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Measuring Fluorescence Quantum Yield Using a Tube Inside an Integrating Sphere

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Abstract: A method for measuring the fluorescence quantum yield Φ_f of samples using a soft transparent tube with 0.58 mm inner diameter inside an integrating sphere is presented. Based on simple energy balance within the sphere, an equation for determining Φ_f in terms of the optical output signal is derived. In order to determine the feasibility of measuring Φ_f using the present integrating sphere-spectrometer setup, the Φ_f of Rhodamine 6G (R6G) solutions is determined. The measured Φ_f of R6G in water and in ethanol are 0.647±0.005 and 0.881±0.025, respectively. These values are close to those found in literature, with the measured Φ_f of R6G in ethanol within the range of values reported for a similar integrating sphere method. Recommendations are given for measuring the fluorescence quantum yield of low efficiency fluorophores of biomedical interest. *Copyright © 2016 IFSA Publishing, S. L.*

Keywords: Fluorescence quantum yield, Integrating sphere, Rhodamine 6G.

1. Introduction

The absorption of light by target absorbers may result to either radiative or non-radiative energy conversion [1]. In the our previous publication [2], the Grüneisen parameter, the photoacoustic efficiency of absorbing samples mounted using a tube inside the integrating sphere is related to the conversion of absorbed light energy to non-radiative thermal and eventually mechanical energy [3]. In the research presented here, fluorescence, the property of target materials to convert absorbed photons into emitted light is considered. A direct measurement of the efficiency of some atoms and molecules to re-emit absorbed light at (in general) a longer wavelength than the original one is called the fluorescence quantum yield Φ_f. Measuring the ratio of the number of photons emitted to the number of photons absorbed directly indicates Φ_f of the fluorescent material. The different procedures and techniques for absolute measurements, or measurements relative to a fluorescent standard material with a known Φ_f by optical or calorimetric methods, under various environmental conditions, have already been described and reported over the past twenty-five years [4-5]. The technique of using an integrating sphere for measuring Φ_f has also been previously explored [6-7]. The integrating sphere method has the advantage of measuring the absorption of scattering samples in the same experimental setup.

This paper presents the method of measuring Φ_f of samples injected into a tube inside an integrating sphere setup. A simple derivation is demonstrated of the equation for determining Φ_f based on energy conservation and using the optical output signal from the integrating sphere measured by a spectrometer, similar to that found in related literature [6, 8]. The feasibility of the method using the present integrating sphere-spectrometer setup is initially investigated by measuring Φ_f of Rhodamine 6G solutions.

2. Brief Theoretical Description

An energy balance inside the integrating sphere (illustrated in Fig. 1) can be written as follows

$$E_{in}(\lambda) = E_a(\lambda) + E_w(\lambda) + E_{out}(\lambda), \tag{1}$$

where E_a is the magnitude of the energy absorbed by the absorber inside the tube and E_{out} is the optical energy that leaves the system through the output port. E_w represents all the other energy losses within the integrating sphere system, for example, energy absorbed by the medium filling up the entire sphere cavity and wall of the tube. If there is no sample (ns) inside the tube, $E_a = 0$ and $E_{out} = E_{out,ns}$ and for incident wavelengths in the absorption region of the sample, $\lambda = \lambda_a$

$$E_{in}(\lambda_a) = E_{w,ns}(\lambda_a) + E_{out,ns}(\lambda_a) \tag{2}$$

On the other hand, if there is a sample (s) inside the tube,

$$E_{in}(\lambda_a) = E_a(\lambda_a) + E_{w,s}(\lambda_a) + E_{out,s}(\lambda_a) \tag{3}$$

For a fluorescent sample with fluorescence quantum yield Φ_f, the absorbed energy E_a is linearly related to the energy of the emitted (fluorescent) light

$$E_a(\lambda_a) = \frac{\lambda_f E_f(\lambda_f)}{\lambda_a \Phi_f}, \tag{4}$$

where λ_f and λ_a are the fluorescence and absorption wavelengths, respectively, since Φ_f is defined as a ratio of number of fluorescence photons to that of absorbed photons. The fluorescence can be taken as another source of energy within the sphere cavity, which can also be absorbed by the sample, by other parts or by the output port. For these fluorescence wavelengths λ_f, another energy balance can be written as

$$E_f(\lambda_f) = E_{a,f}(\lambda_f) + E_{w,s}(\lambda_f) + E_{out,s}(\lambda_f) \tag{5}$$

Here $E_{a,f}(\lambda_f)$ is the fluorescence emission energy absorbed by the fluorophore (reabsorption term), $E_{w,s}(\lambda_f)$ is energy absorbed by the wall and $E_{out,s}(\lambda_f)$ is energy of light reaching the output port, due to fluorescence. Since the fluorescence emission is Stokes-shifted relative to the absorption peak of the fluorophore, the volume of the fluorophore as well as the concentration are small, we can neglect the effect of re-absorption and the first term can be approximated to be zero, i.e. $E_{a,f}(\lambda_f) = 0$.

