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Application News

No.A496

Spectrophotometric Analysis

Measuring the Fluorescence Quantum Efficiency of Liquid Samples

Fluorescent substances absorb a characteristic wavelength of that substance and then emit a fluorescent light with a wavelength longer than the absorbed light. The ratio of photons absorbed versus emitted as fluorescent light is referred to as the fluorescence quantum yield or quantum efficiency and is associated with the fluorescence intensity of fluorescent substances.
Two methods are used to determine this ratio, either a relative or absolute method. The relative method involves comparison of fluorescence intensity to a standard fluorescent substance with a known ratio to calculate a relative value. In this article, the ratio determined using this method is referred to as fluorescence quantum yield. On the other hand, the absolute method involves calculating the ratio directly from a fluorescence spectrum measured from the fluorescent substance using an integrating sphere. In this article, the ratio determined by this method is referred to as fluorescence quantum efficiency. The absolute method only involves the measurement sample, which makes it easier than the relative method.
This article describes using an RF-6000 spectrofluorophotometer with an integrating sphere attached to determine the fluorescence quantum efficiency and measure quinine sulfate.

Method of Determining Fluorescence Quantum Efficiency

The RF-6000 spectrofluorophotometer system used for measurements is shown in Fig. 1. A diagram of how fluorescence quantum efficiency is determined is shown in Fig. 2. To determine the fluorescence quantum efficiency, a fluorescence spectrum is measured first from a blank sample (plain solvent for samples in solution) and then from the measurement sample, but the instrument function must be removed from these spectra. With conventional models, it was difficult to obtain such corrected fluorescence spectra easily. However, the RF-6000 is able to provide fluorescence spectra automatically corrected during measurements, which is especially beneficial when determining fluorescence quantum efficiency.
Quantities related to fluorescence quantum efficiency include absorption factor, internal quantum efficiency, and external quantum efficiency. These quantities are defined by the expressions shown in Table 1. The absorption factor indicates the percent of incident photons that are absorbed by the sample. The internal quantum efficiency indicates the percent of absorbed photons that are emitted as fluorescent light. The external quantum efficiency indicates the percent of incident photons that are emitted as fluorescent light. These quantities are calculated from the area values for ranges (1), (2), (3), and (4) indicated with the two pairs of vertical lines in Fig. 2. Based on these definitions, fluorescence quantum efficiency corresponds to internal quantum efficiency.
The LabSolutions RF software included standard with the RF-6000 system is able to calculate all these quantities, including absorption factor, internal quantum efficiency (fluorescence quantum efficiency), and external quantum efficiency.

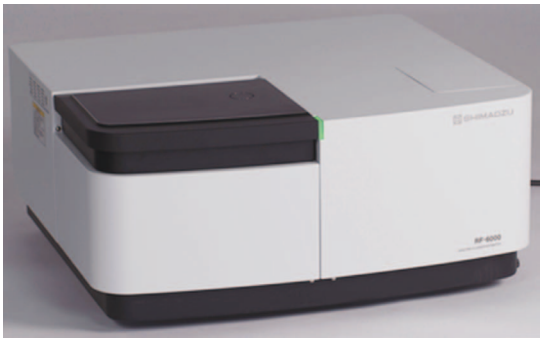

Fig. 1 RF-6000 Spectrofluorophotometer

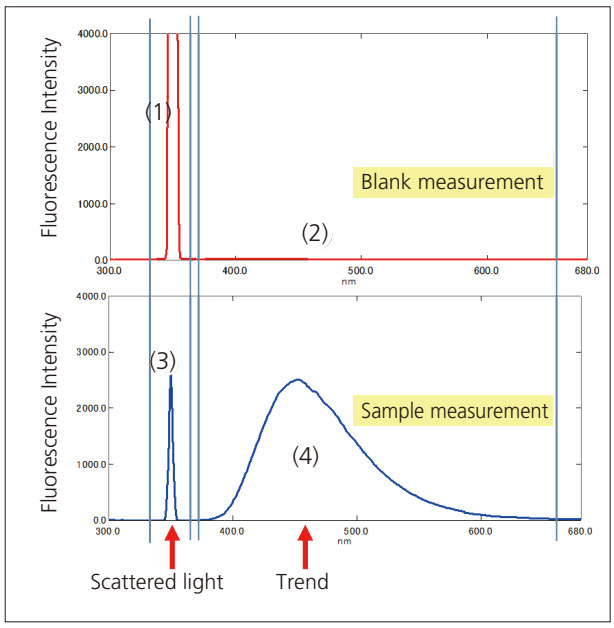

Fig. 2 Measurement Method of Fluorescence Quantum Efficiency

Table 1 Formulas Related to Fluorescence Quantum Efficiency

Absorption factor = Absorbed/Incident = ((1) - (3)) / (1)
Internal quantum efficiency = Emitted/Absorbed = ((4) - (2)) / ((1) - (3))
External quantum efficiency = Emitted/Incident = ((4) - (2)) / (1)
External quantum efficiency = Internal quantum efficiency × Absorption factor
(1) : Number of incident photons
(2) : Number of photons included in baseline
(3) : Number of photons not absorbed
(4) : Number of photons in fluorescent and baseline light
(1) - (3) : Number of photons absorbed
(4) - (2) : Number of photons emitted as fluorescent light

Measuring the Fluorescence Quantum Efficiency of Quinine Sulfate

A 200 mg/L quinine sulfate solution (1.0 N sulfuric acid solvent) was prepared using quinine sulfate dihydrate (special grade reagent from Wako Pure Chemical).
The following procedure was used to determine the fluorescence quantum efficiency of quinine sulfate.

