

Fluorescence Quantum Yields and Their Relation to Lifetimes of Rhodamine 6G and Fluorescein in Nine Solvents: Improved Absolute Standards for Quantum Yields[†]

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ABSTRACT

Absolute fluorescence quantum yields are reported for the rhodamine 6G cation and the fluorescein dianion dyes in nine solvents. This information is combined with previously reported fluorescence lifetimes to deduce radiative and nonradiative decay rates. Along the alcohol series from methanol to octanol, rhodamine 6G displays an increasing radiative rate, in parallel with the square of the refractive index increase, and a slightly decreasing nonradiative rate. Fluorescein is different: the apparent radiative rate actually decreases, suggesting that the emissive species is perturbed in some fashion. For both dyes, fluorescence yields are enhanced in D₂O, rising to 0.98, in parallel with a corresponding increase in lifetimes. Protonated solvents invariably give shorter lifetimes and lower quantum yields, contrary to some previous speculation. From this work and an analysis of existing literature values, more precise values have been obtained for two previously proposed absolute quantum yield standards. The yield of fluorescein in 0.1 N NaOH(aq) is 0.925 ± 0.015 , and for rhodamine 6G in ethanol, it is 0.950 ± 0.015 . In both cases, the solutions are assumed to be in the limit of low concentration, excited close to their long-wave absorption band and at room temperature but may be either air-saturated or free of oxygen.

INTRODUCTION

Recently, in this journal (1), we reported fluorescence lifetimes, that is, lifetimes of the lowest excited singlet states, for five xanthene dye species in nine solvents, including water, deuterium oxide, methanol (MeOH), ethanol (EtOH), isopropanol, normal propanol, butanol, hexanol (HxOH) and

octanol (OcOH). The rhodamine B cation lifetime ranged from 1.5 ns in H₂O up to about 3.2 ns in OcOH. The rhodamine B zwitterion showed a similar trend, but in every solvent the lifetime was longer by 0.2–0.5 ns. This variation, more than a factor of two, must be primarily because of changes in nonradiative processes that compete with fluorescence.

The other three dyes, rhodamine 6G, rhodamine 101 and fluorescein, displayed lifetimes that were near 4 ns and almost constant but with small systematic trends. The cause of those small variations was not obvious. State lifetimes, τ , alone are insufficient to distinguish changes in radiative rates from changes in nonradiative rates. Measurements of fluorescence quantum yields, ϕ , reported here, are needed to distinguish the two contributions. Then the radiative rate, k_r , and the nonradiative rate, k_{nr} , are calculated using Eqs. 1 and 2.

$$k_r = \phi/\tau \quad (1)$$

$$k_{nr} = (1 - \phi)/\tau \quad (2)$$

Of course, k_{nr} represents the sum of several processes, some of which may be negligible. The array of 45 different lifetimes was already useful for several purposes, as outlined earlier (1); but the addition of quantum yield information for two species adds further utility.

As an alternative to measuring quantum yields, k_r might be calculated using the Strickler–Berg relation (2). There are, however, reasons (3) to doubt that adequate precision could be obtained. At the time of that report, we were equally dubious about measuring ϕ accurately because of well-known difficulties (3) in measuring quantum yields. It is a challenge to measure a relative yield to 5% precision, and the standards to which relative yields are referred are themselves uncertain by 5% or more. Determining absolute yields is often thought to be even more difficult.

We subsequently realized that we, of all people, should have recognized that there might be a way (using calorimetry) to measure ϕ to about 1% precision and accuracy, at least for xanthene dyes that have $\phi \approx 1$. Calorimetric methods determine the heat produced by the nonradiative decay. If ϕ is greater than 90%, then a calorimeter measurement of heat deposited need only be accurate to about 10% to achieve 1% accuracy in ϕ . Long ago, in this very journal, one of us introduced a classical approach to using calorim-

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Abbreviations: EtOH, ethanol; F²⁻, sodium fluorescein dianion; HxOH, hexanol; MeOH, methanol; OcOH, octanol; R6G⁺, rhodamine 6G chloride cation.

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etry to obtain fluorescence quantum yields (4), and a decade later another among us introduced thermal blooming (also known as thermal lensing) as a newer calorimetric approach to the same end (5). We decided to try thermal blooming for the present project in the hope that with the improved instrumentation available today, we could measure ϕ well enough to compare with the lifetime data. Initially, we had no particular ambition to relate this work to the quest to improve fluorescence quantum yield reference standards; but two significantly improved dye–solvent standards emerged naturally. In addition, any of the other dye–solvent combinations can be used as a standard accurate to better than 5%. Because one of the more troublesome aspects of relative measurements involves a correction for solvent index of refraction, there is considerable advantage in having standards known for the range of refractive indexes provided here.