Equation (4) and (5) can be inserted in Equation (3) to obtain

$$E_{in}(\lambda_a) = \frac{\lambda_f}{\lambda_a \Phi_f}\left\{E_{w,s}(\lambda_f) + E_{out,s}(\lambda_f)\right\} + E_{w,s}(\lambda_a) + E_{out,s}(\lambda_a)$$

$$E_{in}(\lambda_a) = \frac{\lambda_f}{\lambda_a \Phi_f} E_{w,s}(\lambda_f) + \frac{\lambda_f}{\lambda_a \Phi_f} E_{out,s}(\lambda_f) + E_{w,s}(\lambda_a) + E_{out,s}(\lambda_a) \tag{6}$$

Grouping terms together gives

$$E_{in}(\lambda_a) = \left\{\frac{\lambda_f}{\lambda_a \Phi_f} E_{w,s}(\lambda_f) + E_{w,s}(\lambda_a)\right\} + \left\{\frac{\lambda_f}{\lambda_a \Phi_f} E_{out,s}(\lambda_f) + E_{out,s}(\lambda_a)\right\} \tag{7}$$

Here the first right-hand term between brackets is the total energy absorbed by the sphere wall and the second term is the total energy of light incident on the output port, with a fluorophore inside the sphere. Comparing Equation (7) to Equation (2) yields the following equalities

$$E_{w,ns}(\lambda_a) = \left\{\frac{\lambda_f}{\lambda_a \Phi_f} E_{w,s}(\lambda_f) + E_{w,s}(\lambda_a)\right\} \tag{8a}$$

$$E_{out,ns}(\lambda_a) = \left\{\frac{\lambda_f}{\lambda_a \Phi_f} E_{out,s}(\lambda_f) + E_{out,s}(\lambda_a)\right\} \tag{8b}$$

Equation (8a) is particularly valid for a spectrally neutral integrating sphere system. Equation (8b) gives the equation for the energy of light reaching the output port, which is the relevant equation in determining the fluorescence quantum yield using optical measurements from the integrating sphere. Rearranging terms gives

$$E_{out,ns}(\lambda_a) - E_{out,s}(\lambda_a) = \frac{\lambda_f}{\lambda_a \Phi_f} E_{out,s}(\lambda_f)$$

Thus,

$$\Phi_f = \frac{\lambda_f E_{out,s}(\lambda_f)}{\lambda_a \left\{E_{out,ns}(\lambda_a) - E_{out,s}(\lambda_a)\right\}} \tag{9}$$

In order to measure the fluorescence quantum yield Φ_f of a sample inside the tube in the integrating sphere, two main measurements are required:

1) Optical output signal when there is no sample inside the tube;

2) Optical output signal when there is a fluorescent sample inside the tube, $E_{out,s} = E_{out,s}$ (λ_a, λ_f), where λ_a is the range of wavelengths in the absorption (excitation) region and λ_f is the range in the fluorescence (emission) region. $E_{out,s}$ (λ_f) is summed over the emission wavelengths, whereas the denominator $E_{out,ns}$ (λ_a) − $E_{out,s}$ (λ_a) should be summed over the incident absorption wavelengths.

Equation (9) should be used to determine the fluorescence quantum yield Φ_f with careful consideration that $E_{out,ns}$ and $E_{out,s}$ are energy magnitudes at a particular wavelength, similar to that in [6-8]. For fluorophores with substantially overlapping absorption and emission spectra, a correction procedure must be done by varying fluorophore concentrations, as in [9].

