Investigation of Fluorescence Lifetime Quenching of $Ru(bpy)_3^{2+}$ by Oxygen Using a Pulsed Light-Emitting Diode

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The measurement of fluorescence lifetime is a valuable experiment for an undergraduate instrumental analysis or physical chemistry laboratory because it highlights relative rates of electronic transitions in molecules and introduces students to data collection of a pulsed, as opposed to continuous, signal. The experiment has several advantages over the more common measurement of fluorescence intensity. Specifically, lifetime measurements are not sensitive to changes in excitation intensity, optical alignment, or detector gain. However, lifetime measurement is more complicated than intensity measurement because it requires triggered data acquisition at MHz sampling frequencies. It also typically employs a pulsed laser or flashlamp *(1),* which introduces additional complexity and safety issues. Two experiments in which the lifetime of I_2 vapor was investigated have appeared previously in this *Journal (2, 3).* One of these used an Nd:YAG for excitation, the other used a N_2 laser to pump a dye. In contrast, our experiment is done with aqueous solutions and uses a light-emitting diode as an excitation source. The long fluorescence lifetime of $Ru(bpy)_3^{2+}$, around 600 ns, allows data to be collected on a modest oscilloscope.

Oxygen is a well-known quencher of fluorescence. Previous qualitative studies of fluorescence quenching by oxygen using steady-state fluorescence intensity measurements have appeared in this *Journal (4, 5).* In contrast, our experiment is quantitative (an O_2 electrode monitors $[O_2]$ as sodium sulfite is added to the solution) and pulsed (lifetime is measured rather than intensity). Using sodium sulfite to remove O_2, allows us to vary $[O_2]$ from an initial value of ~280 μM to a final value of ~25 μM.

Background

The fluorescence lifetime is the quantity of time required for the fluorescence intensity to decay to 1/e of its initial value following an excitation pulse. Measurement of this lifetime can yield data of interest to the physical or analytical chemist. Specifically, because dynamic quenching reduces this lifetime, fluorescence lifetime can be used to measure collision rates. Similarly, measurements of the lifetime can yield information about variables such as temperature, pH, polarity, $[O_2]$, and viscosity *(6–8).*

Diatomic oxygen, a ground-state triplet, is a particularly efficient quencher of fluorescence. In dynamic quenching by oxygen, an excited-state fluorophore collides with a triplet-state O_2 molecule to produce the ground-state fluorophore and a low-energy excited singlet O_2 *(4).* We should point out that the luminescence of the $Ru(bpy)_3^{2+}$ used in this experiment arises from a ligand-to-metal charge-transfer in which the emitting state has some degree of triplet character. However, owing to the high atomic number of Ru, these emitting states can be described as spin-orbit states rather than triplets or singlets *(9).* Thus, there has been discussion of whether the light-emitting process should be termed "fluorescence", "phosphorescence", or simply "luminescence". Fluorescence and luminescence are the terms that appear most often in the literature.

A complete discussion the "log slope" method of fluorescence lifetime determination, derivation of the Stern–Volmer equation, and calculation of the diffusion-controlled bimolecular rate constant appears in the Supplemental Material.[W] Briefly, fluorescence lifetimes can be determined from the slope of plots of natural log of fluorescence intensity versus time. Stern–Volmer plots (τ_0/τ vs [Q]) yield the quenching rate constants, and diffusion-controlled bimolecular rate constants are calculated using the Stokes–Einstein equation.

Commonly, quenching rate constants are less than the calculated diffusion-controlled bimolecular rate constants. In aqueous solutions at room temperature the bimolecular collision rate is about 10^{10} M^{-1} s^{-1} *(10).* If all collisions result in quenching, the quenching rate constant approaches this value, but steric and energetic considerations usually lower the quenching rate constant significantly.

