

Reevaluation of absolute luminescence quantum yields of standard solutions using a spectrometer with an integrating sphere and a back-thinned CCD detector†

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We reevaluate the absolute fluorescence and phosphorescence quantum yields of standard solutions by using a novel instrument developed for measuring the absolute emission quantum yields of solutions. The instrument consists of an integrating sphere equipped with a monochromatized Xe arc lamp as the light source and a multichannel spectrometer. By using a back-thinned CCD (BT-CCD) as the detector, the sensitivity for spectral detection in both the short and long wavelength regions is greatly improved compared with that of an optical detection system that uses a conventional photodetector. Using this instrument, we reevaluate the absolute fluorescence quantum yields (Φ_f) of some commonly used fluorescence standard solutions by taking into account the effect of reabsorption/reemission. The value of Φ_f for 5×10^{-3} M quinine bisulfate in 1 N H_2SO_4 is measured to be 0.52, which is in good agreement with the value (0.508) obtained by Melhuish by using a modified Vavilov method. In contrast, the value of Φ_f for 1.0×10^{-5} M quinine bisulfate in 1 N H_2SO_4 , which is one of the most commonly used standards in quantum yield measurements based on the relative method, is measured to be 0.60. This value is significantly larger than Melhuish's value (0.546), which was estimated by extrapolating the value of Φ_f for 5×10^{-3} M quinine bisulfate solution to infinite dilution using the self-quenching constant. The fluorescence quantum yield of 9,10-diphenylanthracene in cyclohexane is measured to be 0.97. This system can also be used to determine the phosphorescence quantum yields (Φ_p) of metal complexes that emit phosphorescence in the near-infrared region: the values of Φ_p for $[\text{Ru}(\text{bpy})_3]^{2+}$ (bpy = 2,2'-bipyridine) are estimated to be 0.063 in water and 0.095 in acetonitrile under deaerated conditions at 298 K, while that in aerated water, which is frequently used as a luminescent reference in biological studies, is reevaluated to be 0.040.

1. Introduction

The fluorescence or phosphorescence quantum yield, which is defined as the ratio of the number of emitted photons to that of absorbed photons, is one of the most fundamental and important photophysical parameters of emissive compounds.^{1–3} The luminescence quantum yield measured for a molecule in solution varies depending on the experimental conditions,

including the kind of solvent, the concentrations of sample molecules and dissolved oxygen in the solution, temperature, and excitation wavelength. When the physical conditions are fully specified, the absolute quantum yield can, in principle, be precisely determined. However, even if these parameters are specified, a number of pitfalls exist, which must be considered explicitly to determine reliable quantum yields. These include polarization effects, refractive index effects, reabsorption/reemission effects, internal reflection effects, and the spectral sensitivity of the detection system.^{4,5}

Over the past several decades considerable efforts have been made to develop reliable methods for determining luminescence quantum yield.^{4,5} They can be classified into absolute (or primary) methods and relative (or secondary) methods. The first reliable absolute method was developed by Vavilov,⁶ in which a solid scatterer (magnesium oxide) was used to calibrate the detector/excitation system absolutely. In the Vavilov method, the detector first monitors the sample luminescence generated by total absorption of the excitation light focused to a point in the cell. The detector then records

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the light that is diffusely scattered from the magnesium oxide surface, which was substituted for the original cuvette. The absolute quantum yield of the sample can be calculated by substituting these data together with some additional information into complicated equations.⁴ The Weber and Teale method⁷ compares the fluorescent light with light from a scattering solution. This method has the advantage that errors resulting from self-absorption and quenching of fluorescence can be eliminated by extrapolating measurements to zero concentration.⁸ Calorimetric methods^{9–12} and photoacoustic (optoacoustic) methods^{13,14} detect the fraction of the absorbed energy that is lost by nonradiative processes in a luminescent sample, *i.e.*, the complement of the luminescence energy yield. These photothermal methods generally require making the assumption that the relaxation processes of excited molecules involve no photochemical reactions.

The absolute methods require performing various complex corrections to obtain accurate quantum yields. Therefore, in most laboratories relative (secondary) methods are used to determine quantum yields. In secondary methods, the quantum yield of a sample solution is determined by comparing the integrated fluorescence intensity with that of a standard solution under identical conditions of incident irradiance. Thus, it is critical to correct for the spectral sensitivity of the instrument, and the measured quantum yield is only as accurate as the certainty of the quantum yield of the fluorescence standard. One of the most widely used secondary standards is quinine bisulfate (QBS) in 1 N H₂SO₄ at 298 K ($\Phi_f = 0.546$ for infinite dilution) reported by Melhuish.^{15,16} This value was estimated by extrapolating the Φ_f value (0.508) of 5×10^{-3} M QBS solution, which was determined by absolute measurements based on the Vavilov method, to infinite dilution using the self-quenching constant.¹⁵ There is a limited amount of data available for such a widely used reference.^{8,10,12,14,17,18} 9,10-Diphenylanthracene (DPA) has also been employed as a popular fluorescence standard because of its high quantum yield. However, the published quantum yields of DPA vary widely from 0.86 to 1.06.^{11,19–22}

Reevaluation of absolute phosphorescence quantum yields (Φ_p) of metal complexes is also necessary for the following two reasons: (i) the sensitivity of conventional photon detectors, such as photomultiplier tubes, is quite low at long wavelengths, where phosphorescence is generally observed, and (ii) the reported Φ_p values are generally obtained by using some fluorescence standards, the quantum yields of which also need to be reevaluated. In the excited states of transition metal complexes, the efficiency of intersystem crossing is enhanced because of the heavy atom effect. This results in the appearance of phosphorescence even at room temperature. As a representative phosphorescent metal complex, ruthenium(II) tris(bipyridine) complex, $[\text{Ru}(\text{bpy})_3]^{2+}$ (bpy: 2,2'-bipyridine), has been widely investigated and many of its derivatives have been synthesized.^{23,24} $[\text{Ru}(\text{bpy})_3]^{2+}$ shows a metal-to-ligand charge transfer (MLCT) band at around 450 nm in the absorption spectrum and exhibits a broad phosphorescence band with a peak at 600–650 nm; the positions of these bands are slightly dependent on the solvent used. In spite of its relatively low phosphorescence quantum yield (less than 10%), $[\text{Ru}(\text{bpy})_3]^{2+}$ is frequently

used as a standard, probably because it is readily available and has been well investigated.

However, several different values for the phosphorescence quantum yield of $[\text{Ru}(\text{bpy})_3]^{2+}$ have been reported by some research groups: 0.042–0.055 in H₂O^{25–30} and 0.059–0.090 in CH₃CN.^{27,30–32} The most cited value is 0.042 in H₂O, which was measured by Van Houten and Watts based on a relative method using fluorescein in 0.1 N NaOH as a standard.^{25,26} In particular, the quantum yields in organic solvents (*e.g.*, 0.062 in CH₃CN), which were obtained based on the value (0.042) in H₂O, are also frequently quoted.³¹ Recently, Nozaki *et al.* reported that the quantum yield of $[\text{Ru}(\text{bpy})_3]^{2+}$ in CH₃CN is 0.090, which was evaluated by using a back-thinned CCD (BT-CCD) detector with the Φ_f value (0.91 in deaerated cyclohexane) of 9,10-diphenylanthracene as the standard.³²

Recently, integrating sphere instruments^{18,33–42} have received considerable attention as they provide a simple and accurate means for determining the absolute luminescence quantum yield. By using an integrating sphere, much of the optical anisotropy is eliminated by multiple reflections on the inner surface of the integrating sphere. We have developed an instrument for determining the absolute luminescence quantum yield of solutions, solids,⁴³ and thin films⁴³ by utilizing an integrating sphere for a sample chamber to eliminate the effects of polarization and refractive index from measurements. In this present study, we reevaluate the absolute quantum yields of fluorescent standard solutions using this integrating sphere instrument. We also report the reevaluated Φ_p value of ruthenium(II) tris(bipyridine) complex, which has often been employed as a standard in measurements of the relative quantum yield of metal complexes.