3. Description of Experimental Setup

The experimental setup for measuring fluorescence quantum yield Φ_f using an integrating sphere is given in Fig. 1. An integrating sphere (Thorlabs IS200) with a radius of 25.4 mm and a sphere wall reflectance of 0.99 was used in the setup. A polyethylene tube (Portex) with inner diameter of 0.58 mm and outer radius of 0.96 mm was inserted through two opposite ports. The tube axis was located approximately 5 mm above the center of the sphere. The absorber was injected into this tube. Injecting the absorber until it flowed out on the other side of the tube ensured that the tube was completely filled. A Tungsten Halogen light source (Avantes AvaLight-Hal) was used for illumination and was connected to a converging lens by an optic fiber (Thorlabs M37L01, core diameter of 550 µm, 0.22NA). This ensured that incident light could not directly hit the tube and was reflected diffusively on the sphere wall at least once before hitting the tube. The lens was connected to the input port of the sphere. A filter is added to the input port of the integrating sphere to allow only certain wavelengths of light to pass through. For the measurements presented here, a filter (Semrock 475/23 nm BrightLine® single-band bandpass filter (FF01-475/23-25)) that only allows a certain region (for this filter wavelength range from 450 nm to 500 nm) in the absorption spectrum of Rhodamine 6G solutions is used. A fiber optic spectrometer (Avantes AvaSpec-2048) was used for detection of the outgoing light and was connected to the output port of the sphere by another optic fiber of the same type. There are two main measurements:

1) Measurement of optical output signal without the sample inside the tube;

2) Measurement with the fluorescent sample inside the tube.

Correction for the dark noise in the spectra is not necessary since the noise floor is very low as can be seen in the results of measurements in the next Section.

The samples used in the measurements presented here are dilute solutions of Rhodamine 6G chloride (R-634 Life Technologies) with 0.1 g of the dye powder dissolved in 100 ml purified water. Fluorescent samples of R6G are prepared with a small amount of this stock solution diluted 500 times with water and with ethanol.

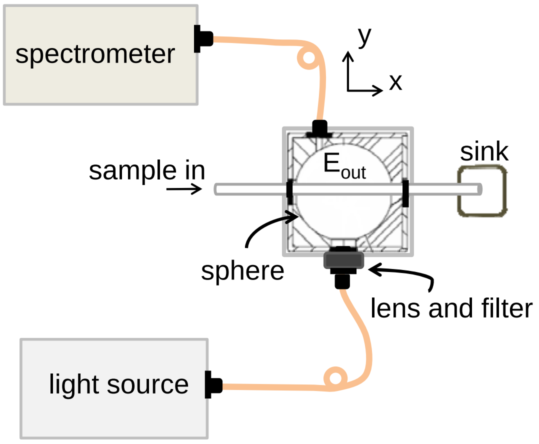


Fig. 1. Schematic diagram of the experimental setup (top-view) for measuring the fluorescence quantum yield Φ_f of an absorbing liquid sample inside a tube (inner diameter = 0.58 mm) mounted through an integrating sphere (diameter = 50.8 mm). The axis of the tube is positioned approximately 5 mm above the center plane which contains the optical ports. A lens converges the incident beam at the center of the spherical cavity, whereas a filter only allows wavelengths at the absorption region of the sample. For the case of Rhodamine 6G, the filter allows the wavelength range from 450 nm to 500 nm to pass through the sphere's input port.

4. Results and Discussion

Fig. 2 shows an example of the optical output signal detected by the spectrometer when there is no filter connected to the input port of the sphere.

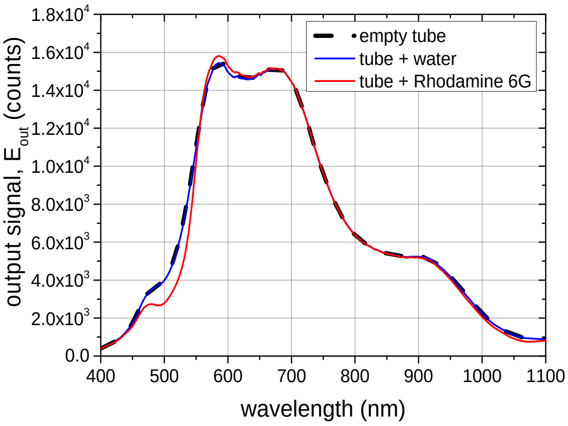


Fig. 2. Example of detected optical output signal when there is no filter connected to the input port.

Three sets of detected signals are shown:

1) When there is air inside the tube;

2) When there is water inside the tube;

3) When there is Rhodamine 6G sample (R6G) inside the tube.

It can be observed that the detected signal with an empty tube coincides with that with water inside. With the R6G, there is a dip around 500 nm wavelength which implies absorption and a peak around 575 nm which can be due to fluorescence. In order to differentiate the fluorescence signal, a filter is connected to the input port so that only the wavelength in the absorption region of R6G is allowed to pass through. Typical absorption and emission spectra [10] are given in Fig. 3.

