

Absolute Quantum Yield of Room-Temperature Phosphorescence Using the Time-Resolved SSTD Mode of a Fluorolog[®]-QM with Integrating Sphere



Introduction

The interest in room temperature phosphorescence (RTP) has been steadily growing over the years with numerous applications in the fields of medicine, organic electronics, protein studies and biological imaging. The characterization of RTP is often severely handicapped due to a strong fluorescence overlap, especially when organic materials are involved. This fluorescence interference makes it difficult to determine some basic photophysical properties, such as the phosphorescence spectrum and the quantum yield. In this note we employ a time-resolved methodology, which utilizes the Single-Shot Transient Digitizer (SSTD) mode of the HORIBA Fluorolog-QM equipped with a pulsed microsecond Xe lamp and an integrating sphere to determine both the phosphorescence decay and the gated phosphorescence spectrum of a sample. In addition, with the time gate positioned over the excitation pulse time range, a spectral scan is performed across the Rayleigh peak for both the sample and the blank. Using the results of the RTP decay fit analysis, the integrated intensity of the time-resolved phosphorescence is then extrapolated to the onset time of the excitation pulse. This approach makes the standard integrating sphere calculation formula for the quantum yield applicable to the results obtained from the time-gated experiment. The SSTD/PLQY method was applied to determine the RTP quantum yield of a deoxygenated solution of human serum albumin (HSA) protein, as well as crystalline samples of BN-substituted xanthene derivatives.

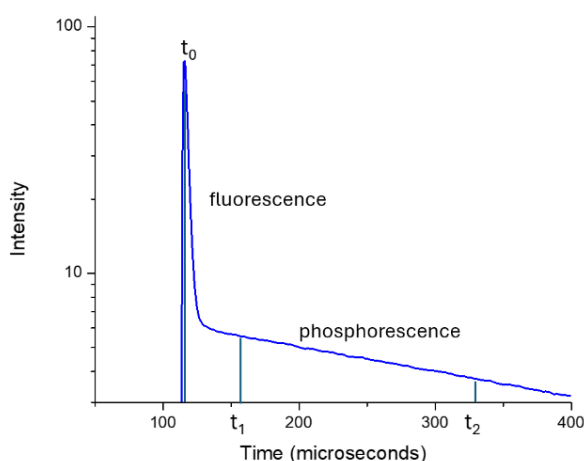


Figure 1. Gating out fluorescence and integrating phosphorescence when both emissions spectrally overlap

Methodology

The measurements were carried out using the SSTD mode of the Felix FL software, which controls all functions of the Fluorolog-QM spectrometer, and provides analytical tools needed to perform the required calculations. The QM-400 Phos-pulsed Xe lamp accessory was used as the light source. The SSTD mode controls the repetition rate of the lamp (2-1000 Hz, user selectable), records a complete decay after each pulse and averages individual decays over a user-specified number of pulses. When operated in the time-resolved emission spectral mode (TRES), the software scans the emission monochromator while collecting a complete decay, and integrates it over the user-selected time window (t_1 , t_2) at each wavelength (Fig. 1).

In order to determine the phosphorescence quantum yield of a sample with a strong fluorescence interference, the following protocol was used:

1. The sample decay was measured at a phosphorescence wavelength and the RTP lifetimes and pre-exponential factors were determined from a multi-exponential tail fit using the PowerFit decay analysis package of Felix FL. The fitting range should exclude any fast fluorescent component in the decay.
2. Based on the decay curve, an appropriate time gate range (t_1 , t_2) for TRES phosphorescence acquisition was selected (Fig. 1).
3. The sample was placed in the integrating sphere and its phosphorescence TRES measured within the (t_1 , t_2) time gate.
4. The TRES of the sample was subsequently measured across the excitation peak with an earlier time gate (t_{ex1} , t_{ex2}) which included the IRF. Neutral density filters with known transmittance were used to avoid signal saturation.
5. The blank sample was then placed in the sphere and steps 3 and 4 were repeated.

A 'partial' quantum yield (Φ_1) can be calculated from the collected TRES traces using the standard Quantum Yield Calculator function for the integrating sphere in Felix FL. Φ_1 represents only that fraction of RTP intensity which is contained within the integration time gate (t_1 , t_2). Knowing the RTP lifetimes and pre-exponential factors, the total RTP quantum yield Φ can be calculated by integrating the RTP

decay from t_0 (the onset of the excitation pulse determined by the peak of the IRF) to ∞ , then integrating the RTP decay within the (t_1, t_2) time gate and calculating the ratio of the two areas, i.e.:

$$(1) \quad R = \frac{\int_{t_0}^{\infty} D(t-t_0)dt}{\int_{t_1}^{t_2} D(t-t_0)dt}$$

where $D(t)$ is the RTP decay function based on the fit parameters determined in step 1. The total RTP quantum yield Φ is thus given by

$$(2) \quad \Phi = R\Phi_1$$

Results

Instrument Verification

First, the Fluorolog-QM spectrometer and the sphere were tested with a rhodamine 101 PLQY standard using the 75W Xe lamp excitation in the steady-state mode. The verification test yielded $\Phi = 0.92 \pm 0.03$ being in good agreement with the expected value of 0.93 ± 0.05 and thus ensuring that the instrumental setup has proper calibration and emission correction factors.

