

Application News

No. A504

Spectrophotometric Analysis

Relative Quantum Yield Measurement of a Sample in Solution

■ Introduction

Quantum yield is the ratio of the number of photons of excitation light adsorbed by a fluorescent material to the number of photons emitted by that material as fluorescent light. Quantum yield is used to evaluate the luminous intensity and luminous efficiency of fluorescent materials used in lighting and displays, and of fluorescent probes used in life sciences.

Quantum yield can be calculated for unknown samples by a relative method that uses a fluorescent material of known quantum yield as a standard sample, or by an absolute method that uses an integrating sphere to calculate quantum yield directly (values calculated by the absolute method are called absolute quantum yield or quantum efficiency).

We describe using the RF-6000 spectrofluorophotometer to measure and calculate quantum yield by the relative method.

■ Formula for Calculating Relative Quantum Yield

By the relative method, the absorbance measured using a UV-VIS spectrophotometer "A", the area of the FWHM of the corrected emission spectrum peak "F", the solvent refractive index "n", and the dilution ratio of the sample during emission spectrum measurement compared to its concentration during UV-VIS spectrum measurement "D" are used to calculate the quantum yield " ϕ_x " of an unknown sample using formula (1).

$$\phi_x = \phi_{st} \cdot \left(\frac{A_{st}}{A_x}\right) \cdot \left(\frac{F_x}{F_{st}}\right) \cdot \left(\frac{n_x^2}{n_{st}^2}\right) \cdot \left(\frac{D_x}{D_{st}}\right) \quad (1)$$

In formula (1), the suffix "st" represents the standard sample and "x" represents the unknown sample.

■ Matters of Note for Calculating Quantum Yield by the Relative Method

Matters of note for calculating quantum yield by the relative method according to formula (1) are shown below.

(1) Selection of standard sample and excitation wavelength

- Choose a fluorescent material of known quantum yield with an excitation spectrum in a wavelength range that overlaps the excitation spectrum of the unknown sample, as the standard sample.
- Choose a wavelength in the wavelength range over which the excitation spectrum of both the standard sample and unknown sample overlaps. The standard sample and the unknown sample should be excited by the same wavelength. If either the standard sample or unknown sample have a peak in the overlapping wavelength range, choose that peak wavelength as the excitation wavelength.

(2) Absorbance measured using a UV-VIS spectrophotometer

- Place each of the solvents dissolving the standard sample and unknown sample on the reference beam side of the UV-VIS spectrophotometer when obtaining their UV-VIS spectra.
- Read absorbance at the excitation wavelength on the UV-VIS spectrum of the standard sample and unknown sample.

(3) Sample concentration

- Using a high-concentration sample without dilution can result in concentration quenching when measuring the emission spectrum. If the absorbance measured in (2) is high, dilute the standard sample and unknown sample so absorbance at the excitation wavelength is ≤ 0.05 .¹⁾ Insert the dilution ratio used for this adjustment into formula (1).

The RF-6000 spectrofluorophotometer used for calculating quantum yield by the relative method is shown in Fig. 1, and the UV-2600 UV-VIS spectrophotometer used for measuring absorbance is shown in Fig. 2.

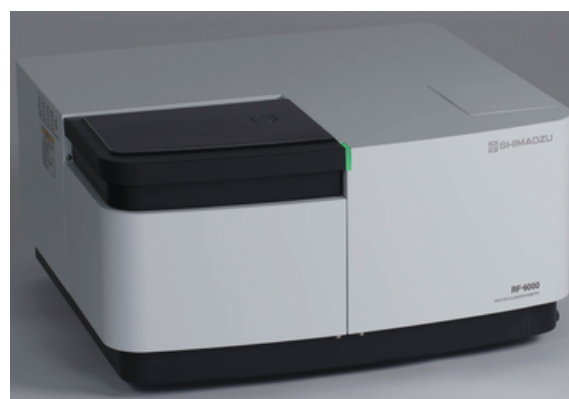


Fig. 1 RF-6000 Spectrofluorophotometer

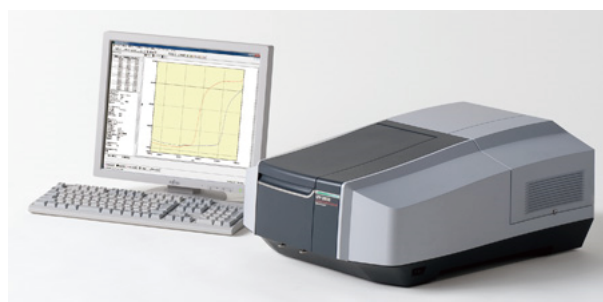


Fig. 2 UV-2600 UV-VIS Spectrophotometer

Measuring Relative Quantum Yield of Rhodamine B

Rhodamine B is fluorescent red pigment used to dye textiles and to color paper and other materials. As shown in Fig. 3, a wavelength region on the excitation spectrum of uranine (sodium fluorescein) in ethanol, a substance of known quantum yield, overlaps with the excitation spectrum of rhodamine B in ethanol. Based on this, uranine in ethanol was chosen as the standard sample for calculating the quantum yield of rhodamine B. The analytical conditions used to obtain the spectra shown in Fig. 3 are shown in Table 1.