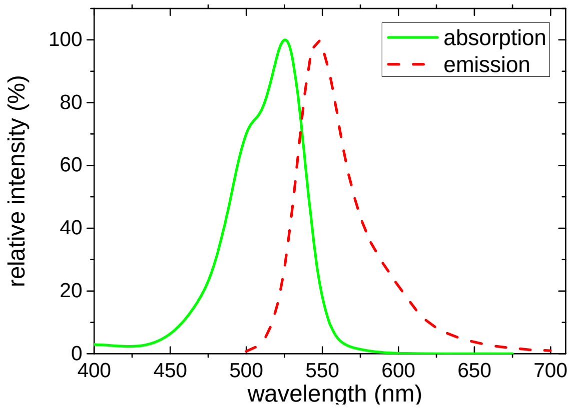


Fig. 3. Typical absorption and emission spectra of Rhodamine 6G [10].

There is an overlap in the absorption and emission of R6G between 500 nm and 600 nm. Filtering out wavelengths higher than 500 nm from the incident beam ensures that the detected light with wavelength higher than 500 nm is due to fluorescence. An example of the detected signals when the specified filter that transmits light in the wavelength range 450 nm to 500 nm, is connected to the sphere input port is given in Fig. 4 (the spectrometer integration time is 140 ms). The black and green lines are the detected signals for blank $E_{out,ns}$ (no sample, only water inside the tube) and sample $E_{out,s}$ (R6G solution inside the tube) measurements, respectively. An inset shows the zoomed in region of the emission spectrum above 500nm which is used for determining E_f in Equation (6). The absorption and emission regions are indicated on the figure. The detected signals are measured in terms of counts which are actually proportional to the energy of the light incident on the detector with varying sensitivity per wavelength. Thus, the detected value must be multiplied by the wavelength divided by the spectral sensitivity of the detector at that wavelength. Multiplication with the wavelength is necessary in order to obtain a value proportional to the number of photons since a typical spectrometer measures a quantity proportional to the incident power or energy. An estimated spectral sensitivity curve of the integrating sphere-spectrometer system based on a theoretical black body radiation light source is shown in Fig. 5(a).

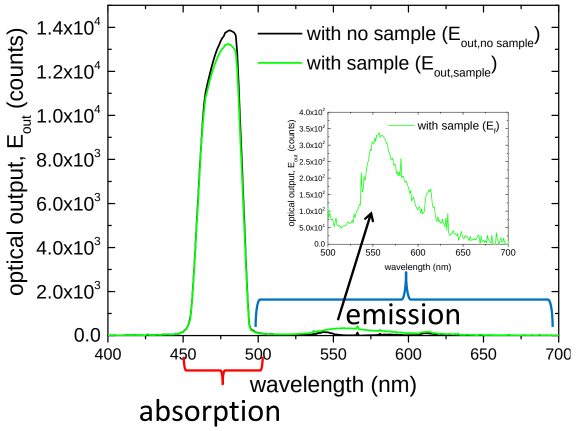
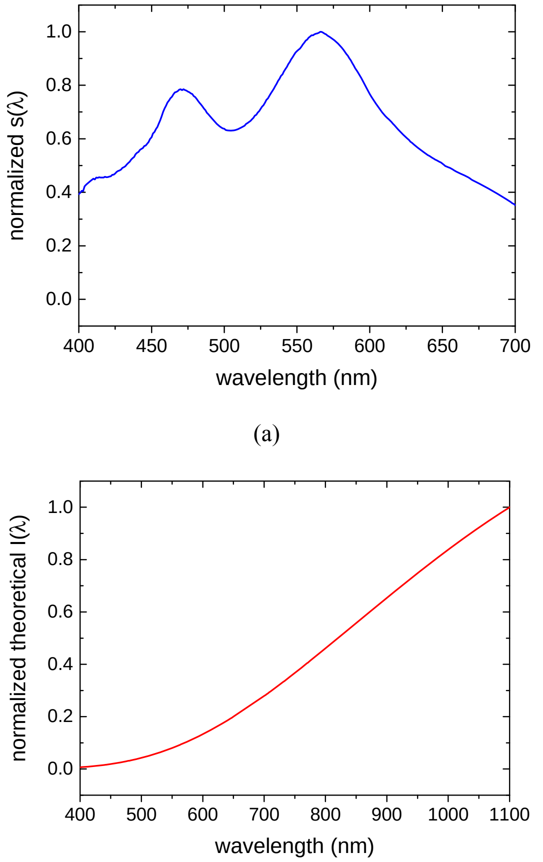


Fig. 4. Example of detected signals when there is a 450-500 nm passband filter connected to the input port of the integrating sphere. The black and green plots are used for determining the values of $E_{out,ns}$ and $E_{out,s}$ in Equation (6). In the inset the green plot above above 500 nm is zoomed in to visibly show the emission profile used for determining E_f used in Equation (6).