Method Validation

As the next step, the correctness of the SSTD/PLQY method was tested with an aqueous solution of terbium chloride. The Tb^{3+} solution exhibited a single-exponential decay with a lifetime of $430 \mu s$, thus ensuring it represented a single emitting species with no detectable short-lived contaminants (Fig. 2).

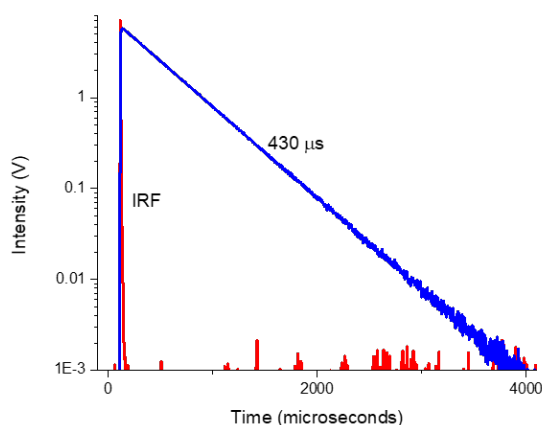


Figure 2. PL decay of Tb^{3+}

Therefore, it was expected that the PL quantum yield of Tb^{3+} measured with the conventional steady-state setup should yield the same result as the quantum yield determined by the SSTD/PLQY with the pulsed Xe lamp, and thus will prove the equivalence of the two methods.

The quantum yields from both methods produced very similar results: $\Phi = 6.1 \pm 0.4\%$ (steady-state) and $6.3 \pm 0.5\%$ (SSTD). The SSTD quantum yield was obtained by scaling the result obtained from direct integration of the time-gated Tb^{3+} spectrum (Fig. 3) by a factor $R = 1.217$, which accounts for extrapolation of the decay to the peak of

IRF at $t_0 = 115 \mu s$ (Fig. 2) as described in the Methodology section. The quantum yields obtained are in a reasonable agreement with the literature value of $5.3 \pm 0.6\%$ ^[1].

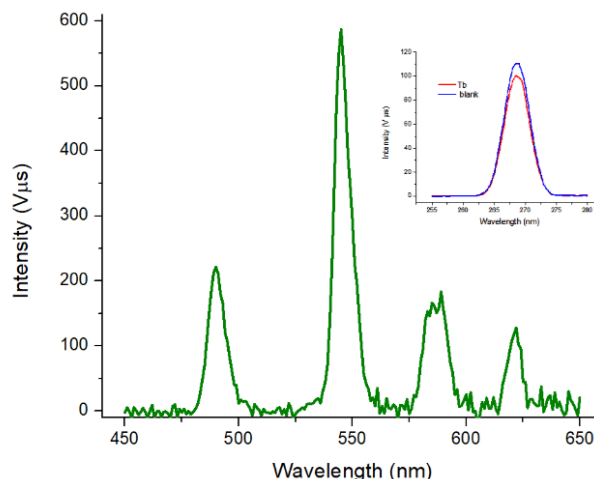


Figure 3. Time-gated Tb^{3+} emission and gated scans across the excitation peak (insert) with SSTD method

RTP Quantum Yield of Human Serum Albumin

RTP of proteins has been used since the 1970's to study conformational transitions, folding-unfolding and changes in local tryptophan environments on a microsecond to second timescale. Human serum albumin (HSA) has a single tryptophan residue (Trp-214), located within the rigid hydrophobic core, which offers some protection against triplet quenching by solvent molecules and molecular motions. Two main obstacles in detecting RTP are oxygen quenching of the triplet state, and the dominance of tryptophan fluorescence, which totally obscures the phosphorescence spectrum (Fig. 4). While oxygen can be removed by bubbling inert gas through the solution or, preferably, using a chemical O_2 scavenger, any spectral/intensity measurements of protein RTP require the use of time-resolved methods which gate out the unwanted short-lived fluorescence. Due to the fluorescence interference, it is practically impossible to use a standard steady-state methodology to measure the basic photophysical property, such as the RTP quantum yield.

