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Corrected Emission Spectra and Quantum Yields for a Series of Fluorescent Compounds in the Visible Spectral Region

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INTRODUCTION

The increased use of steady-state and dynamic fluorescence measurements to address chemical problems is directly attributable to its sensitivity and selectivity as well as innovations and developments in measurement systems. Additionally, during the last two decades fluorescence applications to biochemical measurement problems have increased tremendously, especially through the development of new fluorescent ligands and their inclusion in antibody techniques.

In these developments and applications, however, accurate measurements and comparability of results among various laboratories are critical for continued progress and are dependent on the availability of fluorescence standards for both steady-state and dynamic measurements. It is important that laboratories, especially National Standards Laboratories, develop fluorescence standards that research laboratories can use so that the research laboratories can focus their efforts on measurements, applications and chemical breakthroughs, rather than developing standards to ensure that their measurements are accurate and transferable. The issuance [1] of the first fluorescence Standard Reference Material, Quinine Sulfate Dihydrate (SRM 936) in the late 1970's [2] was a step in this direction and has been followed by reports at workshops that included the continuing need for and research on additional fluorescence standards [3].

Magde *et al*. [4] have recently provided absolute quantum yields by a thermal blooming technique for rhodamine 6g and fluorescein in various solvents. They also used these data with earlier reported lifetimes [5] to calculate radiative and non-radiative decay rates. These data aid in providing cross-checks for the measured parameters of a series of fluorescence standards covering the ultraviolet to red spectral regions that are robust and self-consistent. However, the majority of chemical and biochemical luminescence measurements continues to be made on commercially-available or laboratory-built spectrofluorimeters that produce steady-state excitation and emission spectra. It is thus important that a series of well-characterized, fluorescence standards with carefully measured corrected excitation and emission spectra as well as quantum yields be available for these studies to help provide quality assurance and comparability of the measurements among laboratories. These standards, and the continued measurement comparisons, establish the robustness of the measurements and standards, and their usefulness for general research laboratory applications.

We provide in this paper information on absorbance values, corrected excitation and emission spectra, and the relative quantum yields for a series of fluorescent compounds that cover the wavelength range 395 nm (quinine sulfate) to 675 nm (sulforhodamine 101) at the $1/10^{th}$ spectral power points of the emission spectra. These data were produced on a commercially available spectrofluorimeter using research grade solvents and chemicals. A solution of Standard Reference Material (SRM 936) Quinine Sulfate Dihydrate was used as the basic, comparative standard. The other fluorophors, including SRM 1932 Fluorescein in Borate Buffer currently being developed at the National Institute of Standards and Technology (NIST) [6], were measured relative to the quinine sulfate solution. The data provide supporting evidence to the measurement communities for the self-consistency, robustness and usefulness of these fluorescent molecules as a series of standards, and extends the wavelength coverage further into the red spectral region.

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EXPERIMENTAL

Solvents and Reagents

Solvents and reagents were reagent grade except where noted. In general, the fluorophores were specially purified materials.

Quinine Sulfate: Two samples of quinine sulfate were used: NIST SRM 936, Quinine Sulfate Dihydrate and Fluka AG 22640 Fluorescence Indicator, >99%. The NIST SRM was dissolved in 0.1 M $HClO_4$ and used as the relative fluorescence standard to determine quantum yields of the other fluorophores [1]. The quantum yield of the Fluka quinine sulfate was measured in 0.05 M H_2SO_4against the NIST SRM 936 to check the sulfate ion quenching effect on the quantum yield and to validate the quinine sulfate used generally as a standard at the Pharmacy Institute, University of Oslo, Norway.

Fluorescein: NIST-ampouled solutions of the proposed fluorescein SRM 1932 and solid fluorescein (Molecular Probes highly purified fluorescein, MPR 71358) [7] were used. The solid fluorescein was dissolved in a borate buffer solution (pH = 9.1) prepared according to Gaigalas [8].

Rhodamine B: Aldrich laser dye grade #18328DR was used to produce rhodamine B solutions in methanol, a solvent suggested to reduce potential polarization problems [9].