2. Experimental

2.1 Materials

Naphthalene (Kanto) and 1-aminonaphthlene (Tokyo Kasei) were purified by vacuum sublimation. Quinine bisulfate (QBS; Wako) was purified by recrystallization three times from water. Anthracene (Tokyo Kasei) was purified by recrystallization from ethanol. 9,10-Diphenylanthracene (Lancaster) was purified by high-performance liquid chromatography. *N,N*-Dimethyl-1-aminonaphthalene (Kanto) was purified by distillation under reduced pressure. Fluorescein (Wako) was purified by column chromatography on a silica-gel column using ethyl acetate as the eluent. Tryptophan (Kanto) was used as received. 2-Aminopyridine (Tokyo Kasei) was purified by recrystallization from cyclohexane. 3-Aminophthalimide (Kodak) and *N,N*-dimethylamino-*m*-nitrobenzene (Tokyo Kasei) were purified by recrystallization from ethanol. 4-Dimethylamino-4'-nitrostilbene (Tokyo Kasei) was purified by recrystallization from chloroform. Cyclohexane (Aldrich, spectrophotometric grade), ethanol (Tokyo Kasei, spectrophotometric grade) and sulfuric acid (Wako, analytical grade) were used without further purification. Benzene (Kishida, spectrophotometric grade) was used as received. $[\text{Ru}(\text{bpy})_3](\text{PF}_6)_2$ was prepared as follows: RuCl₃·*n*H₂O (Wako) and 2,2'-bipyridine (Wako)

were refluxed in aqueous 30%-NaH₂PO₂ solution for 2 h. After filtration, a saturated NaPF₆ solution was added to the filtrate, yielding orange precipitates. The collected solid was washed with benzene and recrystallized in water/acetonitrile. Anal. Calcd for C₃₀H₂₄N₆F₁₂P₂Ru: C, 41.92; H, 2.81; N, 9.78. Found: C, 42.28; H, 2.88; N, 9.89. [Ru(bpy)₃]Cl₂·6H₂O was prepared as follows: RuCl₃·nH₂O and 2,2'-bipyridine were refluxed in ethanol under Ar for 72 h. After filtration, the filtrate was evaporated to yield a brown solid. The solid was washed with benzene and then recrystallized in water to yield red crystals. Anal. Calcd for C₃₀H₃₆N₆O₆Cl₂Ru: C, 48.13; H, 4.85; N, 11.23. Found: C, 48.36; H, 4.52; N, 11.29.

2.2 Methods

The fluorescence quantum yield (Φ_f) was measured with an absolute photoluminescence quantum yield measurement system (Hamamatsu, C9920-02), which is shown schematically in Fig. 1. This system consists of a Xe arc lamp, a monochromator, an integrating sphere, a multichannel detector, and a personal computer. A 10 mm path length quartz cuvette for solution samples is set in the integrating sphere. A monochromatic light source was used as the excitation light source, which mounted a xenon lamp with a lamp rating of 150 W and an output stability of 1.0% (peak to peak). The excitation light was introduced into the integrating sphere by an optical fiber. The integrating sphere had an inner diameter of about 84 mm and contained a baffle between the sample and detection exit positions to prevent direct detection of the excitation light and/or emission from the sample. Spectralon (Labsphere) was mounted on the internal surface of the integrating sphere as a high reflectance material (99% reflectance for wavelengths from 350 nm to 1650 nm and over 96% reflectance for wavelengths from 250 nm to 350 nm). A photonic multichannel analyzer PMA-12 (Hamamatsu, C10027-01) was used as the multichannel detector. It employed a BT-CCD with 1024 × 122 pixels and a pixel size of 24 μm × 24 μm providing a wide spectrum range from 200 nm to 950 nm. By using a BT-CCD, the sensitivity of the detector for fluorescence detection was vastly superior to that of an optical detection system using a conventional CCD (*i.e.*, a front-illuminated CCD), especially at short wavelengths. The sensitivity of this system was fully calibrated for the spectral region 250–950 nm using deuterium and halogen standard light sources. These

standard light sources were calibrated in accordance with measurement standards traceable to primary standards (national standards) located at the National Metrology Institute of Japan. The primary measurement standards are based on the physical units of measurement according to the International System of Units (SI). The transfer accuracy in the sensitivity calibration was between ±2.4 and ±4.9%, depending on the wavelength.

The fluorescence quantum yield Φ_f is given by

$$\Phi_f = \frac{\text{PN(Em)}}{\text{PN(Abs)}} = \frac{\int \frac{\lambda}{hc} [I_{\text{em}}^{\text{sample}}(\lambda) - I_{\text{em}}^{\text{reference}}(\lambda)] d\lambda}{\int \frac{\lambda}{hc} [I_{\text{ex}}^{\text{reference}}(\lambda) - I_{\text{ex}}^{\text{sample}}(\lambda)] d\lambda} \quad (1)$$

where PN(Abs) is the number of photons absorbed by a sample and PN(Em) is the number of photons emitted from a sample, λ is the wavelength, h is Planck's constant, c is the velocity of light, $I_{\text{ex}}^{\text{sample}}$ and $I_{\text{ex}}^{\text{reference}}$ are the integrated intensities of the excitation light with and without a sample respectively, $I_{\text{em}}^{\text{sample}}$ and $I_{\text{em}}^{\text{reference}}$ are the photoluminescence intensities with and without a sample, respectively.

Fig. 2 shows the excitation light profile and the fluorescence spectra obtained by setting quartz cells with and without a sample solution, when a 1 N H₂SO₄ solution of QBS is set inside the integrating sphere. The irradiation of a quartz cell that does not contain the sample solution gives the excitation light spectrum with a peak wavelength at 350 nm, and the excitation of the sample solution exhibits the fluorescence spectrum of QBS in the wavelength range from 380 nm to 650 nm, which is accompanied by a reduction in the excitation light intensity. The spectra in Fig. 2 are fully corrected for the spectral sensitivity of the instrument. The number of photons absorbed by QBS is proportional to the difference of the integrated excitation light profiles, while the number of photons emitted from QBS is proportional to the area under its fluorescence spectrum. Thus, according to eqn (1), the fluorescence quantum yield can be calculated by taking the ratio of the difference of the integrated excitation light profiles to the integrated fluorescence spectrum.