When a collimated laser beam traverses a sample and is slightly absorbed, the refractive index is changed in the illuminated volume. A variety of effects contribute, but for typical liquid solvents, the dominant contribution is thermal expansion leading to lower density and lower refractive index “within” the beam. This has the effect of a diverging lens. If the beam is a Tem_{00} Gaussian beam, and the sample is positioned correctly, the result is a symmetric expansion of the laser spot in the far field. If the beam is “turned on” abruptly (by opening a shutter), the far field spot appears to “bloom” over the time required for thermal equilibration. As a result (6), the intensity measured through a small pinhole on axis decreases with time according to Eq. 3.

$$I(t) = I_0 \left[1 - \theta \left(1 + \frac{t_c}{2t} \right)^{-1} + \frac{1}{2} \theta^2 \left(1 + \frac{t_c}{2t} \right)^{-2} \right]^{-1} \quad (3)$$

where I_0 is the beam intensity on axis immediately after turn on and before blooming, t_c is a characteristic time for thermal diffusion to establish the steady-state profile of the refractive index, t is time after the sudden turn on of the beam and θ is, in the simplest interpretation, the product of the absorbed thermal power and three constants as in Eq. 4.

$$\theta = P_{\text{th}}(dn/dT)/\lambda_L k \quad (4)$$

where P_{th} is the thermal power deposited in the sample, dn/dT is the temperature dependence of the refractive index, λ_L is the laser wavelength and k is the thermal conductivity. This was explained very well by Hu and Whinnery (6) some time ago. They pointed out that thermal blooming is useful for measuring very weak absorption because the experimental observable may be increased virtually without limit by using a more powerful laser. They used the material parameters to calculate how much power is absorbed. For the purpose of calorimetric determinations of nonradiative decay in competition with fluorescence, a variation is better (5). The essential idea is to compare the blooming caused by absorbing a known optical power in a perfect (100%) absorber with the blooming caused by the same or a known multiple of the optical power in the fluorescent solution. Strictly speaking, this is a relative measurement; but because it is possible to find absorbers that are “perfect” to any needed precision, thermal blooming becomes a route to what is effectively an absolute fluorescence yield measurement. This idea was introduced and first applied to fluorescein in an article (5) that

describes an implementation in detail and lists a number of precautions to be observed. Of course, one has to remember that even a 100% quantum yield in a fluorescent solution still deposits some thermal energy proportional to the Stokes shift between the laser frequency $\nu_L = 1/\lambda_L$ and the mean frequency of fluorescence given by Eq. 5.

$$\langle \nu_f \rangle = \sum \nu_f n(\nu_f) / \sum n(\nu_f) \quad (5)$$

where the number of fluorescent photons observed at a particular frequency $n(\nu_f)$ is conveniently recorded with photon-counting electronics, corrected for the relative sensitivity of the fluorimeter. With the assumption of constant laser power, P_L , and defining an absorption fraction (*not* absorbance) for a solution A , we may use the result derived previously (5) and given by Eq. 6.

$$\phi^x = \frac{\nu_L}{\langle \nu_f \rangle} \left[1 - \frac{A^r \theta^x}{A^x \theta^r} \right] \quad (6)$$

where superscript r refers to the nonemissive reference solution and superscript x denotes the fluorescing species that is the unknown to be characterized.

In the present project, we made two changes in the application of Eq. 6 from that used more than two decades ago (5). First, with today’s desktop computers, we could determine θ by numerically fitting Eq. 4 to the entire time trace of the detector output, rather than make further approximations to use only the initial slope or the asymptotic behavior. Second, we changed the general strategy for using Eq. 6. In the earlier implementation, the plan was to keep $A_r \approx A_x$ and measure the differences in the blooming parameter θ . This time, after further consideration of the error budget, we decided to keep the extent of blooming approximately the same in the reference and unknown, while adjusting the ratio of absorptions A^r/A^x to produce that result. The rationale was that one needs reasonably large θ in order to gain precision, but the theory is valid only in the limit of small θ . By treating the measurement of θ as a null (*i.e.* balanced) measurement, higher order terms in the theory and a number of other possible errors should cancel out. This puts the burden of precision and accuracy on the measurement of the absorption values. Although modern spectrophotometers are quite linear so that a reasonable range of A can be measured, quite a large ratio, A^x/A^r , is required for dyes that have quantum yields approaching unity and small Stokes shifts. So the final strategy was to prepare stock solutions for which we could measure absorbance values in the range 0.4–0.6 (corresponding to our $A = 0.6$ –0.75) and then rely on serial dilutions to obey Beer’s Law at the low micromolar concentrations involved. Another factor of two was gained by using shorter cells for blooming than for the absorption measurement.

MATERIALS AND METHODS

The dyes, rhodamine 6G chloride cation (R6G^+) and sodium fluorescein dianion (F^{2-}), and the nine solvents were the same as described in the study of lifetimes (1). The relevant dye structures are displayed in Fig. 1. As before, hydroxide was added to water, and triethylamine was added to alcohols to form F^{2-} . Special effort was taken to try to ensure that the triethylamine added to the higher alcohols was sufficient to produce the F^{2-} dianion as verified by monitoring absorption because the calorimetry measurements do not have the ability to distinguish different species in the way that the lifetime measurements could.

