Application Note

FP-0009

Relative quantum yield measurement using FP-8000 series

Introduction

Fluorescence quantum yield is defined as the ratio of the number of photons emitted from sample as fluorescence to the number of photons in the excited light absorbed. Absolute method and relative method are known measuring methods. Regarding the absolute method, it is necessary to detect all the fluorescence from the sample, requiring the integrating sphere, while relative method can calculate quantum yield of unknown sample by comparing the intensity of standard fluorescence with unknown sample, and accordingly the relative method is easier to get the results of quantum yield. In this experiment, an example will be shown for the calculation of quantum yield of Rhodamine B when fluorescein is used as the standard sample.

<Calculation method for relative quantum yield>

In order to calculate relative quantum yield, 1) Quantum yield of standard sample is required and in addition 2) Absorbance at excitation wavelength and 3) Area of emission spectrum with spectral correction are also required for the standard sample and the unknown sample respectively. Moreover, when the solvent of the standard sample is different from that of unknown sample, 4) Average refractive index value in the wavelength range to calculate the area of emission spectrum is required. When the standard or unknown samples for emission spectrum measurement are diluted, 5) Dilution ratio is required.

Table 1 Required parameters to calculate relative quantum yield

	Item	Samples	Expression
1)	Quantum yield	Standard	ϕ_{st}
2)	Absorbance at Ex Wavelength	Standard Unknown	A_{st} A_x
3)	Area of emission spectrum	Standard Unknown	F_{st} F_x
4)	Average refractive index value of solvent	Standard Unknown	n_{st} n_x
5)	Dilution ratio	Standard Unknown	D_{st} D_x

By using the parameters in the Table 1, relative quantum yield of unknown sample, ϕ_x is shown by the following equation (1).

$$\Phi_x = \Phi_{st} \bullet \left(\frac{A_{st}}{A_x}\right) \bullet \left(\frac{F_x}{F_{st}}\right) \bullet \left(\frac{n_x^2}{n_{st}^2}\right) \bullet \left(\frac{D_x}{D_{st}}\right) \qquad (1)$$

In addition to [Absorbance measurement] program to measure 2)Absorbance at excitation wavelength and [Spectra measurement] program to get 3)Emission spectrum area, [Relative quantum yield calculation] program to calculate relative quantum yield of unknown sample based on equation (1) is installed as standard to the FP-8000 series. So everything from measuring to analyzing relative quantum yield can be performed only by using standard programs.

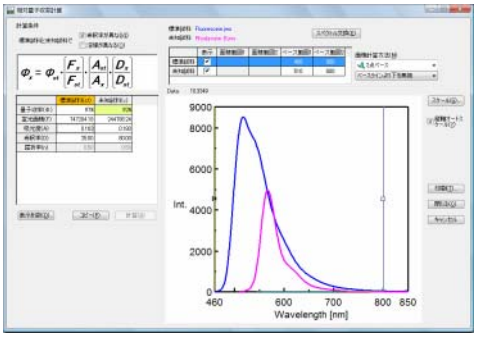

Fig. 1 [Relative quantum yield calculation] program screen

Keyword: Quantum yield, Relative method, and Solution

JASCO INTERNATIONAL CO., LTD

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

<Measuring System>

FP-8500 Spectrofluorometer*1)

FUV-803 Absorbance measurement cell block

*1) Emission spectrum with spectral correction is required for relative quantum yield calculation. The spectral correction was performed using Rhodamine B on Ex side, and ESC-842Calibrated WI light source on Em side for this measurement.

<Samples>

	Name	Concentration	Solvent
Standard	Fluorescein	For Absorbance:5400 µg/L For Emission:72 µg/L	Ethanol
Unknown	Rhodamine B	For Absorbance:7200 µg/L For Emission:36 µg/L	Ethanol

<Calculation procedure of parameters required to calculate relative quantum yield >

1) Quantum yield of standard sample

Published value of 0.97 from literature*2) was used for quantum yield of fluorescein.

*2) Literature: Kazuhiko Kinoshita and Koshin Mihashi, *Fluorescence measurements - Applications for Biochemical Sciences.* (The Spectroscopic Society of Japan, Measurement Method Series 3) Japan Scientific Societies Press, 1983.

2) Absorbance at Ex wavelength

Absorption spectra was measured using [Absorbance measurement] program and FUV-803 Absorbance measurement cell block. Results are shown in Fig. 2. From the results, it was confirmed that absorbance of the fluorescein at Ex wavelength,450 nm was 0.490 and the absorbance of the Rhodamine B at Ex wavelength 500 nm was 0.225.