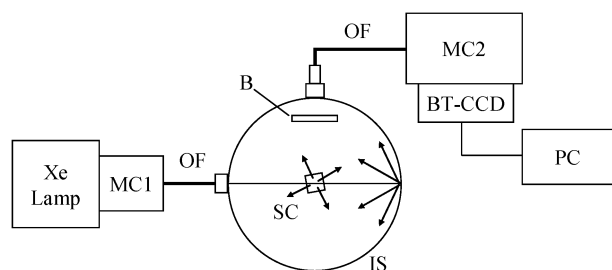


Fig. 1 Schematic diagram of an integrating sphere instrument for measuring absolute fluorescence quantum yields. MC1, MC2: monochromators, OF: optical fiber, SC: sample cell, B: baffle, BT-CCD: back-thinned CCD, PC: personal computer.

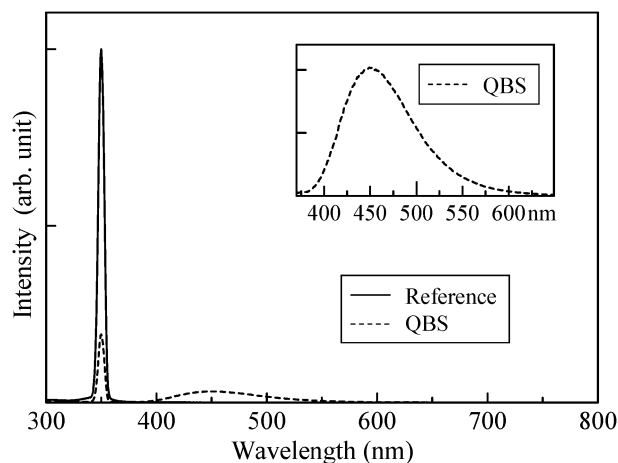


Fig. 2 Excitation light profiles and fluorescence spectrum obtained by 350 nm excitation of reference and QBS in 1 N H₂SO₄. The inset is an expanded fluorescence spectrum of QBS.

Photoacoustic (PA) measurements were made by using the third harmonic (355 nm) of a Nd³⁺:YAG laser (Spectra-Physics, GCR-130, pulse width ~6 ns) as the excitation source.⁴⁴ Argon-saturated sample solutions were irradiated by the laser beam after it had been collimated by a 0.5 mm wide slit, so that the effective acoustic transit time was about 340 ns. The laser fluence was varied using a neutral density filter, and the laser pulse energy was measured using a pyroelectric energy meter (Laser Precision, RJP-753 and RJ7620). The PA signal detected by a piezoelectric detector (Panametrics, V103, 1 MHz) was amplified by using a wide-band high-input impedance amplifier (Panametrics, 5676, 50 kHz, 40 dB) and fed to a digitizing oscilloscope (Tektronix, TDS-540). The temperature of the sample solution was held at ±0.02 K.

Nanosecond laser photolysis experiments were carried out using the third harmonic of a Nd³⁺:YAG laser. Details of the laser photolysis system are reported elsewhere.⁴⁵ The solutions used in the laser photolysis experiments were degassed by freeze–pump–thaw cycles (five times) on a high vacuum line.

In order to examine the temperature dependence of the quantum yields of [Ru(bpy)₃]²⁺, an acetonitrile (or an aqueous) solution of [Ru(bpy)₃]²⁺ in a pyrex glass tube (O.D. = 8 mm, I.D. = 6 mm) was degassed by freeze–pump–thaw cycles on a high-vacuum line, and the tube was then sealed. The sealed tube was soaked in water in a square quartz cuvette (1.0 × 1.0 × 10.0 cm); the temperature of the water was controlled using a thermostat (Eyela, NCB-1200). The phosphorescence quantum yield at each temperature was determined relative to the emission intensities by taking the absorbance changes into account. The relative quantum yields were converted to absolute values by using the quantum yields at 298 K, which were determined by using the integrating sphere instrument. The temperature of the sample solution was monitored with a digital thermometer (Custom, CT-1310) equipped with a thermocouple (LK-310).

3. Results and discussion

3.1 Spectral sensitivity of the instrument

In the absolute fluorescence quantum yield measurements using an integrating sphere, the obtained absorption and fluorescence spectra of the sample solutions need to be corrected for the spectral sensitivity of the entire system, including the integrating sphere, the grating monochromator, and the photon detector. Thus, the spectral sensitivity of our instrument was calibrated both for an integrating sphere and a multichannel spectrometer by using deuterium and halogen standard light sources. Using the calibrated multichannel spectrometer (without the integrating sphere), we first remeasured the absolute fluorescence spectra of some standard solutions: 2-aminopyridine (2-APY; 10⁻⁵ M in 0.1 N H₂SO₄), QBS (10⁻⁵ M in 0.1 N H₂SO₄), 3-aminophthalimide (3-API; 5 × 10⁻⁴ M in 0.1 N H₂SO₄), *N,N*-dimethylamino-*m*-nitrobenzene (*N,N*-DMANB; 10⁻⁴ M in benzene (3:7, v/v)), and 4-dimethylamino-4'-nitrostilbene (4,4'-DMANS; 10⁻³ M in *o*-dichlorobenzene).³ The normalized fluorescence spectra of these standard solutions are displayed in Fig. 3 together with data from the literature.^{3,46}

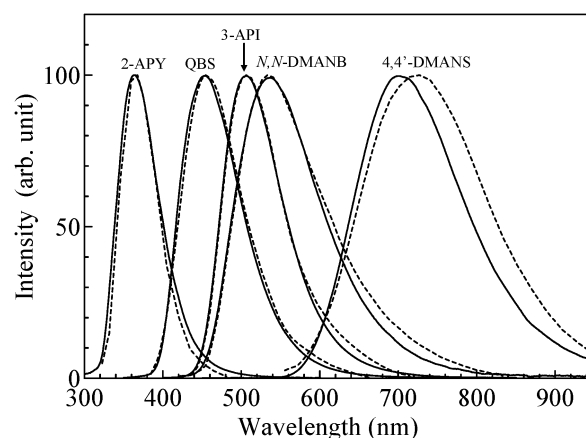


Fig. 3 Corrected fluorescence spectra for 2-aminopyridine (2-APY; 10⁻⁵ M in 0.1 N H₂SO₄), QBS (10⁻⁵ M in 0.1 N H₂SO₄), 3-aminophthalimide (3-API; 5 × 10⁻⁴ M in 0.1 N H₂SO₄), *N,N*-dimethylamino-*m*-nitrobenzene (*N,N*-DMANB; 10⁻⁴ M in benzene-hexane (3:7, v/v)), and 4-dimethylamino-4'-nitrostilbene (4,4'-DMANS; 10⁻³ M in *o*-dichlorobenzene). Solid lines (this study), broken lines (from ref. 3 and 46).

Good agreement was obtained for 2-APY, QBS, and 3-API, while a significant difference is found for the long wavelength region, *i.e.*, the near-infrared region of *N,N*-DMANB and 4,4'-DMANS. Because our instrument uses a BT-CCD as the photon detector, its sensitivity in the near-infrared region is significantly better than that of a conventional photomultiplier tube. A complete set of corrected spectra (in relative quantum per wavelength) is available as Table S1 in ESI†.