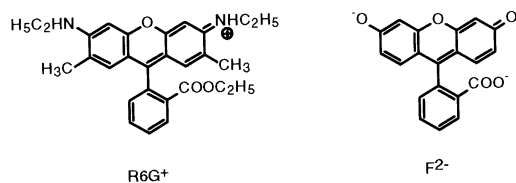


Figure 1. Structures of the dye species examined in the present study.

The nonfluorescent reference absorber was basic fuchsin dye for the alcohol solvents. For water and heavy water, we initially used permanganate ion, but later the results were confirmed by using dichromate ion. An anonymous referee suggests that we point out that proving that a reference does not luminesce is not sufficient; one should also rule out "photochemical" conversions that would absorb (or release) free energy. Irreversible photochemistry at the level of several percent, which would be needed to compromise the present results, should make itself quite obvious because it is something we were watching for. Any species that form and decay rapidly compared with the "blooming" time cause no problem. Most troublesome might be metastable species that last a few seconds but do not accumulate. One would have to be rather unlucky to encounter such processes, but at least two approaches provide protection. One could carry out transient absorption studies and show that the starting material is regenerated in a short time. We chose basic fuchsin on this ground. It is one of the triphenylmethane dyes. Although we are not aware of published data for basic fuchsin, one of us and dozens of other laboratories investigated triphenylmethane dye internal conversion and proved that it is very efficient in fluid media. We had carried out unpublished work with basic fuchsin two decades ago. As an alternative to completing a full study of the photophysics of a reference dye, or to provide extra reassurance, one can use two or more reference absorbers. It is unlikely that all will be plagued with rare metastable photoproducts. This is what we did for the water samples in which both permanganate and dichromate were used as reference absorbers.

The absorption measurements were carried out in cells with 1 cm pathlengths, using an Ocean Optics Chem2000 instrument. Because this is a new and low-cost instrument, the absorbance calibration was checked against two different models of Hewlett-Packard diode array spectrophotometers and a Perkin-Elmer Lambda 18 scanning instrument. For absorbances in the range used, 0.4–0.6, repeatability for each machine and similarity among all machines was better than 0.5% when reasonable care was taken with the cleanliness of cell windows and the positioning of cells. For the protocol used, involving only ratios, absolute accuracy is not required for the absorption measurements. Wavelength calibration was confirmed using a mercury lamp. This is important because the absorptions needed must be determined at the true laser wavelength.

Fluorescence spectra for calculating the Stokes shift were recorded using a homemade photon-counting instrument. Excitation used the same lasers as the thermal blooming measurements detailed below. Samples were dilute solutions in 1 cm² cells, except for the D₂O samples, for which a 3 mm² cell was used. Detection used a Spex 0.5 m monochromator and an RCA 31034 photomultiplier with a Pacific Precision AD6 amplifier-discriminator feeding a Stanford Research SR245 computer interface, which counted photons and also incremented a stepper motor to advance the wavelength. Wavelength was calibrated using a mercury lamp. Data counts were transferred to a desktop computer. Two or more scans were recorded for each dye in every solvent. Wavelengths were converted to wavenumbers and integrated using the spreadsheet included with a ProStat plotting program. Repeated scans gave identical centroids, except for rare, obvious "glitches." When necessary, additional scans were taken. Spectra were corrected for system response, but they are sufficiently peaked that the correction had no effect at all on the trends among solvents and no significant effect even on absolute values. Spectra were recorded over a wavelength range extending to count rates well below 1% of peak rate at both long and short wavelengths. In addition, we attempted to keep the contributions from the truncation equal on both sides, even though that has negligible effect on the final uncertainties. For these dyes it is not difficult to select

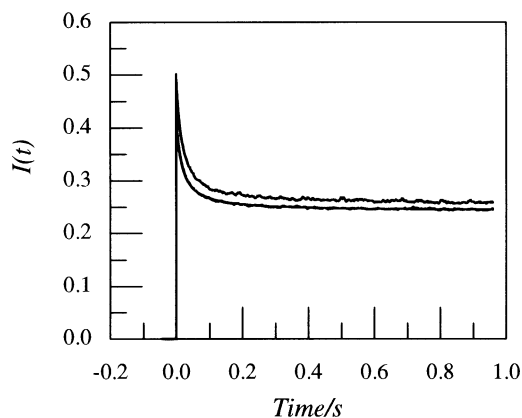


Figure 2. Data for thermal blooming with fits to Eq. 3. Each data file contains 5002 digitized points. Each fit is a continuous curve that is entirely obscured by overlying data points. Top: basic fuchsin in EtOH with absorption = 0.0259; fit has $\theta = 0.71$, $t_c = 0.047$ s, $I_0 = 0.510$ V. Bottom: R6G⁺ in EtOH with absorption = 0.208; fit has $\theta = 0.57$, $t_c = 0.046$ s, $I_0 = 0.424$ V. For both dyes, the laser power is the same, about 2 mW, and the laser wavelength is 532 nm.

concentrations having intensities well above solvent background, while still being dilute enough to avoid the inner filter effect that would reduce the short wavelength component of the spectrum, producing a systematic error.

