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Rhodamine 800 as reference substance for fluorescence quantum yield measurements in deep red emission range

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ABSTRACT

The determination of fluorescence quantum yields (Φ_f) of deep red dyes emitting at 635–900 nm is difficult due to lack of suitable standards. In this work, we propose a commercial dye, rhodamine 800 (Rho800), as reference standard which belongs to the family of xanthenes. The quantum yield of rhodamine 800 in absolute ethanol has been studied using a relative method with cresyl violet (CV) and rhodamine 101 (Rho101) as references, and an absolute fluorometric method by integrating sphere measurements.

1. Introduction

The efficiency of the conversion of absorbed photons into emitted photons by a chromophore is the photoluminescence quantum yield (termed here as fluorescence quantum yield, Φ_f) [1–3].

$$\Phi_f = \frac{N_{em}}{N_{abs}} \tag{1}$$

Φ_f can be determined fluorometrically with a relative method through a properly chosen fluorescent standard which ought to have photophysical properties similar to the sample, with a known fluorescence quantum yield. The experimental conditions should be as similar as possible: if the absorption bands of sample and reference are nearly overlapped, it is possible to use the same excitation wavelength for both; instead, if different excitation wavelengths have to be used, it is necessary to use the correction curve of the excitation channel [4–6]. In this method, the emission quantum yield of the sample is calculated by [7,8]

$$\Phi_x = \Phi_s \left(\frac{I_x}{I_s}\right)\left(\frac{F_s}{F_x}\right)\left(\frac{n_x}{n_s}\right)^2 \tag{2}$$

where I_x and I_s are respectively the integrated emission intensity expressed as photon flux density of the sample (x) and standard (s), F_x and F_s are the fractions of light absorbed and n_x and n_s denote the respective refractive indexes.

The absolute quantum yield can be instead determinated by de Mello's method [9], where the value is given by

$$\Phi_x = \frac{I_{sample,in} - (1-A) I_{sample,out}}{X_{blank,in} A} \tag{3}$$

with

$$A = \frac{X_{sample,out} - X_{sample,in}}{X_{sample,out}} \tag{4}$$

where $I_{sample,in}$ and $I_{sample,out}$ are respectively the integrated emission intensities of the sample when the laser beam directly hits the sample and when it strikes the inner wall of the integrating sphere and the secondary diffuse light hits the sample, $X_{blank,in}$ is the integrated excitation profile of blank that directly hits blank (*blank* is the solvent filled cuvette), $X_{sample,in}$ and $X_{sample,out}$ are respectively the integrated excitation profile of sample as a result of direct and secondary light excitation and A is the fraction of light absorbed, expressed as one minus the transmittance.

The aim of the present work is to provide the fluorescence quantum yield of Rhodamine 800, which is a commercially available deep red emitting dye, as potential fluorescence reference (Table 1).

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2. Experimental

2.1. Materials

Rhodamine 101 (Rho101, CAS number 116450-56-7), Rhodamine 800 (Rho800, CAS number 101027-54-7) were purchased from Sigma. Cresyl violet acetate (CV, CAS number 10510-54-0) was purchased from Acros Organics. These dyes were the highest purity available; the absence of impurities for all the dyes was confirmed by mass spectroscopy (DCI–MS). The solvent used is ethanol absolute anhydrous of spectroscopic grade and purchased from Carlo Erba.

2.2. Instrumentation

Absorption spectra were collected on a Perkin Elmer Lambda 950 spectrophotometer (spectral bandwidth 1 nm, stepsize 1 nm). Fluorescence emission and excitation spectra were measured with Horiba Fluorolog 3 spectrofluorometer. The setup consists of a 450 W Xenon lamp coupled a single monochromator for excitation, a double monochromator for emission iHR320 with holographic rating (150 g/mm for Perltier cooled CCD, Synapse, Horiba Jobin Yvon). In order to correct the temporal fluctuations of the stabilized Xenon lamp source, the emission signal was divided by the signal of a reference diode monitoring the lamp output. For all measurements, the spectral sensitivity of the whole setup (with or without integrating sphere) was corrected through instrument correction files provided and updated by the manufacturer, obtained by calibrated lamps.