We then measured the fluorescence spectra of these standard solutions by using the entire system (including the integrating sphere). The corrected spectra agree very closely with those obtained by the multichannel spectrometer, indicating that the spectral sensitivity of the whole system including the reflectivity of the integrating sphere is properly corrected in the spectral region 250–950 nm.

3.2 Effects of reabsorption and reemission

The fluorescence spectrophotometer equipped with an integrating sphere is useful for compensating the effects of polarization and refractive index in the quantum yield measurements. However, random and multiple scattering of excitation light on the inner wall of the integrating sphere increases the effective optical path length. This increases the effect of reabsorption and reemission on quantum yield measurements, especially in compounds whose absorption and fluorescence bands substantially overlap.

In order to clarify the effects of reabsorption and reemission on the quantum yield obtained using our integrating sphere instrument, we examined the influence of the concentration of anthracene in ethanol on the fluorescence spectrum and quantum yield. The anthracene concentration was varied between 1 × 10⁻⁶ M and 1 × 10⁻³ M at room temperature. The absorption and fluorescence spectra of anthracene overlap significantly with each other in the 0–0 band region.

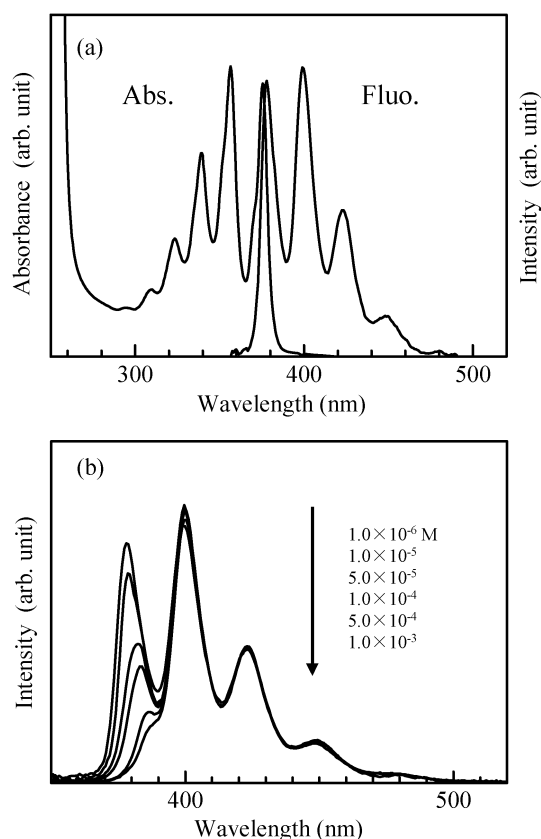


Fig. 4 (a) Absorption and fluorescence spectra of 1.0×10^{-6} M anthracene in ethanol, and (b) dependence of the fluorescence spectra on the anthracene concentration in ethanol.

Table 1 Observed and corrected fluorescence quantum yields of anthracene in ethanol and 9,10-diphenylanthracene in cyclohexane

Concentration (M)	Anthracene			9,10-diphenylanthracene		
	Φ_f^{obs}	a^a	Φ_f	Φ_f^{obs}	a^a	Φ_f
1×10^{-5}	0.278	0.066	0.290	0.972	0.099	0.975
5×10^{-5}	0.262	0.142	0.294	0.966	0.173	0.971
1×10^{-4}	0.252	0.179	0.291	0.963	0.215	0.971
5×10^{-4}	0.235	0.251	0.289	0.963	0.299	0.973
1×10^{-3}	0.220	0.271	0.280	0.962	0.327	0.974

^a Probability of reabsorption.

As shown in Fig. 4, the 0–0 vibrational band around 375 nm is almost absent in the fluorescence spectrum of 1×10^{-3} M solution when the integrating sphere is used. When the concentration is reduced, the intensity of the 0–0 band increases remarkably and reaches a maximum at a concentration of 1×10^{-6} M. The fluorescence spectrum of the 1×10^{-6} M solution obtained using the integrating sphere instrument was almost consistent with that obtained using a conventional fluorescence spectrophotometer. The observed Φ_f values (Φ_f^{obs}) varied from 0.278 for a 1×10^{-5} M solution to 0.220 for a 1×10^{-3} M solution (see Table 1).

We used the method recently reported by Ahn *et al.* to correct the effect of reabsorption and reemission.⁴⁷ They considered a fluorescent system with a quantum yield of Φ_f .

If the probability of an emitted photon being reabsorbed by sample molecules is expressed by a , the photon escape probability is given by $1 - a$. The observed fluorescence quantum yield Φ_f^{obs} is then given by the geometric series

$$\begin{aligned} \Phi_f^{\text{obs}} &= \Phi_f(1 - a)(1 + a\Phi_f + a^2\Phi_f^2 + \dots) \\ &= \frac{\Phi_f(1 - a)}{1 - a\Phi_f} \end{aligned} \quad (2)$$

where the successive terms represent photon escape after successive absorption–reemission cycles. The self-absorption parameter a depends on the overlap between the absorption and fluorescence spectra, and can be estimated by comparing the observed fluorescence spectrum with that of a sufficiently diluted solution (the true fluorescence spectrum).⁴⁷ An equation for calculating the fluorescence quantum yield can be derived from eqn (2) and is given by

$$\Phi_f = \frac{\Phi_f^{\text{obs}}}{1 - a + a\Phi_f^{\text{obs}}} \quad (3)$$

Table 1 gives the fluorescence quantum yields of anthracene solutions corrected for reabsorption/reemission effects using eqn (3) along with the values of the self-absorption parameter a and the uncorrected quantum yield Φ_f^{obs} . The corrected Φ_f gives almost constant values in the concentration range 1×10^{-5} M to 1×10^{-3} M. This correction method is thus useful for determining the Φ_f value of high-concentration sample solutions.

3.3 Fluorescence quantum yields of standard solutions

We measured the quantum yields of representative fluorescence standard compounds dissolved in organic solvents or H_2O . The Φ_f values obtained using our instrument are shown in Table 2 along with the accepted values from the literature.

The compounds in Table 2 are commonly used as fluorescence standards in quantum-yield measurements based on a relative (secondary) method with optically dilute or dense solutions.^{4,5} Because the magnitude of the fluorescence quantum yield depends on the physical conditions, such as the solvent, the sample concentration, and the excitation wavelength, these parameters are also specified in Table 2. Inspection of the Φ_f values in Table 2 reveals that there is excellent agreement between our Φ_f values and the values given in the literature and that they lie within experimental errors, with the exception of 9,10-diphenylanthracene in cyclohexane and 1.0×10^{-5} M QBS in 1 N H_2SO_4 aqueous solution.