A diagram of the thermal blooming apparatus, which is very simple, was displayed previously (5). Measurements on R6G⁺ used a diode-pumped Coherent Nd:YVO₄ laser doubled to give 1 W at 530 nm. Measurements on F²⁻ used a Spectra Physics Ar⁺ ion laser producing about 500 mW at either 514.5 or 488 nm in different runs. Attenuating filters reduced the laser powers in all measurements by factors ranging from 10 to more than 100. Water requires higher power than organic solvents to achieve comparable blooming. Filters introduce small lensing effects of their own; so they must be inserted before the shutter. The lens had a focal length of 200 mm. The shutter was positioned near the beam waist so as to have the shortest possible "opening" time, as determined by monitoring the transition time with an oscilloscope. The shutter was opened for 1 s, repeated at 10 s intervals. The sample should be positioned one confocal distance past the beam focus. The position was optimized by seeking the maximum blooming effect. The sample cell was an ordinary absorption cell with a 5 mm pathlength. The detector was a 2 mm diameter photodiode, operated in photoconductive mode with 50 V of bias. It was positioned about 274 cm from the sample and shielded from stray light. Traces were digitized in a LeCroy 9361 digital oscilloscope. Traces (from 20 to 50) were averaged and then transferred to a microcomputer for curve fitting. Data were fit to Eq. 3, using a nonlinear least squares routine. In every case, we measured the thermal response of the pure solvent, which might contribute an extra, additive contribution to θ . It was always small compared to quoted uncertainties. In most cases it was ignored; in a few cases, involving particularly dilute dye solutions, the solvent contribution was subtracted from both the sample and the reference measurements. We suspect that variations among measurements under ostensibly similar conditions may be the result of slight variations in the transverse mode structure of the beam, even though profiles were good by usual standards, and to slight offsets in the detector position relative to the center of the beam. One may either try to keep everything stable long enough for a comparison to be made; or one may realign frequently in order to convert potential systematic errors into random errors, which are then reduced by averaging. We did both at various times.

RESULTS

A typical example of the photodiode output for thermal blooming as recorded on axis is displayed in Fig. 2. When

Table 1. Photophysical parameters for R6G⁺ in nine solvents

Solvent	ν_a (μm^{-1})	$\langle\nu_f\rangle$ (μm^{-1})	ϕ	τ (ns)*	k_r (ns^{-1})	n^\dagger	k_r/n^2	k_{nr} (ns^{-1})
H ₂ O	1.900	1.772	0.90 ± 0.02	4.08	0.221	1.333	0.124	0.025
D ₂ O	1.903	1.773	0.98 ± 0.015	4.36	0.225	1.3384	0.126	0.005
MeOH	1.896	1.777	0.93 ± 0.005	4.13	0.225	1.3288	0.127	0.017
EtOH	1.888	1.770	0.95 ± 0.005	3.99	0.238	1.3611	0.128	0.013
<i>i</i> -PrOH	1.882	1.775	0.95 ± 0.01	3.96	0.240	1.3850	0.125	0.013
<i>n</i> -PrOH	1.882	1.772	0.95 ± 0.01	3.95	0.241	1.3776	0.127	0.013
BuOH	1.882	1.767	0.96 ± 0.005	3.89	0.247	1.3992	0.126	0.010
HxOH	1.875	1.765	0.95 ± 0.005	3.88	0.245	1.4178	0.122	0.013
OcOH	1.872	1.762	0.96 ± 0.01	3.83	0.251	1.4293	0.123	0.010

*Magde *et al.* (1).

†CRC Handbook (28).

the shutter is opened, the photosignal increases abruptly from zero to a maximum (which is I_0); then heat deposited in the sample imposes a diverging lens that grows in over time, with a consequent decrease in the photointensity on axis, as recorded by the diode. The time course is fit well by Eq. 3 to give a θ characteristic of a particular dye, solvent and concentration.