Looking at Fig. 3, because uranine has a peak at 498 nm (red arrow) that overlaps with the excitation spectrum of rhodamine B in ethanol, this peak wavelength was chosen as the excitation wavelength. The UV-VIS spectra of rhodamine B and uranine in ethanol are shown in Fig. 4, and the analytical conditions used are shown in Table 2. Based on these spectra, at 498 nm rhodamine B and uranine have an absorbance of 0.0216 and 0.0243, respectively. The absorbance of both samples was ≤ 0.05 , so the solutions were used without dilution to obtain emission spectra.

Quantum yield program included as a standard feature of the LabSolutions RF control software for RF-6000 was used to calculate quantum yield according to formula (1). Required parameters for formula (1) relating to uranine in ethanol, including quantum yield and absorbance, were entered into the formula and then the emission spectrum of uranine in ethanol was obtained. Required parameters for formula (1) relating to rhodamine B in ethanol were then entered into the formula and its emission spectrum was obtained. The areas of the FWHM of the peak height in each spectrum, shown in blue in Fig. 5 (I) and (II), were calculated, and finally the quantum yield of the unknown sample rhodamine B in ethanol was calculated automatically and displayed as shown in Fig. 6. The analytical conditions used to obtain Fig. 5 are shown in Table 3. The quantum yield program displays emission spectra with wavelength on the horizontal axis, while the FWHM area is calculated using emission spectra with values proportional to the number of photons on the vertical axis and wavenumber on the horizontal axis.

Taking the quantum yield of the standard sample uranine in ethanol as 0.97,¹⁾ the quantum yield calculated for rhodamine B in ethanol was 0.7149, which is within the 0.69 to 0.97 range seen in literature.¹⁾

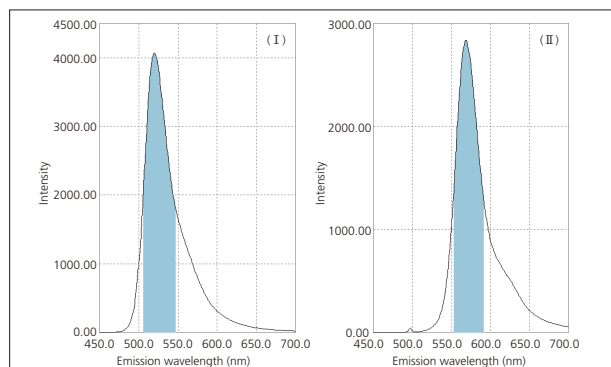


Fig. 5 Emission Spectra of Uranine (I) and Rhodamine B (II) in Ethanol

1) Kinoshita, K. and Mihashi, K., eds. 1983. The Spectroscopical Society of Japan Measurement Method Series 3 "Fluorometry—Applications in Biological Sciences". Japan: Gakkai Shuppan Center.

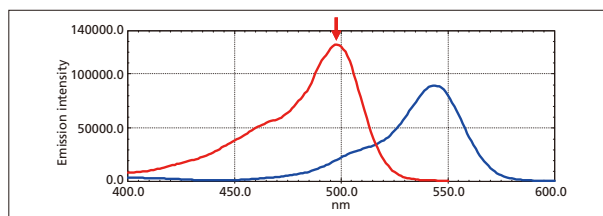


Fig. 3 Excitation Spectra of Rhodamine B (Blue) and Uranine (Red) in Ethanol

Table 1 Analytical Conditions

Measurement Instrument	: RF-6000
Emission Wavelength	: Rhodamine B: 568 nm, Uranine: 520 nm
Scanning Speed	: 200 nm/min
Slit Width	: Ex = 3 nm, Em = 3 nm
Sensitivity	: Low

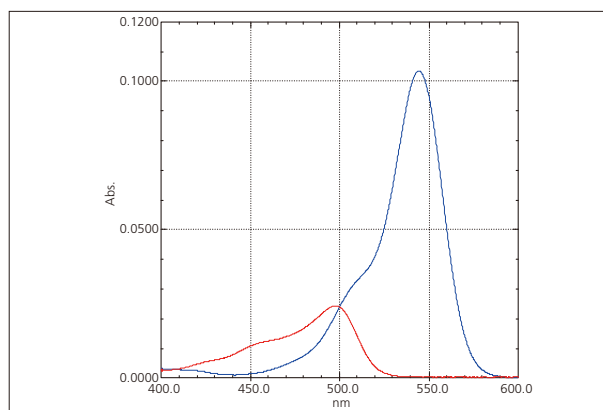


Fig. 4 UV-VIS Spectra of Rhodamine B (Blue) and Uranine (Red) in Ethanol

Table 2 Analytical Conditions

Measurement Instrument	: UV-2600
Spectra Type	: Absorbance
Scanning Speed	: Medium speed
Slit Width	: 2 nm

Table 3 Analytical Conditions

Measurement Instrument	: RF-6000
Excitation Wavelength	: 498 nm
Scanning Speed	: 200 nm/min
Slit Width	: Ex = 3 nm, Em = 3 nm
Sensitivity	: Low

Standard sample table

No.	name	QY	Abs	RI	Memo
0	Uranine	0.9700	0.0243	1.3000	

Unknown sample table

No.	name	QY	Abs	RI	Memo
1	<input checked="" type="checkbox"/> Rhodamine B	0.7149	0.0216	1.3000	

Fig. 6 Quantum Yield of Rhodamine B in Ethanol
QY: Quantum Yield, Abs: Absorbance, RI: Refractive Index of Solution

Conclusion

Because the RF-6000 can obtain corrected emission spectra in real time, using the quantum yield program included as standard in the LabSolutions RF software allows easy calculation of quantum yield for a target substance.