3.4 Fluorescence quantum yield of quinine bisulfate

The fluorescence quantum yield of QBS in sulfuric acid has been widely used as a secondary standard in relative quantum yield measurements.^{4,5} The optical properties of QBS in 0.1 or 1.0 N sulfuric acid make it an ideal quantum-yield standard. Specifically, there is no significant overlap between its

Table 2 Comparison of Φ_f values of some fluorescence standard solutions obtained in this study with values from the literature

Compound	Solvent	Conc. (M)	λ_{exc} (nm) ^a	Φ_f (this work)	Φ_f (literature)
naphthalene	cyclohexane	7.0×10^{-5}	270	0.23 ± 0.01	0.23 ± 0.02^{5b}
anthracene	ethanol	4.5×10^{-5}	340	0.28 ± 0.02	0.27 ± 0.03^5
9,10-diphenylanthracene	cyclohexane	2.4×10^{-5}	355	0.97 ± 0.03	0.9 ± 0.02^{20}
1-aminonaphthalene	cyclohexane	5.7×10^{-5}	300	0.48 ± 0.02	0.465^{48}
<i>N,N</i> -dimethyl-1-aminonaphthalene	cyclohexane	1.0×10^{-4}	300	0.011 ± 0.002	0.011^{48}
quinine bisulfate	1N H ₂ SO ₄	5.0×10^{-3}	350	0.52 ± 0.02	0.508^{15}
	1N H ₂ SO ₄	1.0×10^{-5}	350	0.60 ± 0.02	0.546^{15}
fluorescein	0.1N NaOH	1.0×10^{-6}	460	0.88 ± 0.03	0.87^{8c}
tryptophan	H ₂ O (pH 6.1)	1.0×10^{-4}	270	0.15 ± 0.01	0.14 ± 0.02^5

^a Excitation wavelength. ^b Reference number. ^c Average of values obtained by excitation at 313.1, 365.5 and 435.8 nm.

absorption and fluorescence spectra, it is not appreciably quenched by oxygen, its fluorescence quantum yield is almost constant with excitation at wavelengths from 240 nm to 400 nm.⁴⁹ The most commonly used Φ_f values are given by Melhuish¹⁵: 0.546 for QBS at infinite dilution in 1 N H₂SO₄ at 298 K. It should be noted that Melhuish originally proposed $\Phi_f = 0.508$ for 5×10^{-3} M QBS in 1 N H₂SO₄ at 298 K as a secondary standard because the absolute fluorescence quantum yield measurements were carried out for a 5×10^{-3} M QBS solution on the basis of the modified Vavilov method. The Φ_f value (0.546) at infinite dilution was estimated by using the self-quenching rate constant.¹⁵

Using our integrating sphere instrument we obtained Φ_f values of 0.52 ± 0.02 and 0.60 ± 0.02 for 5×10^{-3} M and 1×10^{-5} M QBS in 1 N H₂SO₄ at 296 K, respectively. Our value for the 5×10^{-3} M solution is in good agreement with the value (0.508) reported by Melhuish (see Table 2). However, the Φ_f value (0.60) for the 1×10^{-5} M QBS solution is significantly larger than that (0.546) reported by Melhuish for a solution at infinite dilution.

Since the reliability of the Φ_f value (0.546) determined by Melhuish for the QBS solution under infinite dilution depends on the accuracy of their self-quenching constant, we remeasured the value of this constant using the Stern–Volmer equations

$$\frac{1}{\tau_f} = \frac{1}{\tau_f^0} + \frac{K_s}{\tau_f^0} [\text{QBS}] \quad (4)$$

$$\frac{1}{\Phi_f} = \frac{1}{\Phi_f^0} + \frac{K_s}{\Phi_f^0} [\text{QBS}] \quad (5)$$

where τ_f^0 and τ_f are respectively the fluorescence lifetimes of QBS for infinite dilution and for the concentration [QBS], Φ_f^0 and Φ_f are respectively the fluorescence quantum yields of QBS for infinite dilution and for the concentration [QBS], and K_s is the bimolecular self-quenching constant. The fluorescence quantum yield and lifetime of QBS were measured in the concentration range between 1×10^{-5} M and 7×10^{-3} M in 1 N H₂SO₄. The observed fluorescence decay profiles $I_f(t)$ were analyzed in terms of two exponential decay terms (eqn (6)): a “fast” component (about 2%) with a lifetime τ_{f1} of about 2 ns and a “slow” component (about 98%) with a lifetime τ_{f2} of about 19 ns.

$$I_f(t) = A_1 e^{-t/\tau_{f1}} + A_2 e^{-t/\tau_{f2}} \quad (6)$$

The observation of non-exponential fluorescence decay for QBS in H₂SO₄ solutions is consistent with the results of Phillips *et al.*^{20,50} Although the long decay time represents the major portion of the emission, we used the intensity-averaged decay time $\langle\tau_f\rangle$ expressed in eqn (7) for the Stern–Volmer analyses.²

$$\langle\tau_f\rangle = \frac{A_1\tau_{f1}^2 + A_2\tau_{f2}^2}{A_1\tau_{f1} + A_2\tau_{f2}} \quad (7)$$

The concentration dependences of Φ_f and τ_f for QBS in 1 N H₂SO₄ are given in Table S2 in ESI†.

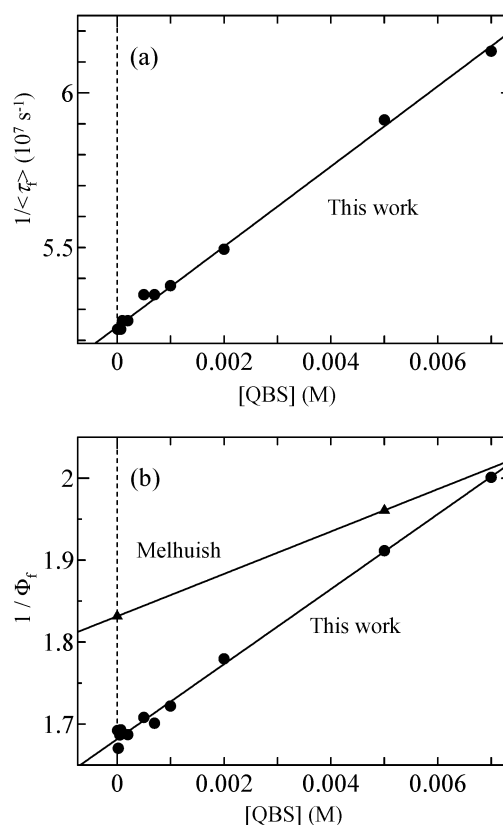


Fig. 5 Concentration dependences of (a) the mean fluorescence lifetime ($\langle\tau_f\rangle^{-1}$) and quantum yield (Φ_f) of quinine bisulfate (QBS) in 1N H₂SO₄. The data reported by Melhuish (ref. 15) are denoted by closed triangles.

Table 3 Comparison with the reported Φ_f values of quinine bisulfate in H_2SO_4

Solvent	Conc. (M)	λ_{exc} (nm) ^a	Temp. (K)	Φ_f	Method	Reference
1N H_2SO_4	5.0×10^{-3}	350	296	0.52 ± 0.02	integrating sphere	This work
1N H_2SO_4	1.0×10^{-5}	350	296	0.60 ± 0.02	integrating sphere	This work
1N H_2SO_4	5.0×10^{-3}	366	298	0.508	Vavilov method	Melhuish ^{15b}
1N H_2SO_4	infinite dilution	366	298	0.546	Vavilov method	Melhuish ¹⁵
0.1N H_2SO_4	infinite dilution	350	295	0.577	Weber-Teale method (ludox colloidal silica)	Eastman ¹⁷
1N H_2SO_4	infinite dilution	365	296	0.54 ± 0.02	Weber-Teale method (ludox colloidal silica)	Dawson and Windsor ⁸
3.6N H_2SO_4	infinite dilution	365	296	0.60	Weber-Teale method (ludox colloidal silica)	Dawson and Windsor ⁸
1N H_2SO_4	5.0×10^{-3}	366	298	0.561	calorimetric method	Gelernt <i>et al.</i> ¹⁰
0.1N H_2SO_4	10^{-3} – 10^{-2}	366		0.53 ± 0.02	photoacoustic method	Adam <i>et al.</i> ¹⁴
0.2N H_2SO_4	1.0×10^{-6}	350		0.65	integrating sphere	Gaigalas and Wang ⁴²

^a Excitation wavelength, ^b Reference number.