Sulforhodamine 101: Solid and solution samples of sulforhodamine 101 were supplied by Molecular Probes S-359 Lot 0181-2, Eugene, Oregon, USA and prepared in ethanol.

Equipment/Procedures

Absorbance Instrumentation/Measurements: Absorbances of solutions were measured on a Shimadzu UV-2101PC UV-VIS Scanning Spectrophotometer [10] with automatic wavelength calibration and baseline correction. Solvent was placed in two, matched quartz spectrophotometer cells in the sample and blank cell positions. The baseline was "zeroed" and measured at 1-nm intervals. At no wavelength between 220 and 700 nm did the measured baseline deviate from 0.000 by more than ±0.001 absorbance units; in fact at greater than 99% of the wavelengths, the deviation was no more than ±0.000 absorbance units ($+0.000_4 \geq m \geq -0.000_4$). The solvent in the sample beam was then replaced with the diluted solution of fluorophore and the absorbance spectrum was measured at 1 nm intervals using a slow or medium scan and a 1 nm bandpass.

Absorbance/Fluorescence Solutions: The stock QS SRM standard and other solutions were prepared in volumetric flasks followed by the addition of the appropriate solvent to the mark. Dilutions were made either gravimetrically or volumetrically to provide solutions with absorbances of approximately 0.4 at the peaks that were to be used for excitation in the fluorescence studies.

Fluorescence Solutions: After the absorbance measurements were made on the diluted stock solutions, weighed amounts or analytical volumes of the solutions were transferred quantitatively to volumetric flasks. Dilutions were made gravimetrically or volumetrically to make working fluorescence solutions with absorbances generally ∼0.01 such that fluorescence measurements with minimal errors (<1.0%) due to the "inner-filter effect" [11] were obtained.

Fluorescence Instrumentation/Measurements: Fluorescence spectra were measured on a PTI modular Fluorescence System using Felix™ for Windows software. The general characteristics and main components of the modules of the instrument used to make steady-state measurements are as follows. The excitation source is a 75 watt, sealed xenon excitation lamp with an elliptical reflector (f/4.5) to collect and focus radiation on the excitation monochromator entrance slit. Model 101 monochromators (f/4 0.2-m Czerny-Turner configuration) with continuously adjustable entrance and exit slits are used to select excitation and emission bands with the gratings blazed at 300 nm and 400 nm for excitation and emission, respectively. A portion of the excitation radiation is sampled by a beam splitter and measured with an energy sensitive reference detector to monitor the lamp intensity when measuring spectra and provide a correction when automatically determining the corrected excitation spectrum or in quantum yield determinations. The sample compartment has an automated, 4-cuvette sample holder with temperature control (all measurements were made at 25.0 ± 0.1°C), and is equipped with removable Glan-Thompson polarizers for the excitation and emission sides. The wavelength accuracy of each monochromator was checked. The emission monochromator with a bandpass of 1 nm was checked using several known lines from a low pressure mercury lamp placed in the sample compartment. The accuracy of the excitation monochromator was checked by setting the excitation monochromator to a wavelength and scanning the calibrated emission monochromator set at a 1 nm bandpass. The xenon arc and a reflecting surface set at 45° in the sample compartment were used.

Corrected Excitation Spectra: An excitation correction function is called automatically when the appropriate software functions are enabled. This correction function was checked by measuring the emission spectrum of a rhodamine B quantum counter [12] in a triangular cell in the

sample compartment using an appropriate, red, long-pass filter placed at the exit port of the sample compartment.

Corrected Emission Spectra: The response function of the detection system necessary to provide corrected emission spectra was determined at the PTI factory using a standard lamp (traceable to the National Institute of Standards) and a diffuser in the sample position. The use of a standard lamp, which has been calibrated by a black body, is the same method that was used in our earlier work to calibrate a laboratory-built spectrofluorimeter [1]. Use of the correction function gives the corrected emission spectrum in energy (E) units. During the course of the measurements reported here, a new, red-sensitive photomultiplier was installed and the detection system correction function was re-determined in this laboratory using the standard lamp procedure. For quantum yield measurements, the corrected spectrum in energy units must be multiplied by the wavelength to give the corrected emission spectrum in photon units (Ep) according to Equation 1 [13]:

$$E(\lambda) = Ep(\lambda)/\lambda = E(\nu)/\lambda^2 = Ep(\nu)/\lambda^3 \qquad (1)$$

where $E(\lambda)$ is the signal for the emission spectrum in energy units per bandpass in wavelength units; $Ep(\lambda)$ is the signal for the emission spectrun in photon units per bandpass in wavelength units; and the other designations are for the emission spectrum in energy or photon units per bandpass in frequency (ν) units.

