

Halide (Cl⁻) Quenching of Quinine Sulfate Fluorescence: A Time-Resolved Fluorescence Experiment for Physical Chemistry

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The physical chemistry laboratory is a good place to introduce the latest instrumentation. However, because the physical chemistry curriculum includes important classical topics, such as kinetics, it is important not to exclude experiments related to these topics. The ideal solution is to introduce experiments using modern techniques that incorporate classical concepts. Time-resolved laser-induced fluorescence experiments meet these criteria. They incorporate modern equipment (lasers, computers, and fast data collection) and research topics (e.g., time-dependent spectroscopy), while the time-dependent behavior can still be analyzed and explained in terms of simple kinetic processes. This article describes an experiment investigating the halide quenching of fluorescence from quinine sulfate in water. Quinine is safer and easier to handle than most samples used in similar experiments.

Other time-resolved fluorescence experiments used in undergraduate laboratories range from those using fluorescein mercuric acetate glasses (1) and acridine yellow in glasses (2) at room temperature, where the decays can be monitored on a time scale of seconds, to 2-acetylnaphthalene in water, where the fluorescence lifetime, τ , is approximately 2.0 ns (3). Other systems that have been used include *N*-methylacridinium iodide ($\tau \approx 35$ ns) (3), 1-pyrenesulfonic acid ($\tau \approx 59$ ns) (3), pyrene in hexane and tetradecane ($\tau \approx 400$ ns) (4), pyrene in cyclohexane (τ in 400–500 ns region)

(5), and Ru(II) complexes ($\tau \approx 1.1$ ms) (6). In contrast to these systems quinine is stable in acidic water solutions for at least a year and the fluorescence is not quenched by oxygen, thus the samples do not need to be degassed. Because of the ease of handling the system up to nine students can complete the experiment in one three-hour laboratory using a single fluorescence apparatus, and all students get a chance to use the equipment.

Although sophisticated equipment is required for time-resolved spectroscopy, the quenching may also be investigated without time resolution. Information in the Supplemental Materials^W and refs 7 and 8 give brief descriptions of the kind of analyses that can be done without time resolution. Fraiji et al. (3) give a more thorough discussion of fluorescence intensity measurements and Stern–Volmer analyses (9).

The objectives of this experiment are to: (i) reinforce student understanding of the kinetics of competing pathways; (ii) enhance the student understanding of electronic spectroscopy and the temporal behavior of quantum states; and (iii) make connections with microscopic theories of kinetics through comparison of experimental and theoretical rates of bimolecular reactions in solution. The kinetics of competing pathways and the temporal behavior of quantum states are observed in time-resolved fluorescence decays. Static fluorescence spectra and absorption spectra allow comparison of absorption and emission from closely related states. The collisional quenching rate measured in the experiment can be compared to the theoretical value for diffusion-limited processes.

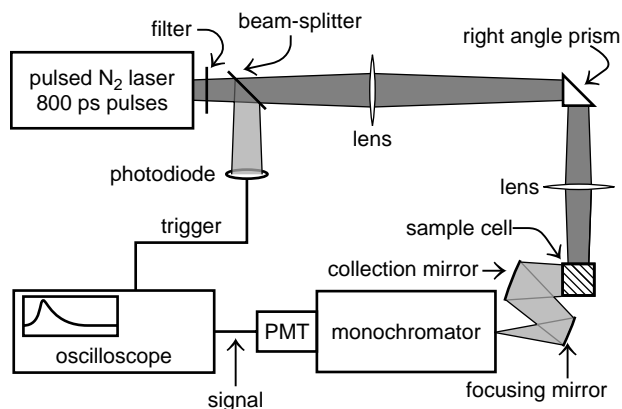


Figure 1. Block diagram of the time-resolved fluorescence apparatus. All optics are fused silica to limit fluorescence from the optics. A UG11 filter is used to block light other than 337 nm produced by the laser.