In Fig. 5, $\langle\tau_f\rangle^{-1}$ and $(\Phi_f)^{-1}$ are plotted as a function of QBS concentration. Linear relationships are observed for both $\langle\tau_f\rangle^{-1}$ and $(\Phi_f)^{-1}$, and their self-quenching constants (K_s) were calculated from the slopes to be 28.5 M^{-1} and 24.8 M^{-1} , respectively. Melhuish has determined the magnitude of the self-quenching constant (K_s) of QBS to be 15.0 M^{-1} based on quantum yield measurements.¹⁵ The results of Melhuish are compared with our data in Fig. 5. It clearly shows that the K_s value obtained by Melhuish is significantly smaller than our values. The disagreement in the Φ_f values of QBS for infinite dilution can thus be ascribed to the difference in the self-quenching rate constant.

Some published values for the quantum yield of QBS in H_2SO_4 measured by different methods are presented in Table 3 together with the experimental conditions. Dawson and Windsor⁸ and Eastman¹⁷ have measured the quantum yield of QBS at infinite dilution based on the Weber–Teale method. They obtained somewhat higher Φ_f values (0.54–0.60) than that (0.546) reported by Melhuish, although their values seem to depend on the concentration of H_2SO_4 . Gelernt *et al.*¹⁰ have also obtained a higher value (0.561) even for a $5 \times 10^{-3} \text{ M}$ solution of QBS in 0.1 N H_2SO_4 by using a calorimetric method. Very recently, Gaigalas and Wang⁴² have measured the Φ_f value of QBS in 0.2 N H_2SO_4 by using an integrating sphere instrument and reported a value of 0.65, which is much higher than previously published values.

3.5 Fluorescence quantum yield of 9,10-diphenylanthracene

9,10-Diphenylanthracene has also been employed as a popular fluorescence standard because of its high quantum yield. Table 4 presents representative values of published quantum yields for DPA in cyclohexane and benzene. Several researchers

have reported a fluorescence quantum yield of unity or greater for DPA in cyclohexane, while Meech and Phillips²⁰ and Hamai and Hirayama²¹ have reported very similar values, 0.91 and 0.90, on the basis of different methods.

With our integrating sphere instrument, we obtained a value of 0.97 for DPA in cyclohexane. The 0–0 absorption and fluorescence bands of DPA overlap substantially, in a similar manner as in anthracene solutions, and the shape of the fluorescence spectrum varies remarkably when the concentration is increased (see Fig. S1 in ESI†). Therefore, we first examined the effect of reabsorption/reemission on the measured quantum yield. The results are summarized in Table 1 together with those of anthracene. The probability of reabsorption is found to become much greater in higher concentration solutions, and at each concentration the reabsorption probability of DPA in cyclohexane is greater than that of anthracene in ethanol. Despite the higher reabsorption probability of DPA, the effect of concentration on the observed quantum yield is extremely small. This clearly demonstrates that the absolute quantum yield of DPA is very close to unity, because if the quantum yield of a solution is unity, the observed quantum yield coincides with the absolute quantum yield, *i.e.*, the reabsorption/reemission effect can be neglected (see eqn (2)). According to eqn (2), if the absolute quantum yield of DPA ($1.0 \times 10^{-3} \text{ M}$) is 0.90, the observed quantum yield should be 0.86, while if the actual quantum yield is 0.97, the measured quantum yield should be 0.96, which is consistent with the results in Table 1.

We also attempted to measure the fluorescence quantum yield of DPA using two complementary methods: transient absorption and time-resolved PA measurements. To determine the fluorescence quantum yield by the PA method, we require data on the quantum yield of intersystem crossing (Φ_{isc}).

Table 4 Published values of some fluorescence quantum yields of 9,10-diphenylanthracene in cyclohexane or benzene

Solvent	Conc. (M)	λ_{exc} (nm) ^a	Temp. (K)	Φ_f	Method	Reference
cyclohexane	2.4×10^{-5}	355	296	0.97 ± 0.03	absolute (integrating sphere)	This work
cyclohexane	infinite dilution		298	1.06 ± 0.05	relative (integrating sphere)	Ware and Rothman ^{34e}
cyclohexane	4.0×10^{-6}	342.5		$0.86 (0.95)^c$	relative	Morris <i>et al.</i> ¹⁹
cyclohexane		366	298	0.95	calorimetric	Mardelli and Olmsted III ¹¹
cyclohexane		^b		0.91 ± 0.02	relative (integrating sphere)	Meech and Phillips ²⁰
cyclohexane	1.6×10^{-5} – 4.7×10^{-5}	325	298	0.90 ± 0.02	actinometric	Hamai and Hirayama ²¹
benzene		308	RT	$0.88 \pm 0.03 (0.97)^d$	thermal lensing	Suzuki <i>et al.</i> ²²

^a Excitation wavelength. ^b The quantum yield is reported to be independent of the excitation wavelength over the first absorption band. ^c After corrections for refractive index. ^d Calculated using the average energy dissipated by fluorescence from the S_1 state (see text). ^e Reference number.

Hence, we first measured the transient absorption spectra of DPA, and determined the Φ_{isc} value to be 0.02 in cyclohexane at room temperature (see Fig. S2 in ESI†). This value is in good agreement with the published values of 0.02 in cyclohexane²² and 0.04 in benzene.⁵¹ We then performed PA measurements for DPA in cyclohexane by using 2-hydroxybenzophenone as a photocalorimetric reference.

The PA signal amplitude H produced after the absorption of a light pulse essentially results from two processes that occur during the heat integration time,¹³ thermally induced volume change in the solution ΔV_{th} and structural volume change ΔV_{r} , so that H can be written as

$$H = k(\Delta V_{\text{th}} + \Delta V_{\text{r}}) \quad (8)$$

where k is an instrumental constant that depends on the geometrical arrangement and on some solution constants such as density ρ and sound velocity v_{a} . ΔV_{th} is the contraction or expansion of the solvent due to the heat released by non-radiative processes and it is given by

$$\Delta V_{\text{th}} = k' \alpha \left(\frac{\beta}{c_{\text{p}} \rho} \right) E_{\text{a}} \quad (9)$$

where α is the fraction of the absorbed energy released as thermal energy within the response time of the detector, β is the thermal expansion coefficient, c_{p} is the heat capacity of the solution, and E_{a} is the absorbed energy. In the following analyses, the contribution of the structural volume change ΔV_{r} was neglected, because in the present system photo-excitation produces no bond dissociation and/or formation and the solvation change due to triplet formation is expected to be negligibly small in cyclohexane.