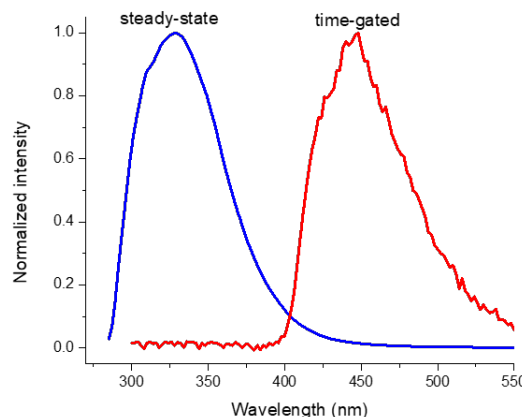
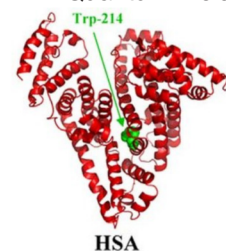


Figure 4. Steady-state and time-gated (0.3 – 10 ms) emission spectrum of deoxygenated HSA (ex= 280 nm)

Figure 4 shows a comparison between the steady-state and time-gated emission spectra of HSA over the combined fluorescence and phosphorescence spectral range. The steady-state spectrum does not exhibit any discernable phosphorescence, while the time-resolved measurement with the pulsed Xe lamp excitation and the 0.3-10 ms time gate clearly detects the long-lived RTP without any fluorescence interference. The time gate was selected based on the HSA phosphorescence decay measured at 450 nm (Fig. 5). The lifetime analysis resulted in 3 vastly different phosphorescence lifetimes reflecting the conformational diversity of the protein.

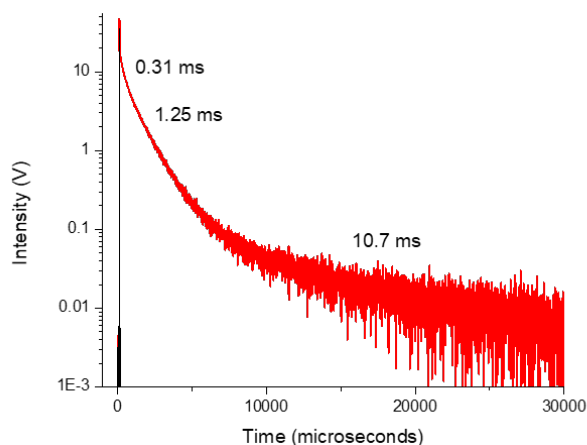


Figure 5. Phosphorescence decay of deoxygenated HSA ($\lambda_{\text{ex}} = 280 \text{ nm}$) resulting in 3 lifetimes: 0.31 ms (51.6%), 1.25 ms (47.7%) and 10.7 ms (0.7%)

To determine the RTP quantum yield for HSA, the integration time gate of 0.3 to 10 ms was set and the time-resolved spectrum measured in the QuantaPhi-2 integrating sphere (Fig. 6).

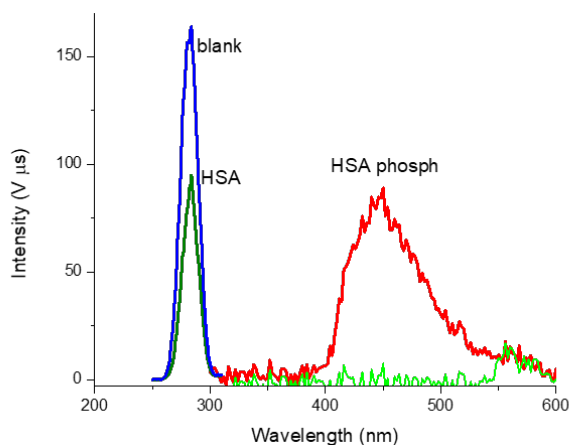


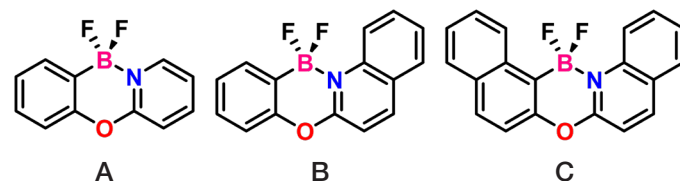
Figure 6. Gated phosphorescence emission scans with 0.3-10 ms time gate and scans across the excitation peak with 100-150 μs time gate (calibrated ND filters were used for scans across the excitation peak)

Subsequently, the gating was changed to the 100-150 μs range and the time-resolved spectrum was acquired across the excitation peak for both the HSA sample and the blank. Based on the results, a 'partial' quantum yield within the selected time gate for the emission, was calculated as $\Phi_1 = (7.3 \pm 0.3) \times 10^{-3} \%$. Using Equation 1 and the results of the HSA RTP decay analysis, the time-gate scaling factor was

calculated ($R = 1.28$) and applied to Equation 2 resulting in total quantum yield of $\Phi = (9.4 \pm 0.4) \times 10^{-3} \%$.