(a)

(b)

Fig. 5. (a) Estimated spectral sensitivity curve of the integrating sphere-spectrometer system and (b) normalized theoretical output spectrum of the light source.

This curve is measured by taking the ratio of the signal detected by the integrating sphere-spectrometer ensemble when there is no sample inside the tube to that of a normalized theoretical spectrum (shown in Fig. 5 (b)) of the light source assumed to be a black body radiator (such as the halogen lamp used in the measurements [11-12]). It can be observed that the spectral sensitivity of the integrating sphere-spectrometer detection system is not constant over all wavelengths which indicates that the detected signal should be normalized by this sensitivity per wavelength. $E_{out,ns} - E_{out,s}$ in Equation (6) is determined over all the absorption wavelengths, whereas the E_f is the sum over all the emission wavelengths. For the measurements presented here, only 51 spectral datapoints around the peak are included in the calculation, since only a small portion of the absorption region is used. Using the spectral sensitivity and detected signals in Equation (6), the measured Φ_f of R6G in water is 0.647 ± 0.005 (mean ± standard error), whereas the Φ_f of R6G in ethanol is 0.881 ± 0.025. These measured values are average from at least three measurements with the same R6G solution. The measured Φ_f of R6G in ethanol is within the recently reported values ranging from 0.87 to 1 using an integrating sphere method [7]. The measured Φ_f of R6G in water is close to the one reported for dimerization and trimerization of R6G in aqueous solutions which is 0.70 [13].

These initial results indicate the feasibility of measuring Φ_f using the present integrating sphere system with a mounted tube used as the sample holder. Although the measured values are close to those in literature, more measurements must be done to investigate the accuracy of the method. The method should also be validated by comparing the measured values with those in literature as well as with those obtained from a calibrated fluorimeter.

Aside from using other concentrations of R6G solutions, other fluorescent dyes which are relevant to biomedical imaging can also be investigated. Results with R6G samples indicate that the emission spectra of fluorophores with relatively high quantum efficiency can be detected in the present integrating sphere detection system. For those with low Φ_f, the fluorescent signal must be distinguished from the noise level of the system. Attaching relevant filters to both input and output ports of the integrating sphere can filter out the noise on the absorption and emission regions of the target fluorophore. Based on the measured spectra with R6G solutions, it can be anticipated that the emission spectra of fluorophores with 10 times lower Φ_f can still be distinguished on the detected output signal of the present integrating sphere setup. Moreover, adjusting the integration time of the spectrometer can also increase the detection sensitivity for very weak output signal. However, for a too long integration time the excitation part of the spectrum might saturate, leading to a saturation artefact sometimes called 'blooming'. A solution might be to split the detection fiber, and to use two spectrometers in parallel, one for the excitation light, and one (with an appropriate filter to block excitation light) for the fluorescent light.

5. Conclusions

An integrating sphere setup commonly used for measuring the optical absorption of scattering samples is developed to measure fluorescence quantum yield Φ_f. A soft transparent tube mounted through the sphere cavity is used as the target sample holder. An equation for determining Φ_f in terms of the optical output signal from the integrating sphere is derived based on a simple energy balance within the sphere. Similar integrating sphere methods for measuring Φ_f are also found in literature [6, 7, 9]. Initial measurements with Rhodamine 6G (R6G) solutions result to Φ_f values of R6G in water and in ethanol of 0.647 ± 0.005 and 0.881 ± 0.025, respectively which are close to those found in literature. In particular, the measured Φ_f of R6G in ethanol is within the range of values reported for a similar integrating sphere method.

Although the measured Φ_f values of R6G solutions are close to those in literature, it should be noted that the measurements here can be further improved by determining the spectral sensitivity of the system more accurately, for example by using a calibrated light source. Also, validation of the method described here can be done by comparing measurements with a reliable and calibrated fluorimeter that measures Φ_f of similarly prepared fluorophores. After implementing these improvements, the method presented here can be further explored on the feasibility to measure the fluorescence quantum yield of fluorophores relevant to biomedical imaging. Moreover, simultaneous measurements of the Grüneisen parameter and absorption coefficient can be done using a similar integrating sphere setup [2].

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