The PA signals of DPA and the photocalorimetric reference 2-hydroxybenzophenone in cyclohexane at 293 K are displayed in the inset of Fig. 6. The difference between the first maximum and minimum in the PA signal was taken as the signal amplitude H . The signal amplitude H^{S} of DPA is related to the incident laser energy E_0^{S} by

$$H^{\text{S}} = K \alpha E_0^{\text{S}} (1 - 10^{-A_{\text{S}}}) \quad (10)$$

where K is a constant that depends on the geometry of the experimental set-up and the thermoelastic quantities of the medium and A_{S} is the absorbance of the sample solution at the excitation wavelength. The signal amplitude H^{R} of the photocalorimetric reference conforms to a similar equation, namely

$$H^{\text{R}} = K E_0^{\text{R}} (1 - 10^{-A_{\text{R}}}) \quad (11)$$

where the thermal conversion efficiency α of the photocalorimetric reference 2-hydroxybenzophenone is assumed to be unity. From eqn (10) and (11), the value of α of the sample solution can be obtained as follows:

$$\alpha = \frac{H^{\text{S}} E_0^{\text{R}} (1 - 10^{-A_{\text{R}}})}{H^{\text{R}} E_0^{\text{S}} (1 - 10^{-A_{\text{S}}})} \quad (12)$$

The relationship between the PA signal amplitude and the laser energy was linear for 2-hydroxybenzophenone within the energy range studied, whereas the signal amplitude of DPA showed a nonlinear laser energy dependence (Fig. 6) because two-photon absorption processes occur.²² Thus, the laser

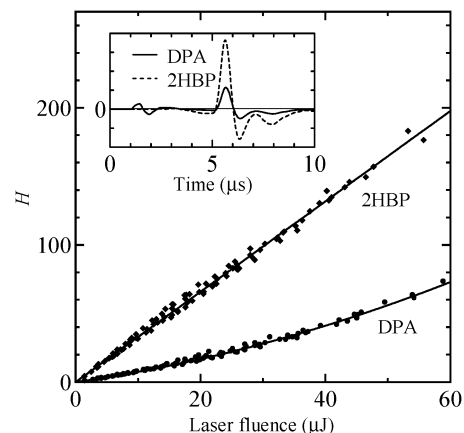


Fig. 6 Laser energy dependence of the photoacoustic signal amplitude of 9,10-diphenylanthracene (DPA) and 2-hydroxybenzophenone (2HBP) in cyclohexane at 293 K. The photoacoustic signals are displayed in the inset.

energy dependence of the sample signal was fitted using the following equation:

$$H^{\text{S}} = c_1 E_0^{\text{S}} + c_2 (E_0^{\text{S}})^2 \quad (13)$$

In the calculation of α , we used the coefficient c_1 in the linear term of eqn (11) instead of $(H^{\text{S}}/E_0^{\text{S}})$ in eqn (10), and calculated the value of α to be 0.198.

With the exception of the decay of the excited triplet state, all other decay processes occur within the heat integration time (about 340 ns), so that the fluorescence quantum yield can be obtained from the following relation:

$$E_{\lambda} = \Phi_{\text{f}} \langle E_{\text{S}} \rangle + \Phi_{\text{isc}} E_{\text{T}} + \alpha E_{\lambda} \quad (14)$$

where E_{λ} is the excitation photon energy ($= 337 \text{ kJ mol}^{-1}$ at 355 nm), E_{T} is the triplet energy (171 kJ mol^{-1}),⁵² and $\langle E_{\text{S}} \rangle$ is the average energy dissipated by fluorescence from the S_1 state, which is given by

$$\langle E_{\text{S}} \rangle = \frac{\int \bar{\nu} I_{\text{f}}(\bar{\nu}) d\bar{\nu}}{\int I_{\text{f}}(\bar{\nu}) d\bar{\nu}} \quad (15)$$

where $I_{\text{f}}(\bar{\nu})$ is the spectral distribution of fluorescence as a function of wavenumber ($\bar{\nu}$). The magnitude of $\langle E_{\text{S}} \rangle$ was calculated to be 275 kJ mol^{-1} . By substituting these quantities into eqn (14), the fluorescence quantum yield of DPA was determined to be 0.97 ± 0.03 . This agrees very well with the value obtained using our integrating sphere instrument.

Recently, Suzuki *et al.*²² have determined the fluorescence quantum yield of DPA in benzene to be 0.88 ± 0.03 by using a time-resolved thermal lensing (TRTL) technique. To calculate the Φ_{f} value, they used the S_1 energy (304 kJ mol^{-1}) of DPA instead of the average energy (275 kJ mol^{-1}) dissipated by fluorescence from the S_1 state given by eqn (15). If one uses the latter value for calculating Φ_{f} based on the TRTL method (eqn (4) in ref. 22), the fluorescence quantum yield of DPA is found to be 0.97. This is in agreement with the Φ_{f} value derived from our measurements based on the integrating sphere.

3.6 Phosphorescence quantum yield of ruthenium(II) tris(bipyridine) complex

Ruthenium(II) tris(bipyridine) complexes exhibit phosphorescence at 550–900 nm when excited at the MLCT band around 450 nm. As the emission spectra of $[\text{Ru}(\text{bpy})_3]^{2+}$ are broad and extend into the near-infrared region, proper corrections for spectral sensitivity are essential when measuring the quantum yield. In particular, as the sensitivity of photomultiplier tubes decreases sharply in the near-infrared region, large correction factors are usually required to obtain true emission spectra. This may be responsible for non-negligible errors in the quantum yield measurements.

We measured the phosphorescence spectra of $[\text{Ru}(\text{bpy})_3]\text{X}_2$ ($\text{X} = \text{PF}_6, \text{Cl}$) in typical solvents of argon-saturated water and acetonitrile and reevaluated the quantum yields (Φ_p) using our integrating sphere instrument equipped with a BT-CCD detector. By using the BT-CCD as a photodetector, the correction factors for the near-infrared region were much smaller than those for spectrofluorometers with conventional detectors. The phosphorescence spectrum of $[\text{Ru}(\text{bpy})_3](\text{PF}_6)_2$ in water has a maximum wavelength at 626 nm which is slightly longer than that (621 nm) in acetonitrile (Fig. 7a). The quantum yields at 298 K were 0.063 ± 0.003 in H_2O and 0.095 ± 0.003 in acetonitrile. The maximum wavelengths for $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ were 625 nm in H_2O and 621 nm in CH_3CN (Fig. 7b), and the quantum yields were 0.063 ± 0.002 in H_2O and 0.094 ± 0.004 in CH_3CN . Thus, there is no difference between the photophysical properties of hexafluorophosphate and chloride salts, showing negligible counter anion effects. The phosphorescence maximum wavelengths and the quantum yields for $[\text{Ru}(\text{bpy})_3]^{2+}$ at 298 K in argon- and air-saturated H_2O and acetonitrile are listed in Table 5, together with values reported in the literature.

