Application Note

FP-0008

Absolute quantum yield measurement of solution 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 as measuring methods. Relative method is comparing the intensity of standard fluorescence with unknown sample to calculate quantum yield of the unknown sample. Therefore obtained results depend on the accuracy of standard sample's quantum yield value. On the other hand, quantum yield can be obtained directly by the absolute method, because the absolute method allows to detect all the fluorescence from the sample and integrates using integrating sphere, enabling more accurate quantum yield measurement.

In this experiment, some examples will be shown for the calculation of solution sample's quantum yield of which published values from literature are known by the absolute method.

Keyword: Quantum yield, Absolute method, Solution

<Measuring system>

FP-8500 Spectrofluorometer*1)
ILF-835 100mmF Integrating sphere unit
1 mm pathlength solution cell
FWQE-880 Quantum yield calculation program

*1) Emission spectrum to which spectral correction is performed is required for quantum yield calculation. The spectra correction was performed using Rhodamine B on EX side ,and was also performed on EM side using standard white plate for synchronous spectrum (250-450 nm) and ESC-842 (450-700 nm).

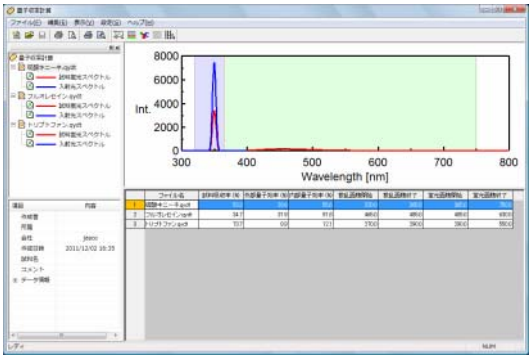


Fig.1 Quantum yield calculation program screen

<Samples>

-200 ppm Quinine sulfate (Solution: 1.0 N H_2SO_4)
-15 ppm Fluorescein (Solvent: 0.1 N $NaOH_{aq}$)
-200 mg/mL tryptophan (Solvent:Ultra pure water)

<Measuring method for absolute quantum yield>

1) Measuring incident light
   Confirm nothing is set on the sample cell holder in the integrating sphere, and measure spectrum of the incident light. Obtained peak area is defined as area from incident light, S_0 (equivalent number of photons in the incident light).
2) Measuring sampleExcitation lightExcitation light
   Set the sample on the sample holder, and measure scattering and emission spectra of the sample. Obtained excitation wavelength peak area is defined as area scattered from sample, S_1(equivalent number of photons which were not absorbed), and peak area in the emission wavelength range is defined as area emitted from sample, S_2.

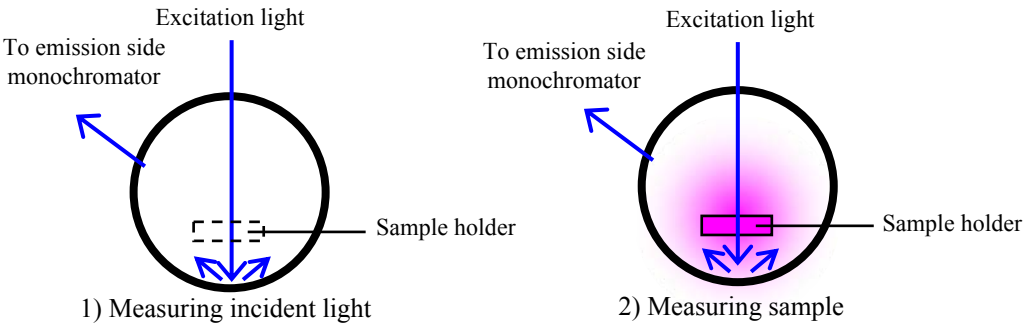


Fig.2 Diagram of integrating sphere for measuring incident light and measuring sample

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) Calculating quantum yield
Calculate in accordance with the following.
Sample absorption[%]=$(S_0-S_1)/S_0$ x 100
External quantum yield[%]=S_2/S_0 x 100
Internal quantum yield[%]=$S_2/(S_0-S_1)$ x 100

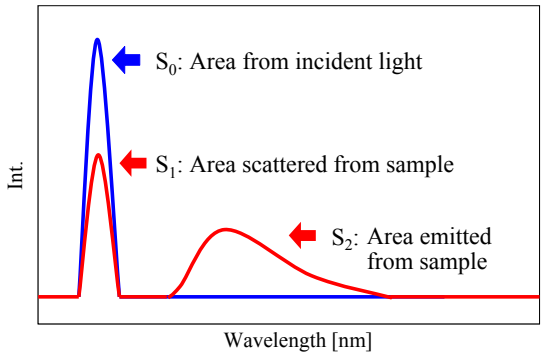
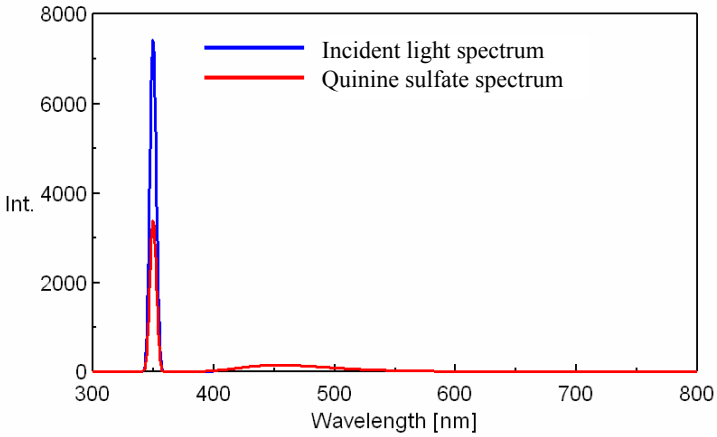


Fig. 3 Image of S_0, S_1, S_2

<Measurement results>

Sample spectra measurement results are shown in the Fig 4 ~ 6.