Quantum Yields: Quantum yields were determined by the relative comparison procedure, using NIST SRM 936 Quinine Sulfate in 0.1 M $HClO_4$ as the main standard. The corrected emission spectra were measured for the quinine sulfate standard ($\lambda_{ex} = 347.5$ nm; absorbance ≤ 0.01; quantum yield $= 0.60_4$ [1]), the selected fluorophors, followed by the re-measurement of the quinine sulfate standard solution to check instrument stability during measurements. The general equation used in the determination of relative quantum yields from earlier research is given in Equation 2 [14].

$$QY_u = \frac{(QY_s)(FA_u)(A_s)(\lambda_{exs})(\eta_u^2)}{(FA_s)(A_u)(\lambda_{exu})(\eta_s^2)} \qquad (2)$$

where QY = quantum yield; FA = integrated area under the corrected emission spectrum (in Ep units); A = absorbance at the excitation wavelength; λ_{ex} = the excitation wavelength; η = the refractive index of the solution; and the subscripts u and s refer to the unknown and the standard, respectively.

Polarization and Oxygen Quenching: Measurements made earlier (1) or in the current research shoed no significant polarization or oxygen quenching for these molecules in these solvents.

Table I. Relative Absorbance Values for Quinine Sulfate, SRM 936

Peak, nm	Ratio [15][a]	Ratio [1]
250.0	1.000	1.0000
316.0	0.146	0.1476
347.5	0.182	0.1842

[a] This work.

RESULTS/DISCUSSION

Absorbance Spectra

Quinine Sulfate: The relative ratios of the absorbance peaks compared well (within ~2%) to earlier work (Table I).

Fluorescein: The absorbance spectrum of fluorescein was measured using solid and NIST-prepared solution samples in the borate buffer (potential NIST SRM 1932 [6]). For the NIST fluorescein-borate buffer solution, replicate volumes of the proposed SRM 1932 solution (from different, sealed ampoules) were diluted with the borate buffer to 10 mL. Absorbance data measured at 490.5 nm for the solutions from the sealed ampoules gave a molar absorptivity of 86,590 ± 1200 $[(mol/kg)(cm)]^{-1}$, in quite good agreement with the preliminary value obtained from NIST (86,700 ± 590 $[(mol/kg)(cm)]^{-1}$[16]). A typical series of measurements is given in Table II.

Typical peak absorbance values for the solid fluorescein material dissolved in the borate buffer and the other fluorophors in their respective solvents are summarized in Table III.

Fluorescence Spectra

Figure 1 gives the normalized corrected excitation and emission spectra for quinine sulfate, fluorescein, rhodamine B, and sulforhodamine 101 as measured with the PTI fluorescence system.

Table II. Relative Absorbance Values for Fluorescein, potential SRM 1932

λ, nm	A s1	A s2	A s3	avg A ± s
491.5	0.305	0.310	0.311	$0.308_7 \pm 0.003_2$
490.5	0.306	0.311	0.312	$0.309_7 \pm 0.003_2$
489.5	0.304	0.310	0.310	$0.308_0 \pm 0.003_5$
485.0	0.276	0.281	0.282	$0.279_7 \pm 0.003_2$
480.0	0.222	0.226	0.227	$0.225_0 \pm 0.002_6$
475.0	0.171	0.174	0.175	$0.173_3 \pm 0.002_1$
322.0	0.031	0.031	0.032	$0.031_3 \pm 0.000_6$
284.5	0.050	0.048	0.050	$0.049_3 \pm 0.001_1$
238.5	0.172	0.167	0.171	$0.170_0 \pm 0.002_6$