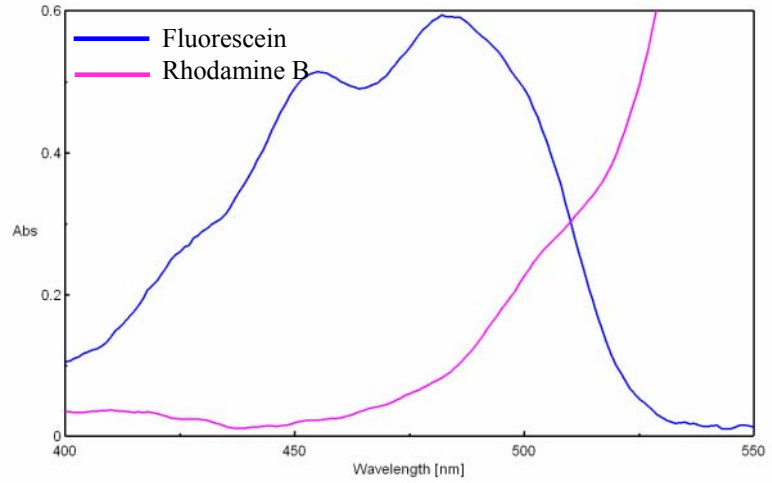

Fig. 2 Absorption spectra

Table 2 Absorbance at Ex wavelength

Standatrd (Fluorescein)	Unknown (Rhodamine B)
0.490	0.225

[Measurement conditions]

Ex bandwidth	2.5 nm	Em bandwidth	10 nm
Scan speed	200 nm/min	Data interval	1 nm
Response	0.5 sec	PMT voltage	230 V
Filter	Used		

Attenuator B was used.*3)

*3) The attenuator B is available as a standard of spectrofluorometer.

JASCO INTERNATIONAL CO., LTD

4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512

3) Area of emission spectrum

Emission spectra were measured using [Spectra measurement] program. In order to prevent from reabsorption of fluorescence, sample solution was diluted so that the absorbance of the sample solution becomes less than 0.02 for this measurement.(Details of dilution ratio will be described in the item 5).) Obtained emission spectra were shown in Fig. 3.

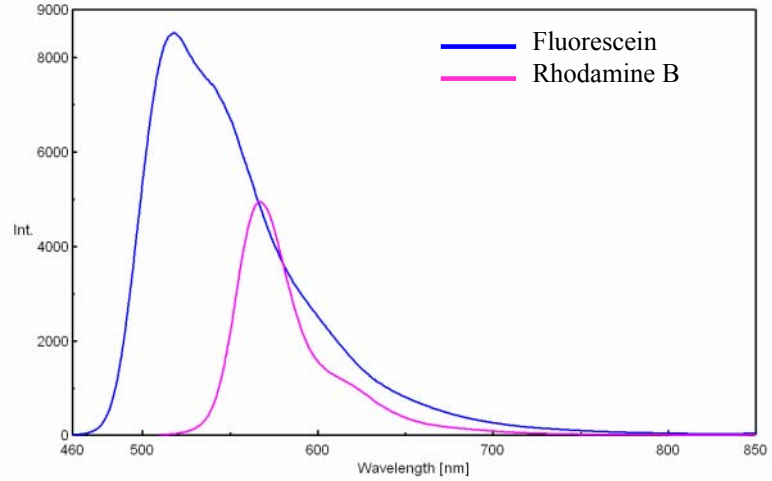

Fig. 3 Emission spectra

Table 3 Area of emission spectrum

Standard (Fluorescein)	Unknown (Rhodamine B)
747204	243513

[Measurement condition]

Ex bandwidth	5 nm	Em bandwidth	5 nm
Scan speed	200 nm/min	Data interval	0.5 nm
Response	0.5 sec	PMT voltage	430 V
Filter	Used	Spectral correction	ON

4) Average refractive index of solvent

As ethanol is used on both standard and unknown samples for this measurement, average refractive index of the solvent is not required. When the solvents used are different from each other, published value is used.

5)Dilution ratio

Comparing with absorbance measurement in the item 2), standard sample solution was diluted by 75 times and unknown sample, by 100 times in the item 3) emission spectra measurement.

Table 4 Dilution ratio

Standard (Fluorescein)	Unknown (Rhodamine B)
75	100

<Analysis results>

Quantum yield of Rhodamine B was calculated by applying parameters obtained by "calculation methods 1)~5) to get parameters which are needed to calculate relative quantum yield" to the equation (1). As a result, quantum yield of 92 % which is within the range of published value, [*3)] 69~97% was obtained.

Table 5 Quantum yield of rohdamine B

Published value	Measured value
69~97%	92%

JASCO INTERNATIONAL CO., LTD
4-21, Sennin-cho 2-chome, Hachioji, Tokyo 193-0835, Japan
Tel: +81-42-666-1322, Fax: +81-42-665-6512