2.3. Measurement of molar absorption coefficient

The molar absorption coefficient $\varepsilon(\lambda)$ was determined from two separately weighted and dissolved stock solutions in a repeat determination of each sample ($N=6$ independent measurements). The dilution was chosen in such a way that the absorbances of the sample solutions equaled 2.5 at the maximum of the first absorption band. The quartz cells (Hellma 110-QS, 110-50-40) were sealed with Teflon stops after each filling step.

2.4. Relative measure of Φ_f

All fluorescence spectra presented here are corrected for emission from the solvent and dark counts from the detector (blank correction), and for instrument specific contribution

Table 2
Steady-state fluorescence properties and the Stokes shift of Rho800 in absolute ethanol.

Dye	$\lambda_{abs\ max}$ (nm)	$\lambda_{em\ max}$ (nm)	$\Delta\omega_{abs\text{-}em}$ (cm^{-1})	r
Rho101	564	588	723	0.015
CV	600	619	512	0.025
Rho800	682, 623	700, 774	377	0.018

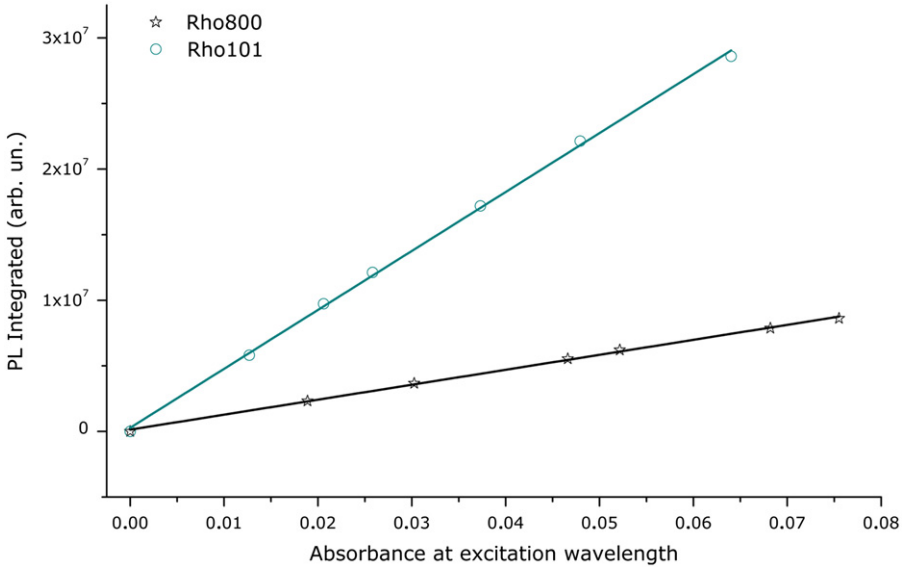
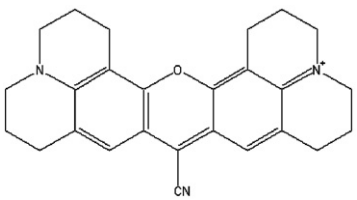
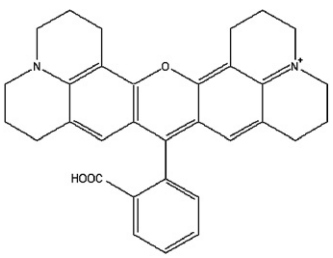


Fig. 1. Linear plots of emission intensity vs. absorbed photons. Conversion into an absolute quantum yield is achieved through the knowledge Φ_f of the reference.

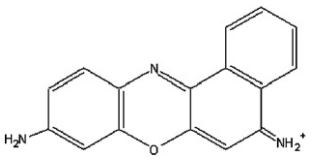
Table 1
Chemical structures of dyes investigates.



Rhodamine 800 (Rho800)



Rhodamine 101 (Rho101)



Cresyl violet acetate (CV)