Table III. Comparison of the Relative Corrected Excitation and Absorbance Spectra Peak Values for Quinine Sulfate; Fluorescein, Rhodamine B, and Sulforhodamine 101

Compound	λ[a]	Absorbance	Ratio	Fluorescence	Ratio[b]
Quinine Sulfate[c]	347.0	0.133	1.000	19.56	1.000
	317.5	0.107	0.803	16.19	0.828
	250.0	0.730	5.489	100.00	5.112
Fluorescein[d]	490.5	0.777	1.000	5.022	1.000
	322.0	0.079	0.102	0.465	0.093
	284.5	0.119	0.153	0.807	0.161
	239.0	0.393	0.506	2.673	0.532
Rhodamine B[e]	544.5	0.707	1.000	13.65	1.000
	396.5	0.023	0.033	0.344	0.025
	355.0	0.062	0.088	1.064	0.078
	304.5	0.098	0.139	1.715	0.126
	285.5	0.092	0.130	2.074	0.152
	258.0	0.202	0.286	3.976	0.291
	237.0	0.163	0.231	3.080	0.226
Sulforhodamine 101[f]	577.0	0.687	1.000	15.67	1.000
	368.5	0.041	0.060	0.824	0.053
	314.0	0.090	0.131	1.950	0.124
	300.0	0.088	0.128	1.920	0.123
	267.0	0.178	0.259	4.069	0.260

[a] Absorbance wavelength maximum values; corrected excitation wavelength maxima are usually within 2 nm of this value.
[b] Flourescence spectra were normalized using 1.000 for the major peak maximum.
[c] Dissolved in 0.1 M $HClO_4$.
[d] Dissolved in borate buffer, pH = 9.1.
[e] Dissolved in methanol [9].
[f] Dissolved in ethanol

Corrected Excitation Spectra: Corrected excitation spectra measured for the four fluorophores were normalized and the relative peak values compared with the normalized values from the absorbance spectra to provide a check on the correction functions and procedures. These data are also summarized in Table III. In general, agreement is acceptable and within 5% for the comparisons of the values for the absorbance and corrected excitation spectra at the major peaks, although some of the smaller peaks have agreement generally to within only 10%. This can be understood when considering that stock solutions having peaks with an absorbance of 0.1 or less give absorbance values of 0.01 or lower when diluted for fluorescence measurements. A 1-digit difference in the third decimal place for the absorbance of the non-diluted solutions could easily produce a 10% difference in the absorbance value for the diluted solutions, essentially due to rounding errors. Additionally, rhodamine B tends to have ratio differences that are somewhat higher than the other three fluorophores. This can be attributed to the observed shifts in peak maxima and small changes in optical properties upon dilution in polar organic solvents [18].

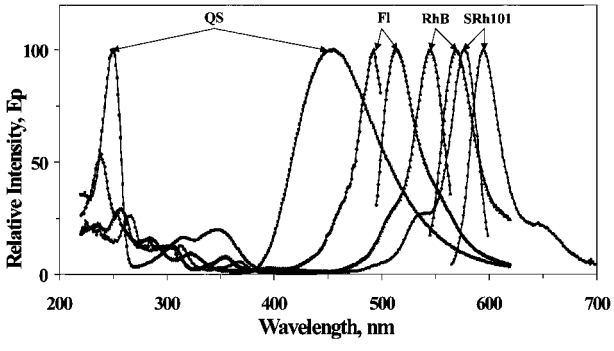

Fig. 1. Corrected excitation and emission spectra: QS = quinine sulfate (squares); Fl = fluorescein (diamonds); RhB = rhodamine B (triangles); and SRh101 = sulforhodamine 101 (circles).

Corrected Emission Spectra: The normalized, corrected emission spectra in Ep units obtained for quinine sulfate, fluorescein, rhodamine B and sulforhodamine 101 with the PTI instrument are shown in Fig. 1 and given in Table IV at 5-nm intervals [19]. Comparison of these values with corrected emission spectra from various sources and their ratios to the NIST values are made in Figs. 2–5. These results are discussed individually in more detail below.

