092	150078	0.087	101482

Table 1: Data from absorption measurements and integrated fluorescence spectra

In the comparative method, the quantum yield is calculated using the slope of the line determined from the plot of the absorbance against the integrated fluorescence intensities. In this case, the quantum yield can be calculated using:

$$Q = Q_R\left(\frac{m}{m_R}\right)\left(\frac{n^2}{n^2_R}\right)$$

where m is the slope of the line obtained from the plot of the integrated fluorescence intensity vs. absorbance. n is the refractive index of solvent and the subscript $_R$ refers to the reference fluorophore of known quantum yield.[2]

If the Ethanol is used for both reference and unknown sample as a solvent, (n^2/n^2_R) will be 1, so the fluorescence quantum yield(Q) can be obtained from the quotient of the two slopes.

The graph of the absorbance vs. the area of fluorescence obtained from the experiment and the resulting linear fits are shown in Figure 5.

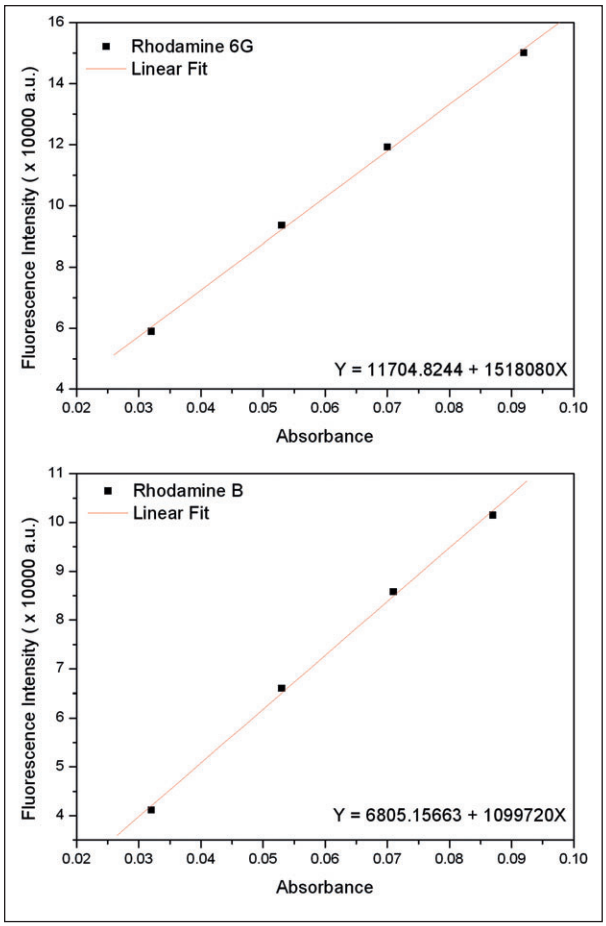

Figure 5: Absorbance vs. Area of fluorescence

The Q value 0.95 well-known (fluorescence quantum yield of Rhodamine 6G as a reference) is inserted into the equation and then calculated as below.

$$Q_B = 0.95\left(\frac{1099720}{1518080}\right)(1) = 0.69$$

As a result, the fluorescence quantum yield of Rhodamine B is obtained as 0.69 which is very close to the Literature Quantum yield 0.7.

Single-Point vs. Comparative Method

Using single-point measurements to determine the fluorescence quantum yield has the advantage of getting results faster than the comparative method. In the single point method, the quantum yield of the unknown sample is calculated using:

$$Q = Q_R\,\frac{I}{I_R}\,\frac{OD_R}{OD}\,\frac{n^2}{n^2_R}$$

where Q is fluorescence quantum yield, I is the integrated fluorescence intensity, n is the refractive index of solvent, and OD is the optical density (absorption). The subscript $_R$ refers to the reference fluorophore of known quantum yield. Shown below are three single-point calculations of quantum yield.[1]

$$Q_B = 0.95\,\frac{41162}{58903}\,\frac{0.032}{0.032}(1) = 0.66$$

$$Q_B = 0.95\,\frac{85799}{119226}\,\frac{0.07}{0.071}(1) = 0.67$$

$$Q_B = 0.95\,\frac{101482}{150078}\,\frac{0.092}{0.087}(1) = 0.68$$

The single-point method produced reasonable results when compared to the literature value, however, the accuracy of the individual single-point measurements is slightly worse than the comparative method.

Conclusions

The fluorescence quantum yield of Rhodamine B was measured using the Lumina fluorescence spectrometer and Evolution™ Array™ UV-Vis spectrophotometer. Two methods for determining the relative quantum yield were investigated: the comparative method and the single-point method. Even though it is difficult to measure the absolute fluorescence quantum yield, the data provided from relative measurements is useful for many applications and calculations including fluorescence brightness and energy transfer measurements.

References

1. J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Ed., Kluwer Academic, New York, 1999.
2. A.T.R. Williams, S.A. Winfield and J.N. Miller. *Analyst*. **108, 1067, 1983**.
3. H. Du, R. A. Fuh, J. Li, A. Corkan, J. S. Lindsey. *Photochemistry and Photobiology*. **68, 141-142, 1998**

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