The representative Φ_p value for $[\text{Ru}(\text{bpy})_3]^{2+}$ in H_2O is 0.042 reported by Van Houten and Watts.^{25,26} They determined the phosphorescence quantum yield relative to the value (0.90) of 0.1 M NaOH solution of fluorescein as a standard. Nakamaru also reported an identical value by using the same standard.^{27,28}

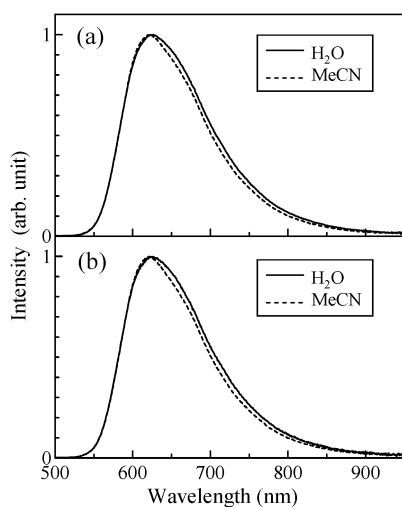


Fig. 7 Emission spectra of (a) $[\text{Ru}(\text{bpy})_3](\text{PF}_6)_2$ and (b) $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$ in H_2O and CH_3CN under Ar saturated conditions at 298 K.

Table 5 Emission spectral maxima and quantum yields (Φ_p) for $[\text{Ru}(\text{bpy})_3]\text{X}_2$ in water and acetonitrile at 298 K with their literature values

X	Solvent	λ_{em} (nm) ^a	Φ_p	Reference
Cl	H_2O	n.d.	0.042 ± 0.002	Watts ^{25,26c}
Cl		608	0.055^b	Harriman ²⁹
Cl		628	0.042 ± 0.002	Nakamaru ^{27,28}
Cl			0.028 ± 0.002 (air)	Nakamaru ²⁸
n.d.		n.d.	0.053	Tazuke ³⁰
Cl		625	0.063 ± 0.002	This work
Cl			0.040 ± 0.002 (air)	This work
PF_6		626	0.063 ± 0.003	This work
Cl	CH_3CN	611	0.059 ± 0.002	Nakamaru ²⁷
PF_6		620	0.062 ± 0.006	Meyer ³¹
n.d.		620	0.086	Tazuke ³⁰
n.d.		n.d.	0.090	Nozaki ³²
Cl		621	0.094 ± 0.004	This work
PF_6		621	0.095 ± 0.003	This work
PF_6			0.018 ± 0.002 (air)	This work

n.d.: not described. ^a Emission maximum wavelength. ^b The measurement temperature is not described. ^c Reference number.

After the study by Van Houten and Watts, their value (0.042) has been most frequently cited for the Φ_p value of $[\text{Ru}(\text{bpy})_3]^{2+}$ in H_2O . In particular, the quantum yields of $[\text{Ru}(\text{bpy})_3]^{2+}$ in organic solvents have been determined by using their value as a standard.³¹

However, some different values have also been reported. For instance, Harriman reported a slightly higher quantum yield of 0.055 without providing a detailed description of the methodology used to obtain this value.²⁹ Tazuke *et al.* have also reported higher values (0.053 in water and 0.086 in acetonitrile) using a spectrometer equipped with a photomultiplier tube, where the spectral response was calibrated by Lippert's method.³⁰ In this present study, the quantum yields were obtained to be 0.063 in H_2O and 0.095 in CH_3CN , which are higher than previously reported values. Nozaki *et al.* reported the Φ_p value of $[\text{Ru}(\text{bpy})_3]^{2+}$ in CH_3CN as 0.090 by using 9,10-diphenylanthracene ($\Phi_f = 0.91$ in deaerated cyclohexane) as a standard.³² If our value (0.97) is used to calculate the fluorescence quantum yield of 9,10-diphenylanthracene, Nozaki's value is recalculated to be 0.096, which agrees well with our value within the experimental error.

Emission from $[\text{Ru}(\text{bpy})_3]^{2+}$ is significantly quenched by molecular oxygen because it is phosphorescence from the triplet excited state. The intrinsic quantum yield should thus be measured under deaerated or oxygen-free conditions. On the other hand, the emission quantum yield of $[\text{Ru}(\text{bpy})_3]^{2+}$ under aerated conditions is important as a standard for luminescent probes in biological systems.^{53,54} Until now, a value of 0.028 for Φ_p has been used for $[\text{Ru}(\text{bpy})_3]^{2+}$ in aerated water.²⁸ In this present study, we reevaluated these values and found them to be 0.040 in aerated water and 0.018 in aerated CH_3CN .

The emission from ruthenium tris(bipyridine) complex is strongly temperature-dependent⁵⁵ because of a non-emissive $^3\text{d-d}$ excited state lying slightly above the $^3\text{MLCT}$ state. With increasing temperature, the non-radiative processes through the $^3\text{d-d}$ state become predominant due to thermal population of the $^3\text{d-d}$ state. The energy gap between the $^3\text{MLCT}$ and $^3\text{d-d}$ states for $[\text{Ru}(\text{bpy})_3]^{2+}$ depends on the solvent, and is

reported to be 37.6–45.7 kJ mol⁻¹ (3140–3820 cm⁻¹).³¹ Hence, the emission intensity and lifetime of [Ru(bpy)₃]²⁺ are influenced by ambient temperature. In this present study, the temperature dependence of the phosphorescence quantum yields of [Ru(bpy)₃]²⁺ has been measured from 283 to 333 K in H₂O and CH₃CN (see Fig. S3 in ESI†). The temperature dependence was observed in both CH₃CN and H₂O, and it was more prominent in CH₃CN: the quantum yields decreased from 0.150 (283 K) to 0.020 (333 K) in CH₃CN, while they decreased from 0.074 (283 K) to 0.029 (333 K) in H₂O. This large temperature dependence indicates that temperature control as well as measurements under deaerated conditions will be important when [Ru(bpy)₃]²⁺ is used as a standard for photoluminescent probes.

4. Conclusions

An instrument for measuring the absolute luminescence quantum yield of solutions has been developed by using an integrating sphere as a sample chamber. By utilizing a BT-CCD for the photodetector, a spectrophotometer with high sensitivity from the ultraviolet to near-infrared region was developed, and the whole system was fully calibrated for spectral sensitivity. By using this system, the fluorescence quantum yields of some standard solutions were reevaluated. For the quantum yield of 1.0 × 10⁻⁵ M quinine bisulfate in 1 N H₂SO₄, a revised value of 0.60 was suggested, instead of 0.546 reported in earlier papers by Melhuish. The fluorescence quantum yield of 9,10-diphenylanthracene was determined to be 0.97, which was supported by complementary experiments based on the photoacoustic method. A quantum yield close to 1.0 for 9,10-diphenylanthracene was consistent with the negligible reabsorption/reemission effects observed in the quantum yield measurements. The phosphorescence quantum yields (Φ_p) of ruthenium tris(bipyridine) complexes [Ru(bpy)₃]X₂ (X = PF₆, Cl) have also been measured by using our integrating sphere instrument. The quantum yields for [Ru(bpy)₃]Cl₂ in deaerated water and acetonitrile at 298 K were measured to be 0.063 and 0.095 respectively, which are significantly higher than the previously accepted values (0.042 in water and 0.062 in acetonitrile). The Φ_p value in aerated water was determined to be 0.040, which is also much higher than the previously reported value (0.028). The significant temperature dependence of the phosphorescence from [Ru(bpy)₃]²⁺* suggests that careful temperature control is required when using the complex as a standard.

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