Quinine Sulfate: The digital values of the corrected emission spectra for quinine sulfate in Ep units for this and earlier work [1,20] are given in Table V and the curves and the curves and ratios for these data and the corrected emission spectrum supplied by Molecular Probes [21] are given in Fig. 2. Excellent agreement for the values from this work compared to those from NIST [1,20] was obtained over the wavelength emission range, with values being well within the ranges reported by 9 other laboratories in an international round-robin study carried out in 1979 [22].

The corrected emission spectrum in Ep units for quinine sulfate obtained from the Molecular Probes' website showed excellent agreement for the blue edge of the emission spectrum. Some deviation is noted starting at approximately 440 nm and the emission maximum is found at 460.6 nm while the NIST and PTI reported maxima are at 455 nm and 457 nm, respectively. Additionally, the emission spectrum near the maximum shows some structure for the MP data, while emission spectra reported here and in the literature for quinine sulfate are generally smooth. The ratio (MP/NIST) shows larger deviations at the extremes of the spectra from a ratio of 1 than the PTI/NIST ratio does, but values are still within the typical deviations reported in the round robin study [22]. The excellent agreement for the blue edge of the MP emission spectrum with earlier reported spectra suggests

Table IV. Normalized, Corrected Emission Spectra for Quinine Sulfate (QS), Fluorescein (Fl), Rhodamine B (RhB), and Sulforhodamine 101 (SRh101)

WVL, nm	QS	Fl	RhB	SRh101	WVL, nm	QS	Fl	RhB	SRh101
360	0.075				535	26.05	57.88	—	—
365	0.083	—	—	—	540	23.28	49.57	—	—
370	0.160	—	—	—	545	20.45	43.82	17.55	—
375	0.427	—	—	—	550	18.40	39.09	32.70	—
380	1.10	—	—	—	555	16.03	34.49	54.69	—
385	2.52	—	—	—	560	14.02	30.08	77.96	—
390	5.06	—	—	—	565	12.41	25.49	95.02	4.01
395	9.43	—	—	—	570	10.87	21.16	99.85	9.40
400	15.69	—	—	—	575	9.63	17.32	92.86	22.24
405	24.16	—	—	—	580	8.29	14.34	79.99	43.72
410	33.95	—	—	—	585	7.37	11.86	65.65	70.22
415	45.13	—	—	—	590	6.42	9.94	52.92	91.87
420	56.68	—	—	—	595	5.74	8.47	42.59	100.00
425	67.76	—	—	—	600	5.05	7.21	35.40	92.50
430	77.53	—	—	—	605	4.54	6.30	30.63	77.13
435	85.47	—	—	—	610	3.75	5.40	28.00	61.56
440	92.33	—	—	—	615	3.42	4.59	26.08	47.82
445	96.45	—	—	—	620	—	—	—	36.40
450	99.04	—	—	—	625	—	—	—	29.40
455	99.65	—	—	—	630	—	—	—	25.08
460	99.10	—	—	—	635	—	—	—	22.83
465	96.62	—	—	—	640	—	—	—	22.24
470	92.61	—	—	—	645	—	—	—	22.47
475	87.34	—	—	—	650	—	—	—	21.50
480	82.03	—	—	—	655	—	—	—	20.02
485	75.47	—	—	—	660	—	—	—	17.54
490	68.84	—	—	—	665	—	—	—	15.16
495	62.91	30.82	—	—	670	—	—	—	11.91
500	57.02	56.43	—	—	675	—	—	—	9.87
505	52.06	82.00	—	—	680	—	—	—	8.01
510	46.80	97.00	—	—	685	—	—	—	6.44
515	42.15	99.56	—	—	690	—	—	—	5.07
520	37.48	92.91	—	—	695	—	—	—	6.25
525	33.29	81.00	—	—	700	—	—	—	5.00
530	29.44	68.46	—	—					

that instrument calibration could be the cause for the differences noted near the maximum and the red portion of the spectrum, and not an acid strength effect or the well-known red shift for the total emission spectrum exhibited for quinine sulfate when excited at 360 nm or above [23].