For each particular sample, at least two curves were recorded. For each dye–solvent combination, three different concentrations were prepared and measured, interleaved with three dilutions of the reference absorber. From the three dye θ^* and the three reference θ , nine evaluations of ϕ may be calculated from Eq. 6. There were small systematic variations in such determinations. This scatter, however, could be reduced significantly by using (or interpolating to) only evaluations for which $\theta \approx \theta^*$. In this way, and as proposed earlier in the introduction, the thermal blooming measurement simply identifies situations in which the deposition of heat is equal, and the ratio of A^y/A^x for that situation, along with the correction for the Stokes shift, determines ϕ according to Eq. 6. Some dye–solvent combinations were measured a second time, some weeks later, as a check for long-term repeatability. Tables 1 and 2 list frequencies for peak absorbance (not centroids) ν_a , mean emission frequencies $\langle\nu_f\rangle$ and quantum yields ϕ for the 18 dye–solvent combinations.

In both dyes, the largest fluorescence quantum yield occurs in D₂O, and it is very close to 100%. No single run ever quite reached 100%; yet no run was much lower than 98%. It is difficult to think of a systematic error that would yield 98% consistently but erroneously. The fact that even with a mean of 98%, no determination ever exceeds 100%

Table 2. Photophysical parameters for F²⁻ in nine solvents

Solvent	ν_a (μm^{-1})	$\langle\nu_f\rangle$ (μm^{-1})	ϕ	τ (ns)	k_r (ns^{-1})	k_{nr} (ns^{-1})
H ₂ O	2.040	1.900	0.92 ± 0.02	4.16	0.221	0.019
D ₂ O	2.042	1.900	0.98 ± 0.01	4.36	0.225	0.005
MeOH	2.014	1.885	0.91 ± 0.015	4.28	0.213	0.021
EtOH	1.997	1.842	0.91 ± 0.02	4.25	0.214	0.021
<i>i</i> -PrOH	1.980	1.869	0.92 ± 0.01	4.36	0.211	0.018
<i>n</i> -PrOH	1.988	1.871	0.91 ± 0.015	4.42	0.206	0.020
BuOH	1.988	1.867	0.91 ± 0.02	4.40	0.207	0.020
HxOH	1.982	1.865	0.93 ± 0.01	4.48	0.208	0.016
OcOH	1.982	1.862	0.94 ± 0.01	4.52	0.208	0.013

provides some added evidence that random errors are not large. At the same time, the fact that all the other dye–solvent combinations are slightly but significantly less fluorescent than the D₂O solutions provides convincing evidence that past speculations that some xanthene dyes dissolved in nondeuterated alcohols achieve $\phi = 100\%$ must have been in error.

For R6G⁺, in Table 1, the H₂O solution has the smallest ϕ , and the MeOH solution shows the smallest ϕ among alcohols, presumably because MeOH is most similar to H₂O. The higher alcohols all give quantum yields for R6G⁺ that are very similar to each other. For F²⁻, in Table 2, all solvents other than D₂O give yields that are almost within error of each other, although there seems to be a slight increase in ϕ for the higher alcohols.

DISCUSSION

Relation of quantum yields to fluorescence lifetimes

Included in Tables 1 and 2 along with the newly measured quantities are the lifetimes reported previously. In addition, the radiative and nonradiative rates, calculated using Eqs. 1 and 2, are displayed, along with refractive indices.

We now have the answer to the question raised previously (1) as to whether the decrease in fluorescence lifetime in R6G⁺ along the series of alcohols from MeOH to OcOH is because of an increase in competitive nonradiative processes (which would reduce ϕ) or reflects an increase in radiative probability (which would increase ϕ very slightly). Because ϕ increases as τ becomes shorter, it is the latter, as predicted (1). Nonradiative processes in R6G⁺ are small and sufficiently constant from EtOH to OcOH that radiative decay is controlling the state lifetime. The radiative rate should scale with the square of the refractive index based on a simple density-of-states argument. Table 1 shows that the quantity k_r/n^2 is more nearly constant than the state lifetimes and that what little scatter does exist in that quantity appears to be random.

Not only do the present data rule out the possibility that the decrease in τ for R6G⁺ along the series of alcohols could be because of a substantial increase in k_{nr} , they even suggest that k_{nr} may decrease along the series. Because there is a dramatic decrease in k_{nr} between H₂O and D₂O, it is reasonable to conclude that the dominant contribution to k_{nr} involves protons. The solvent D₂O produces the largest ϕ and

longest τ of all. Ordinary water occupies approximately the position it would hold as the limit of the alcohol series. Along the alcohol series, the nonradiative rate seems to be decreasing in parallel with the reduced density of solvent O–H groups. The same deuterium effect was observed previously for R6G⁺ in deuterated alcohols (7–9). In the first two of those reports (7,8), the suggestion was made that the deuterium effect might involve hydrogen–deuterium exchange specifically at the amine groups on R6G⁺. However, if we invoke exchange at the amine centers for R6G⁺, we will need a different explanation for fluorescein, which does not have exchangeable amine protons but does show a deuterium effect. It seems more likely that the deuterium effect is similar in the two dyes, and that the solvent O–H group plays a particular role as an accepting mode in an internal conversion process.