RTP Quantum Yield of BN-Substituted Xanthene Derivatives

Three xanthene derivatives in crystalline form were used in this RTP study^[2]. There are two main factors in generating organic RTP: populating the triplet state efficiently (high k_{ISC}), and limiting non-radiative decay of the triplet state (low k_{nr}). The molecular structures of the BN-substituted xanthene derivatives **A**, **B** and **C** were designed to address both challenges.



First, the presence of heteroatoms with non-bonding lone pairs (O, F) is known to increase the n character of the excited triplet state, which leads to increase in k_{ISC} via enhanced spin-orbit coupling in accordance with El-Sayed's rule^[3]. Additionally, the BF_2 functional groups produce several $\text{C-H}\cdots\text{F}$ interactions in each derivative leading to enhanced rigidity of the molecules in the triplet state, which is expected to reduce quenching by thermal motion.

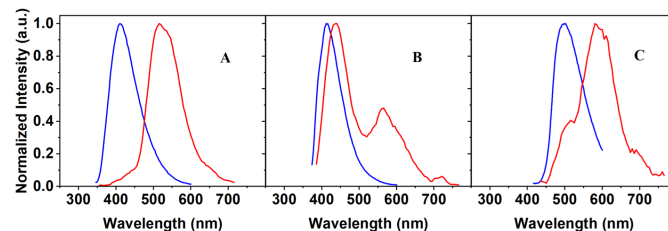


Figure 7. Steady-state PL (blue) and time-resolved RTP spectra with 3 ms time delay (red) of xanthene derivatives.

The steady-state PL data show no sign of phosphorescence, while the time-resolved measurements with 3 ms time gate delay result in distinct phosphorescence peaks in the 500-700 nm range for all three xanthene samples (Fig. 7). In addition, sample B shows a long-lived emission band at 440 nm, most likely the delayed fluorescence.

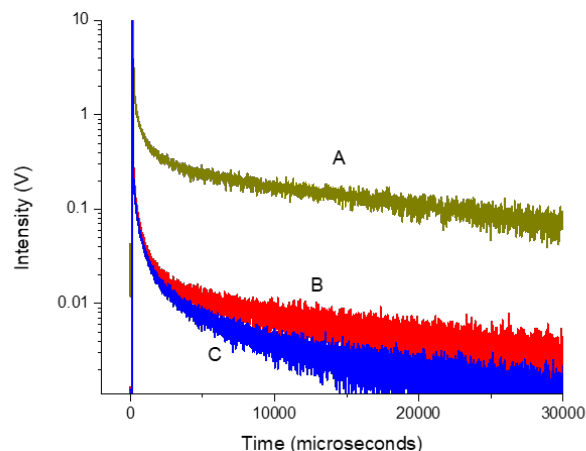


Figure 8. PL decays of crystalline xanthene derivatives

PL decays are presented in Fig. 8, and the results of their lifetime analysis are included in Table 1. All samples required a 2-exponential fit. Quantum yield measurements and calculations were performed using the protocol described in the Methodology section with the integration time gate set at 3 ms after the pulse. The final RTP quantum yield results are included in Table 1. The results suggest that expanding the π -electron ring structure substantially reduces RTP efficiency. This is likely caused by additional vibrational modes in larger structures, which enhance the non-radiative relaxation from the excited singlet state, and compete with the ISC.

Sample	τ_1 , ms	τ_2 , ms	Φ (%)
A	2.87 (21.9%)	24.5 (78.1%)	1.3
B	2.83 (34.4%)	27.8 (65.6%)	0.32
C	2.73 (54.2%)	192 (45.3%)	0.15

Table 1. RTP lifetime and quantum yield results

Conclusion

The Fluorolog-QM is a very useful and powerful platform for determining absolute quantum yields of room temperature phosphorescence, which is often spectrally dominated by much more intense fluorescence. The SSTD mode, a unique feature of the Fluorolog-QM, collects a full sample decay with each excitation pulse, can rapidly accumulate and average a large number of decay traces, and at the same time allows for direct measurement of time-gated spectra, which can eliminate any contamination from sample and background fluorescence, as well as from scattered and stray light. The ability to gate out all these unwanted emissions and artifacts is especially advantageous when using an integrating sphere, which practically captures all scattered and stray light and often prevents measurements of low quantum yields when used in the standard steady-state mode. In this note, with the use of the SSTD/PLQY technique we were able to report RTP quantum yields as low as ~0.01%.

Acknowledgement

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