No major differences were noted for the quinine sulfate emission spectra in perchloric or sulfuric acids in this study within experimental error. The ratio labelled "H_2SO_4" in Fig. 2 was calculated from the emission spectrum for quinine sulfate in sulfuric acid obtained on the PTI instrument and the NIST values for the quinine sulfate in perchloric acid. The ratio labelled "$HClO_4$" was calculated from the quinine emission spectra measured on the PTI instrument and the NIST spectrum in perchloric acid. A small emission maximum difference of approximately 1 nm was observed earlier [1] in the different acids; however that is within the uncertainty of the wavelength measurements for the PTI instrument (± 1 nm).

Fluorescein: Corrected emission spectra from this work and the Molecular Probes' website for fluorescein in the borate buffer at pH 9.1 are given in Fig. 3. In this case, excellent agreement is obtained between the PTI and MP spectra.

Rhodamine B: Corrected emission spectra for rhodamine B in methanol from this work and that reported by the Ultraviolet Standards Group [24] in ethanol are given in Fig. 4. Although the solvents are different (methanol *vs* ethanol), the relative corrected emission spectra agree reasonably well.

Sulforhodamine 101: Corrected emission spectra from this work and the values for sulforhodamine 101 in ethanol from the Molecular probe website are given in

Table V. Corrected Emission Spectrum in Ep Units for Quinine Sulfate in 0.1 M $HClO_4$

Wavelength	This work	NIST [1,19]	Wavelength	This work	NIST [1,19]
375	0.430	0.400	525	33.29	34.93
380	1.102	1.001	530	29.44	30.83
385	2.519	2.402	535	26.05	27.23
390	5.055	4.905	540	23.28	23.92
395	9.430	9.009	545	20.44	21.12
400	15.68	15.02	550	18.40	18.52
405	24.16	22.92	555	16.03	16.22
410	33.95	32.43	560	14.01	14.31
415	45.13	43.04	565	12.41	12.61
420	56.68	54.25	570	10.87	11.01
425	67.76	65.07	575	9.628	9.610
430	77.53	75.08	580	8.291	8.509
435	85.47	83.78	585	7.367	7.407
440	92.33	91.19	590	6.424	6.507
445	96.45	96.60	595	5.743	5.606
450	99.04	99.10	600	5.052	4.905
455	99.65	100.0	605	4.545	4.304
457	100.0	—	610	3.749	3.804
460	99.10	99.60	615	3.422	3.303
465	96.62	97.10	620	3.273	2.903
470	92.61	92.99	625	2.752	2.502
475	87.34	87.79	630	—	2.202
480	82.03	82.68	635	—	1.902
485	75.47	76.88	640	—	1.602
490	68.84	70.97	645	—	1.502
495	62.90	64.97	650	—	1.301
500	57.02	59.66	655	—	1.101
505	52.06	54.05	660	—	1.001
510	46.80	48.75	665	—	0.901
515	42.15	43.84	670	—	0.701
520	37.48	39.24	675	—	0.701

Fig. 5. In this case, the peak maximum for the PTI results is found at 595 nm while the peak maximum for the MP data is found at 593.2 nm. These results are within experimental error.

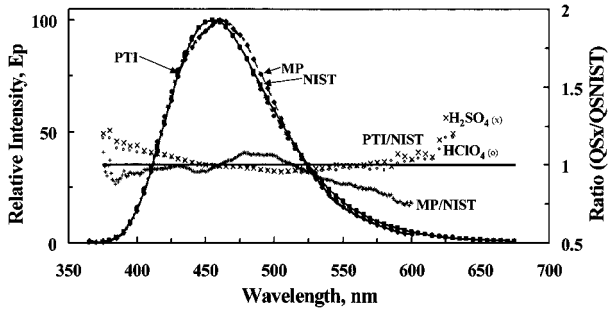

Fig. 2. Comparisons of the corrected emission spectra for quinine sulfate: NIST (squares); MP (diamonds); PTI (circles). The ratios show the ratio for MP/NIST (+), and the ratios PTI/NIST for the quinine sulfate in perchloric (o) and sulfuric (x) acids.