Turning to fluorescein, we see in Table 2 that ϕ is largest for deuterium oxide solvent again because of greatly reduced nonradiative decay. Quantum yield is almost constant for F²⁻ along the series of protonated solvents from H₂O to OcOH, but there seems to be a small increase in ϕ in the two heaviest alcohols. This parallels the change in τ , and so it implies a modest decrease in k_{nr} , as predicted previously (1). At that time we speculated that there might be small, but variable, amounts of triplet formation in fluorescein. Now that we have distinguished radiative and nonradiative rates, it seems likely that the variation of the nonradiative decay involves the same process proposed above for R6G⁺. There must, of course, be some intersystem crossing in both dyes because dye lasers are known to be affected by absorption from a long-lived, metastable state that must be the triplet. Perhaps, intersystem crossing is the residual nonradiative process that suppresses the quantum yield below unity even in D₂O. Fluorescein, however, presents a conundrum. The fact that k_r actually decreases slightly across the entire F²⁻ series requires an explanation because it is contrary to theoretical expectation of an increase proportional to n^2 , as observed in R6G⁺. Perhaps, the character of the solvated dye molecule changes slightly along the alcohol series. Despite our best efforts, we may simply have been unable to prepare identical emitting species across such a wide range of polarity. The F²⁻ dianion cannot be very stable in less polar solvents. There must be some tendency toward ion pairing. We should also consider the effects of the triethylamine base, which was added in increasing amounts along the series, with the higher alcohols requiring substantially larger amounts.

For both dyes, expected errors in all the calculated quantities are dominated by uncertainties in ϕ . Lifetime measurements may be measured to a higher precision than yields; their uncertainty was treated earlier (1). However, we do wish to thank Quentin Hagley (personal communication of unpublished work at the Max Planck Institute for Biophysical Chemistry, Göttingen, Germany) for sharing lifetime measurements he made using frequency domain methods. He found $\tau(\text{R6G}^+, \text{H}_2\text{O}) = 4.11$ ns, $\tau(\text{R6G}^+, \text{MeOH}) = 4.16$ and $\tau(\text{R6G}^+, \text{EtOH}) = 3.94$. These are in quite good agreement with the values we obtained by a different method and reported previously (1) and include in Table 1, both with respect to the variation among the three solvents and even for the absolute values.

Quantum yield standards

We did not set out to refine fluorescence yield standards. However, in the course of a review of the literature, we discovered that there have been other recent absolute determinations for two of the dye–solvent pairs that agree well with our findings. There are also a discouraging number of discordant (but mostly older) data, which we will not review here. We believe there is enough consensus among recent and more convincing determinations to justify considerable confidence about F²⁻ in aqueous base and R6G⁺ in EtOH as precise and accurate quantum yield standards.

Fluorescence quantum yields have been notoriously uncertain. One concern is whether they vary with excitation wavelength. We shall restrict the discussion here to photoexcitation into the lowest energy, visible absorption band. Concentration effects are another concern, and the xanthene dyes are not favorable in this regard. We are considering only the low concentration limit here, that is, not more than a few micromolar. When Demas and Crosby (10) reviewed the situation 30 years ago, they concluded that fluorescein dianion was one of the better standards among the few available. They thought that among the most reliable measurements were probably those of Weber and Teale (11). As early as 1958, they had obtained $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH}) = 0.92$ or 0.93 (depending on excitation wavelength) in a series of studies based on purely photometric measurements that very carefully accounted for the collection efficiency of their detection system. This was corroborated, Demas and Crosby noted, by some other determinations, one of which is among the results obtained by the early calorimetric study (4) mentioned in the introduction section. Whether by coincidence or not, the Weber–Teale value is essentially the same as the result reported here, almost half a century later. However, Demas and Crosby (10) were mindful that several other investigators had obtained values significantly higher or lower (as low as 0.77); they ultimately recommended a conservative $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH}) = 0.90$ “within 10% and probably within 5%.” In hindsight, they might have been justified had they disregarded the extreme values and recommended 0.92 or 0.93 ± 0.05 even with the information available in 1971. Today, we can do better than that.

There have been some additional, independent, absolute determinations since 1971, all based on calorimetry of one form or another. A classical calorimetric method (12) with a claimed limit of accuracy of ± 0.02 found $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH}) = 0.90$. This is slightly lower than our value, but some other quantum yields reported in the same study also appear to be too low. The first use of thermal blooming for fluorescence yield measurements (5) gave $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH}) = 0.95 \pm 0.03$, which is higher than, but within error of, the value reported here. A decade later, another thermal lensing measurement (13), using almost exactly the same procedures, reproduced that result, namely, $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH}) = 0.95$. Only weeks later, however, the same investigators reported (14) another determination that gave a smaller value of $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH}) = 0.92 \pm 0.03$. The second study also employed thermal lensing but with two changes. They used two separate lasers, one for heating the sample and the other as the probe; for the reference compound, they quenched the fluorescein with iodide, rather than