Experimental

To achieve fast LED pulses, a simple electronic circuit, shown and described in the Supplemental Material,[W] is constructed. The circuit, described first by O'Hagan et al. employs a 5-V power supply, a function generator, and two integrated circuits—an AND gate and an inverter *(11).*

A block diagram of the experiment is shown in Figure 1. We use a 470-nm LED (Radio Shack #276-316) to excite

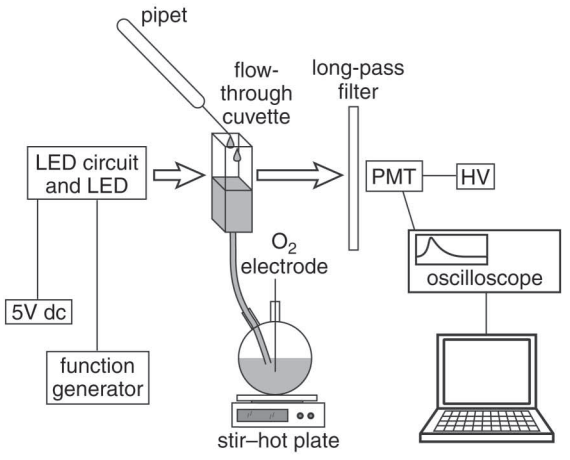

Figure 1. The experimental setup. The dye solution in the round-bottom flask is transferred to and from the cuvette by using a pipet bulb. In this way, solution of known oxygen concentration can be transferred quickly with minimal agitation.

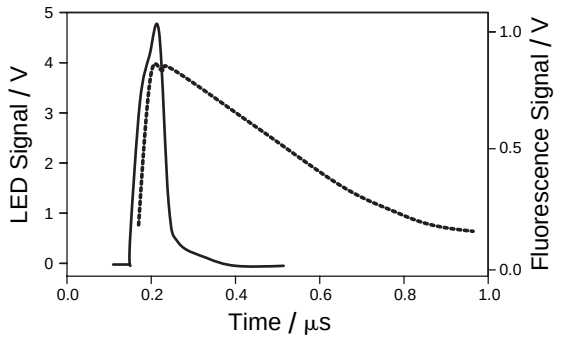

Figure 2. The temporal profiles of the LED pulse and the $Ru(bpy)_3^{2+}$ fluorescence. The fwhm of the LED pulse is 80 ns. The lifetime of the $Ru(bpy)_3^{2+}$ is much longer.

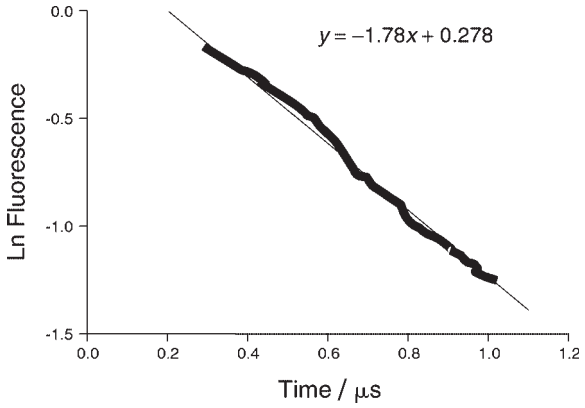

Figure 3. Natural log of fluorescence versus time and the linear fit. The slope is equal to $-1/\tau$. The calculated lifetime and associated error (standard deviation of the lifetime for seven measurements of the same sample) is 562(±16) ns.

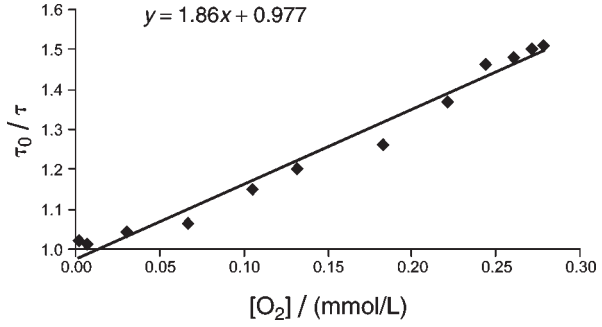

Figure 4. The Stern–Volmer plot for lifetime quenching of $Ru(bpy)_3^{2+}$ by oxygen. The quenching rate constant, given by slope/τ_0, is 3.31 x 10^9 M^{-1} s^{-1}.