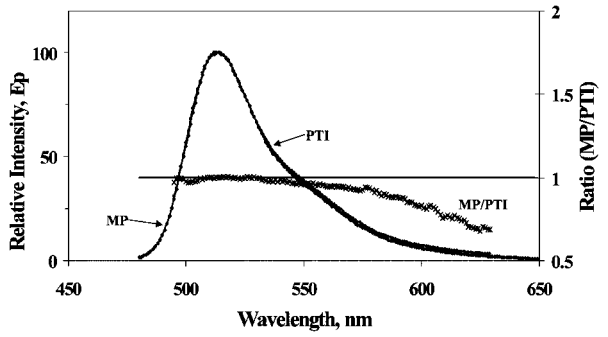

Fig. 3. Comparison of the corrected emission spectra for fluorescein: MP (triangles); PTI (circles). The ratio shows MP/PTI (x).

On an overall basis, however, spectral data suggest that there is a difference in the correction functions used for the PTI instrument and the instrument used to measure the MP spectra reported on their website because the emission peak maxima change relative positions as emission spectra go from the blue to the red regions of the spectrum. In the blue region, the peak maxima followed the order of NIST<PTI≪MP; in the green and "near" red regions PTI ≅ MP; and for the "far" red region, PTI > MP. These differences are typical of maxima reported by several laboratories for quinine sulfate in the international round robin exercise [22], even though ampouled solutions were supplied to the participating laboratories. At that time, the differences were attributed to the instrument correction procedures. In the spectra used for the comparisons in this study, the emission maximum reported for quinine sulfate in Ep units varied from 555 nm to 562 nm. Thus, as we find in this and earlier work (and as expected), the reported corrected emission spectra are a direct result of the correction functions determined and used for any specific instrument. The differences noted here for emission maxima and the spectral envelopes for all the spectra

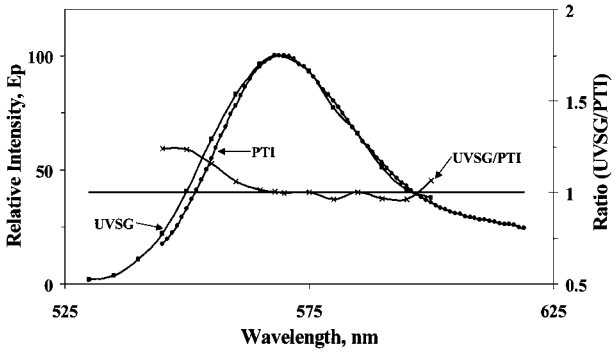

Fig. 4. Comparison of the corrected emission spectra for rhodamine B: UVSG (squares); PTI (circles). The ratio shows UVSG/PTI (x).

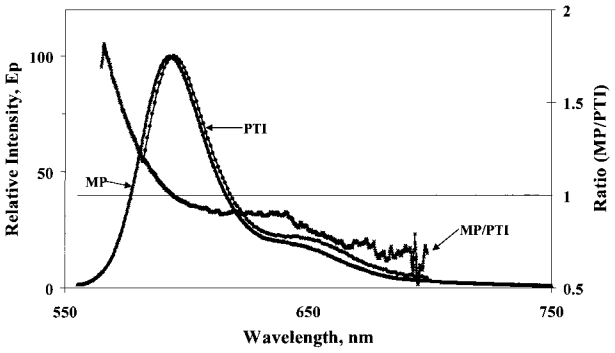

Fig. 5. Comparison of the corrected emission spectra for sulforhodamine 101: MP (triangles); PTI (circles). The ratio shows MP/PTI (x).

reported here are typical of what can be expected and what was observed in the past. On the other hand, emission maxima for the NIST data and our data on the PTI instrument agreed to within ±2 nm, a value that should be achievable with fluorescence equipment currently available.