using a different substance. They cite as a major advantage of quenching that no absorption measurement is needed. We are not convinced that absorption measurements need be problematical, and more recent experimental work (15) suggests the need for some caution in using the quenching method. They did not explicitly address the discrepancy with their own earlier result, but they did note the modest discrepancy (within quoted errors) from the first thermal blooming measurement a decade earlier, and apparently they preferred the lower value. Their second measurement agrees perfectly with our present result, which we also believe should be better than the earliest (and larger) thermal blooming determination (5), for systematic reasons given earlier. Furthermore, with more confidence in the thermal measurement, we put more effort this time into ensuring that the absorption measurements were precise and accurate. We believe that based on the convergence of calorimetric determinations, we can now be quite confident that $\phi(\text{F}^{2-}, 0.1 \text{ N NaOH})$ is almost certainly within the span 0.91–0.94. Fluorescein, however, is somewhat unstable and should be freshly prepared.

There is less information about the fluorescence quantum yield for F^{2-} in alcohols. A calorimetric study (4) found $\phi(\text{F}^{2-}, 0.01 \text{ M KOH in } 95\% \text{ EtOH}) = 0.96$. Another study (16) reported $\phi(\text{F}^{2-}, 0.01 \text{ M KOH in MeOH}) = 1.0$ and $\phi(\text{F}^{2-}, 0.01 \text{ M KOH in EtOH}) = 1.0$ relative to F^{2-} in aqueous base, assumed to be 0.92. A true yield of unity in any of the alcohols is inconsistent with our finding of a substantial deuterium effect. Although both those studies claim less precision and accuracy than the present measurements, they may suggest that if there is any small, systematic error in our results, it may be in the direction to make the fluorescence yield of F^{2-} in alcohols slightly higher than that given in Table 1. Because of the existence of multiple ionic species, with the concomitant need for buffering, we do not think it is worthwhile pursuing F^{2-} as a standard for a range of solvents, although our values can probably serve if accuracy of 4 or 5% is sufficient. A different molecule would be better.

Demas and Crosby (10) pointed out that fluorescein is less than an ideal quantum yield standard for several reasons, and they thought some of the then new xanthene laser dyes might be better, although they are still not ideal because they suffer from undesirable overlap between absorption and emission bands. The laser dyes are available in higher purity than has been customary for dyes in general. In particular, rhodamine 6G was and is attractive because it has only one ionic form, is inexpensive and readily available, and possesses good thermal and photostability. Thirty years ago, there was no well-documented, absolute measurement of R6G^+ ; but in the years since, R6G^+ in EtOH has been the subject of several studies, the most convincing of which agree well with our present result.

One of the most persuasive determinations of $\phi(\text{R6G}^+, \text{EtOH})$ seems to have been neglected for two decades. Butenin and coworkers (9) carried out a calorimetric study using laser excitation and dilatometry measurements to obtain the results: $\phi(\text{R6G}^+, \text{EtOH}) = 0.94 \pm 0.01$ and $\phi(\text{R6G}^+, \text{EtOD}) = 0.98 \pm 0.01$. These are in good agreement with our results and confirm that the deuterium effect is general. The tight confidence intervals seem justified by the data reported. A subsequent study (17), however, using a photo-

metric approach that compared fluorescence to scattering by MgO , along with other tests, suggested that the yield is somewhat higher. Being fully aware of the Butenin result, the authors concluded $\phi(\text{R6G}^+, \text{EtOH}) = 0.94\text{--}0.97$, a range that encompasses our datum of 0.95. A more recent, two-laser thermal lensing approach (18), again obtained $\phi(\text{R6G}^+, \text{EtOH}) = 0.94$.

No discussion of fluorescence efficiencies in laser dyes would be complete without mention of the extensive work of Drexhage. He has published at least three extended reports (7,8,19) that relate fluorescence properties, most notably quantum yields, to dye structures. Although others have pursued the same topic for dyes, like rhodamine B, whose fluorescence yields vary over wide ranges, no one else has done as much to understand small differences in highly luminescent molecules. Drexhage also always paid careful attention to chemical purity. The earlier papers (7,8) reported yields relative to F^{2-} , assumed to have a 0.90 yield. They list $\phi(\text{R6G}^+) = 0.95$, "almost independent of solvent," but also admit in an endnote that there are actually some variations and that one should not expect to know absolute quantum yields to better than 5% accuracy. Probably, part of the reason Drexhage chose the value 0.95 was that he determined that ϕ increased by about 4% in deuterated alcohols. Drexhage neither assigned an estimate of precision nor explained his procedures well enough for us to estimate an uncertainty. The more recent paper (19) should be more definitive. The yield $\phi(\text{R6G}^+, \text{EtOH})$ is still 0.95, but now the source cited for that number is a thermal blooming measurement reported in a Ph.D. dissertation (20). This agrees exactly with our datum. Based on the consistency of all these independent measurements, we believe that $\phi(\text{R6G}^+, \text{EtOH}) = 0.95 \pm 0.015$.