$Ru(bpy)_3^{2+}$. The fwhm of the pulses produced is 80 ns. A long-pass filter (Edmund Optics #NT46-063) is used to remove the excitation wavelength, and the fluorescence signal is detected with a photomultiplier (Hamamatsu R928). The signal is coupled at 50 Ω to an oscilloscope (Tektronix TDS 320). The oscilloscope triggers on the rising edge of the square wave output from the function generator. No lenses are used; the LED and the photomultiplier are placed in close proximity to the dye cuvette.

A solution of 10 μM $Ru(bpy)_3^{2+}$ is made using distilled water in a double-necked 100-mL round-bottom flask. An O_2 electrode (Orion #97-08-00) is placed in one arm of the flask and is used to measure the oxygen concentration of the dye solution continuously. One end of a flow-through cuvette is connected to a pipet that can be used to draw solution from the other neck of the flask. The other end of the cuvette is connected to a pipet bulb. In this way, dye solution of known oxygen concentration can be transferred into the cuvette and returned to the flask quickly and with little agitation.

The oxygen concentration of prepared dye solution is always close to 8 mg/L. By adding dropwise 1×10^{-4} M Na_2SO_3 solution to the round-bottom flask, we can slowly decrease the oxygen concentration as we acquire the lifetime data. Typically we acquire 10–15 data points between 8 mg/L and 0.1 mg/L. The total time required to acquire the data is about 30 minutes.

Hazards

Sodium sulfite and tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate may cause irritation of eyes, skin, digestive tract, and respiratory tract. The toxicological properties of tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate have not been fully investigated. The power supply used to bias the photomultiplier produces high voltage.

Results and Discussion

The LED pulse profile and the fluorescence signal are shown in Figure 2. A plot of the natural log of fluorescence intensity versus time and the linear fit is shown in Figure 3. The fluorescence data acquired before the end of the excitation pulse are removed from this plot so that the fit is not affected. The lifetime in units of μs is given by −1/slope. For the data depicted in Figure 3, the lifetime is −1/−1.78, or 0.562 μs.

A Stern–Volmer plot generated from data taken at different oxygen concentrations is shown in Figure 4. Here, the Stern–Volmer constant is 1.86×10^3 M^{-1}, and the corresponding quenching rate constant is 1.86×10^3 $M^{-1}/0.562$ μs = 3.31×10^9 M^{-1} s^{-1}. Propagating the error associated with the constant and the lifetime for a series of seven measurements gives $1.8(\pm0.1) \times 10^3$ $M^{-1}/0.562(\pm0.016) = 3.3(\pm0.2) \times 10^9$ M^{-1} s^{-1}. This value is consistent with other values obtained for quenching of $Ru(bpy)_3^{2+}$ by oxygen in water. Specifically, Demas et al. obtained a value of 3.2×10^9 M^{-1} s^{-1} in a steady-state experi-

ment *(12)*. For comparison, the calculated diffusion-controlled bimolecular rate constant is $7.4 \times 10^9\ M^{-1}\ s^{-1}$.

Students enjoy using the PMT–oscilloscope to make fast optical measurements and are impressed with the quality of the data achieved in a relatively simple setup. Typically, the Stern–Volmer plots give quenching rate constants that are slightly lower than the calculated diffusion-controlled bimolecular rate constant; students are asked to offer potential explanations. Lifetime quenching as a function of temperature could easily be observed in a nearly identical setup.

[w]Supplemental Material

Instructions for the students, including sections on theory, the circuit, the instrument, and procedure, are available in this issue of *JCE Online*.

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