(1) Place the integrating sphere inside the RF-6000 sample compartment.
(2) Place the quartz cell with four polished sides that contains the blank (1.0 N sulfuric acid solvent) into the integrating sphere cell holder, as shown in Fig. 3. Then close the integrating sphere lid.
(3) Measure the fluorescence spectrum of the blank using the quantum efficiency measurement program in LabSolutions RF. Then replace the cell with a cell containing the quinine sulfate solution and measure its fluorescence spectrum. The excitation light from the excitation monochromator enters the integrating sphere via the inlet window and hits the sample, as shown in Fig. 3. The scattered excitation light and fluorescent light emitted from the sample are scattered uniformly throughout the integrating sphere interior. A portion of that light passes out the integrating sphere outlet window and into the spectrofluorometer, where its fluorescence spectrum is measured.
(4) Area values are calculated from blank and quinine sulfate data in a specified wavelength range. Then the formulas in Table 1 are used to calculate the absorption factor (AF), internal quantum efficiency (QEin), and external quantum efficiency (QEex). The wavelength range used for calculations can be freely specified by moving the two vertical lines shown in Fig. 5.

In this example, the area value corresponding to (1) and (3) in Fig. 2 was determined using the range from 320 nm to 370 nm, as indicated by the blue arrow in Fig. 5, and the area value corresponding to (2) and (4) was determined using the range from 370 nm to 680 nm, as indicated by the green arrow. The fluorescence quantum efficiency calculated from these results for quinine sulfate is 0.5336, which closely matches the 0.50 to 0.57 range indicated in reference.[1)]

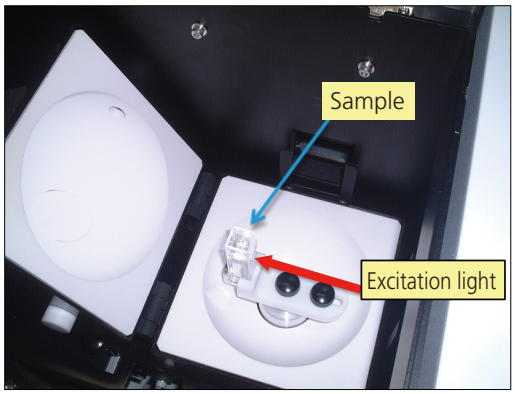

Fig. 3 Sample Mounted to the Integrating Sphere

Table 2 Measurement Conditions

Measuring instrument	:RF-6000 spectrofluorophotometer
Spectrum type	:Fluorescence
Excitation wavelength	:350 nm
Measurement wavelength range	:300 to 680 nm
Data interval	:1.0 nm
Scan speed	:200 nm/min
Bandwidth	:Ex 3 nm, Em 3 nm
Sensitivity	:Auto

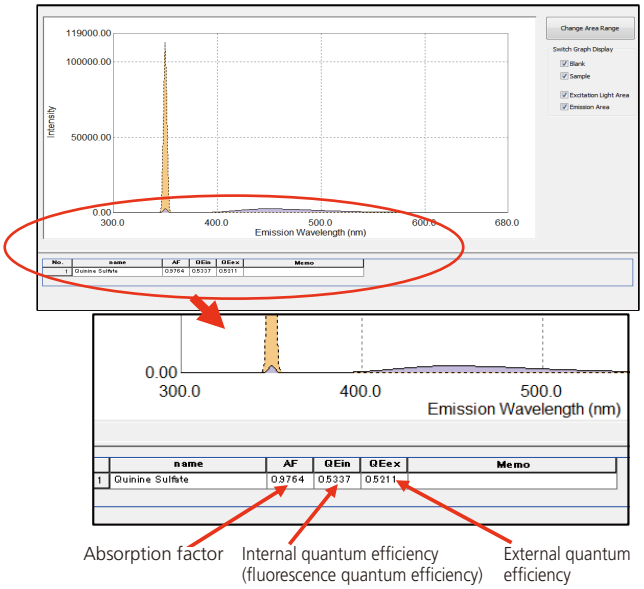

Fig. 4 Fluorescence Quantum Efficiency Calculated with Quantum Efficiency Measurement Mode

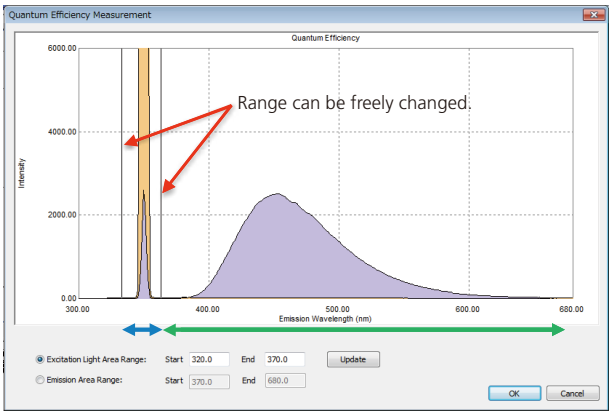

Fig. 5 Setting the Calculation Range

Conclusion

The RF-6000 includes an automatic spectral correction function that makes it easy to obtain corrected fluorescence spectra required for measuring fluorescence quantum efficiency. By using an integrating sphere attachment and the standard LabSolutions RF software, the system can determine the fluorescence quantum efficiency of target substances easily.

1) JASCO Measurement Method Series 3, Applications for Measuring Fluorescence in Biological Sciences (Academic Society Publishing Center)

First Edition: Oct. 2015

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