1. Quinine sulfate

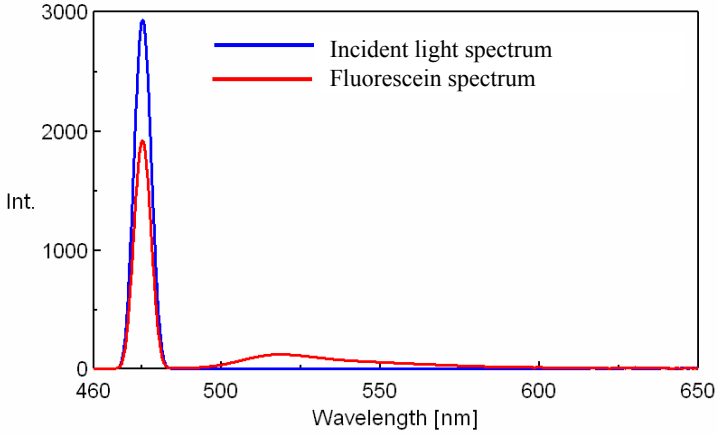


[Measurement condition]

Mode:	Emission
Ex bandwidth:	5 nm
Em bandwidth:	5 nm
Ex wavelength:	350.0 nm
Measurement range:	300 - 800 nm
Scan speed:	200 nm/min
Data interval:	0.1 nm
Response:	0.5 sec
PMT voltage:	350 V

Fig. 4 Emission spectrum of quinine sulfate

2. Fluorescein



[Measurement condition]

Mode:	Emission
Ex bandwidth:	5 nm
Em bandwidth:	5 nm
Ex wavelength:	475.0 nm
Measurement range:	460 - 650 nm
Scan speed:	200 nm/min
Data interval:	0.1 nm
Response:	0.5 sec
PMT voltage:	250 V

Fig. 5 Emission spectrum of fluorescein

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.Tryptophan

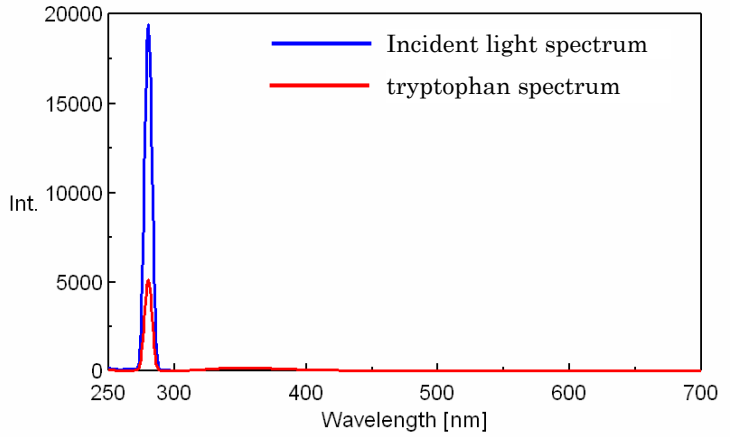


Fig.6 Emission spectrum of tryptophan

[Measurement condition]

Mode:	Emission
Ex bandwidth:	5 nm
Em bandwidth:	5 nm
Ex wavelength:	280.0 nm
Measurement range:	250 - 700 nm
Scan speed:	200 nm/min
Data interval:	0.1 nm
Response:	0.5 sec
PMT voltage:	400 V

<Analysis results>

Table 1 shows area from incident light (S_0), area scattered from sample (S_1), area emitted from sample (S_2) calculated by each of sample's spectra and wavelength range.

Table 1 Detail of quantum yield calculation

Sample name	Area from incident light [S_0]	Area scattered from sample [S_1]	Area emitted from sample [S_2]	Scattered WL range [nm]	Emitted WL range [nm]
Qunine sulfate	48267	22538	14304	320 - 365	365 - 750
Fluorescein	19174	12515	6116	465 - 485	485 - 630
Tryptophan	136135	35842	12101	270 - 290	290 - 550

Calculation results of quantum yield using the values on the Table 1 and equations on the 3) are shown in the Table 2. Obtained results are within the range of published values from literatures for any samples.

Table 2 Calculation results of quantum yield

Sample name	Sample absorbance	External quantum yield	Internal quantum yield	Internal quantum yield [published values]
Qunine sulfate	53.3%	29.6%	55.6%	50-57%*2)
Fluorescein	34.7%	31.9%	91.8%	85-92%*2)
Tryptophan	73.7%	8.9%	12.1%	12-14%*3)

*2) Literature: The Spectroscopical Society of Japan, (Japan Scientific Societies Press)
*3) Literature: Principles of fluorescence spectroscopy, Joseph R. Lakowicz, Springer

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