Relative Quantum Yields (QY)

Quinine sulfate in dilute perchloric or sulfuric acids has been used as a relative quantum yield standard for more than 50 years. The variability of the quantum yield in sulfuric acid was reported earlier [25,26] and verified [1,27]. Perchloric acid (0.1 M) was suggested as the solvent of choice because of the relative constancy of the quantum yield as a function of acid strength [1] and was used in this work. The Pharmacy Institute at the University of Oslo has used commercially available quinine sulfate dissolved in sulfuric acid as a relative quantum yield standard; thus the relative quantum yield of this quinine sulfate in 0.05 M H_2SO_4 was measured *vs* SRM 936 Quinine Sulfate in 0.1 M $HClO_4$ to check previous measurements. Since both solutions were excited at 347.5 nm and the quinine concentrations were both dilute (absorbances $\leq$0.01), the usual Equation 2 for the determination of the quantum yield reduces to Equation 6 with the integrated emission spectrum (FA) in photon units.

$$QY_u = \frac{(QY_s)(FA_u)(A_s)}{(FA_s)(A_u)} \quad (6)$$

The corrected emission spectrum of the commercial quinine sulfate in 0.05M H_2SO_4 was measured and the relative quantum yield calculated to be 0.51 ± 0.02 (Table III), in excellent agreement with the 0.508 value reported previously [28] and the 0.51 measured in our earlier work [1].

The corrected emission spectra of the three other fluorophores were measured and the relative quantum yields were determined relative to the NIST SRM 936 in 0.1 M $HClO_4$. All the quantum yield values are summarized in Table VI. A correction was made for the solvent index of refraction at the emission peak maxima when comparing fluorophores in aqueous and organic solvents; however, corrections were not made for the change of index of refraction as a function of wavelength across the emission spectrum nor for the cell reflectance. These latter corrections average out to be quite small (<0.2%) [1] and are well within typical measurement imprecisions.

Table VI. Relative Quantum Yields of Selected Fluorophores

Compound	Solvent	QY	QY, lit
NIST SRM 936 Quinine Sulfate	0.1 M $HClO_4$	0.60 ± 0.02	Standard [1]
Quinine Sulfate	0.05 M H_2SO_4	0.51 ± 0.02	0.508 [1,28]
Fluorescein	Borate Buffer	0.92_9 ± 0.02	0.90–0.95 [17][a]
Rhodamine B	Methanol	0.69_6 ± 0.02	0.69 [29], 0.71 [30] 0.79 [31], 0.97 [32]
Sulforhodamine 101	Ethanol	0.95_1 ± 0.02	"near 1" [33]

[a] An average for the quantum yield of fluorescein in 0.1 M NaOH of 0.88 was calculated for 16 values reported earlier [1]; the latest reported values are somewhat higher, 0.90–0.95 [17].

As can be seen, quite good agreement with literature values and excellent internal consistency were obtained for all the measured fluorophores. The quantum yields for the fluorescein materials were taken over a one-year period using freshly prepared fluorescein and quinine sulfate solutions as well as determining a new correction function for the detection system. This agreement underscores the robustness of the fluorescein molecule as a standard under specified conditions. The only quantum yield value exhibiting a gross difference is for rhodamine B in methanol. Weber and Teale [32] reported a quantum yield for rhodamine B of 0.97; however, Demas and Crosby in their earlier landmark review [34] suggested that the values of 0.69 and 0.71 were probably more reliable due to the use of instrumentation with increased red-sensitivity relative to that used by Weber and Teale. It is estimated that the measured quantum yields are good to approximately 5%.

CONCLUSIONS

We have presented corrected emission spectra in a digital format and quantum yields for a series of organic fluorophores covering the blue to red spectral regions. The spectra and quantum yields agree well with data reported earlier in the literature. Quinine sulfate in perchloric acid continues to show extreme stability for these fluorescence parameters at specified conditions which only adds

support for its use as a basic fluorescence standard. Additional information on fluorescein in borate buffer is provided to the growing literature, and with the issuance of the fluorescein SRM by NIST, can be used as a fluorescence standard and as a link to the quantitative measurement of fluorescein-tagged biomolecules [35]. The rhodamines continue to be some of the most practical red emitters in use, and are included in this series of potential standards. Unfortunately, the use of organic solvents for these materials presents the necessity for care in handling and measurements.

In summary, the series of fluorescent materials studied here have exhibited a robustness such that research laboratories can use the compounds in this series under the specified experimental conditions to obtain results for other chemical systems in which they can have confidence as well as provide a basis to achieve comparability with results from other laboratories.

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