Although we ignore most relative measurements of ϕ , one study (21) merits mention. It appears to have been carried out with unusual care, even though it makes conservative claims for error limits. The authors measured seven rhodamines relative to quinine sulfate, assumed to have $\phi = 0.55$. They found $\phi(\text{R6G}^+, \text{EtOH}) = 0.95$. This may count as slight added evidence for our consensus value. More important, we mention it because the good agreement may suggest that the values for the other six rhodamines deserve notice.

Solvents for R6G other than EtOH have received much less attention. An early optoacoustic calorimetric method (22) found $\phi(\text{R6G}^+, \text{H}_2\text{O}) = 0.96 \pm 0.02$. A more recent photoacoustic determination (23), however, obtained $\phi(\text{R6G}^+, \text{H}_2\text{O}) = 0.90 \pm 0.02$. One or both of these must be in error by more than the stated uncertainty. The second agrees with our value. A very recent study using a dual-laser thermal lensing method (24) obtained $\phi(\text{R6G}^+, \text{H}_2\text{O}) = 0.95$ and $\phi(\text{R6G}^+, \text{MeOH}) = 0.98$. Both these values are higher than those we measured, but the difference between them is exactly the same as that determined by us. Another report (25) also found $\phi(\text{R6G}^+, \text{MeOH}) = 0.98$. A value as large as 0.98 for MeOH is inconsistent with the deuterium effect found by us (and others mentioned earlier in the article). We conclude that for solvents other than EtOH, information in the prior literature is too scattered to challenge our data, but neither does it lend useful support for a really tight error limit. The trends in different solvents are more secure.

In reviewing the literature on R6G^+ , we found other, fairly

recent papers that cite what initially appear to be additional sources for a quantum yield of R6G⁺. They turned out, however, to be secondary sources, usually involving a chain of citations that led eventually back to Drexhage's earliest work (7,8), except for one poorly described situation that seemed to be another measurement made relative to fluorescein with indeterminate precision and accuracy.

CONCLUSIONS AND CONSEQUENCES

We suggest first that thermal lensing continues to look promising as a calorimetric technique for fluorescence quantum yield determinations. There is some question whether the simplest theory, used here, is adequate. Carter and Harris (26) considered this matter experimentally and found that θ obtained by fits to Eq. 3 are proportional to heat deposition (which is all we care about) even though the interpretation offered by the simple theory and exemplified in Eq. 4 may not be entirely adequate.

The absolute quantum yield for fluorescence of F²⁻ in 0.1 N NaOH(aq) is 0.925 ± 0.015 ; for R6G⁺ in EtOH, it is 0.950 ± 0.015 . These error limits are believed to represent at least a 90% confidence interval. In both cases, the solutions are assumed to be in the limit of low concentration, excited close to their long-wave absorption band, and near room temperature. Whether oxygen is removed or present at ambient partial pressure does not matter at the stated precision. The rhodamine solution is quite stable over time, but the fluorescein solution should be freshly prepared.

Yields for F²⁻ in alcohols and for R6G⁺ in water are given in Table 1, but for them confidence intervals should be slightly larger, although even these are, we believe, well determined when compared with past standards for absolute quantum yield measurements. These systems are not such good solvent-solute pairs. The trends may be used with more confidence than the absolute values. Over the series of alcohols from EtOH to HxOH, the radiative rate constant for R6G⁺ increases proportional to the square of the refractive index, as expected. In F²⁻, some other consideration not only counteracts the refractive index effect but may actually reduce radiative rates slightly.

Nonradiative rate constants are substantially reduced in D₂O, leading to longer lifetimes and fluorescence yields as high as 0.98. The same effect has been observed in deuterated light alcohols (7-9). This suggests that some nonradiative process involves hydrogen bonding. Consistent with that, our results suggest that the nonradiative rate is reduced in higher alcohols, paralleling the reduced density of O-H bonds. An isotope effect on accepting modes in nonradiative decay is characteristic of internal conversion rather than intersystem crossing. In our previous paper treating lifetimes (1), we summarized several of the mechanisms that have been proposed for nonradiative decay in xanthenes. We do not repeat that here. We might, however, mention one additional report that we did not cite previously. Arbeloa and coworkers (27) treat six dye species, including R6G⁺, with particular reference to deuterium effects. They cite useful earlier work. However, their conclusions should be treated with caution. Although the lifetimes are plausible, the fluorescence yields must be in error by more than a third. On the other hand, the authors are correct in their belief that if

one is to test mechanisms for nonradiative decay in xanthene dyes, then dyes with a variety of chemical structures must be examined in a wide range of solvents. A valuable check will be to reproduce in any study, as controls, some of the lifetime or yield data that we believe are now reliably known to quite high precision.

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