Experimental and Equipment Needs

The apparatus used to collect fluorescence decays is shown in Figure 1. The excitation source is a pulsed N₂ laser (800 ps pulses, 337 nm, Laser Photonics LN1000). A small portion of the laser beam is reflected off a quartz window to a fast photodiode (Laser Photonics L-0T) to trigger a digital oscilloscope (Tektronix TDS 744). The sample in a 1-cm quartz fluorescence cuvette is placed in a PTI Alphascan fluorescence spectrometer and the laser beam is directed to the sample through an access port. The fluorescence is collected at 90° to the incoming laser light and resolved using the spectrometer's monochromator set to 480 nm (bandpass of 1–2 nm). The photomultiplier tube (Hamamatsu 928, rise time of 2.5 ns) is housed in a PTI 712 housing with an integral power supply. The raw signal terminated in 50 Ω is averaged over 2400 shots (~2 minutes at 20 Hz) using the oscilloscope's averaging function. After (or before) each decay is collected a background is collected by averaging 2400

shots with the fluorescence blocked. This background is subtracted from the signal to remove the large oscillations caused by radio-frequency (RF) interference from the laser. Some residual RF interference may be seen in the baseline of Figure 2 prior to the rise in the fluorescence. Continuously excited fluorescence spectra are collected using the PTI spectrofluorimeter with an arclamp light source tuned to 337 nm. Absorbance spectra of the solutions are also collected to verify that there is negligible change upon addition of quencher.

This experiment can be performed with a less expensive apparatus. If only fluorescence intensity measurements are to be made the nitrogen laser may be replaced with a continuous UV light source (see the Supplemental Material¹³ and refs 3 and 8). Otherwise, an excitation light source that produces UV pulses with a duration of less than 1 ns is a necessity. At present, N₂ lasers are the least expensive option. The monochromator and collection optics can be replaced by fused silica lenses (Newport Corp. is a good source) and a 480 nm bandpass filter (Omega Optical is a good source). Any room temperature photomultiplier housing and high-voltage power supply may be used. Instead of a digital oscilloscope the data can be collected with a gated boxcar integrator (Stanford Research Systems is a good source). Data collection takes significantly longer using a boxcar because data are collected at only one time delay on each laser shot.

The solutions consist of protonated quinine in water with various quantities of quencher added. Each student prepares and uses one sample; the data are pooled for analysis. The quencher may be any halide ion, but in this experiment Cl⁻ in the form of KCl was used. These solutions may be disposed of down the drain.

Hazards

The major hazards of this experiment are associated with the laser and photomultiplier tube systems. Both use kV power supplies that can produce lethal shocks. The laser produces high intensity invisible radiation. The laser beams should be below eye level and contained within nonreflective walls. All people in the room should wear approved safety goggles that do not allow the UV light to pass. The laser can produce significant quantities of ozone if the N₂ is contaminated with O₂; thus the system should only be used in a well ventilated room.

The reagents used in this experiment are relatively benign. All the solutions may be disposed of down the drain. The stock quinine/sulfuric acid solution the students are provided is high enough concentration that it can cause acid burns if not washed off the skin reasonably quickly, but will not cause instantaneous burns.

Results

The primary task for the students is to decide whether the Cl⁻ ions quench the quinine fluorescence by collisionally deactivating the excited fluorophore (commonly referred to as “dynamic” quenching) or by forming a nonfluorescing complex with the ground-state quinine (commonly referred

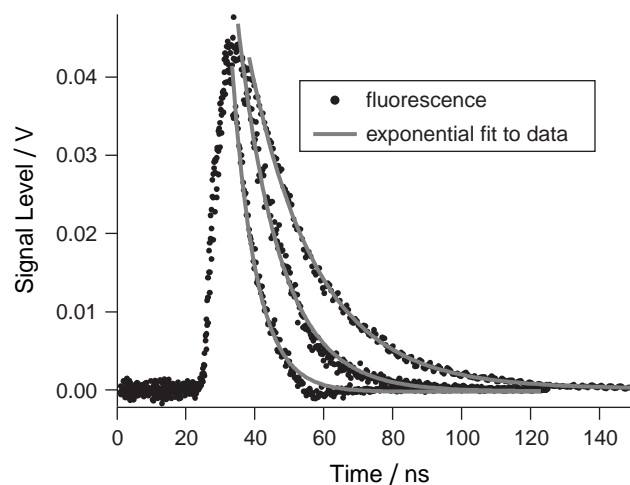


Figure 2. Some of the fluorescence decays collected in one class. The faster decays are for samples of higher chloride ion concentration. The exponential fits to the decays are shown as smooth solid curves. The monochromator slits were adjusted for every sample to keep the maximum intensity about the same. Notice that the single exponential does not fit the faster decay very well.

to as “static” quenching). This is not a question that can be answered using simple fluorescence intensity measurements (3). Thus it is necessary to collect the time-resolved decays of the fluorescence.

A kinetic analysis (see the Supplemental Material¹³) of the two situations leads to the following expressions for the concentration of excited fluorophore, [F*], versus time, *t*, after the laser pulse ends:

dynamic quenching

$$[F^*] = [F^*]_i \exp\left\{-\left(k_f + k_q[Q]\right)t\right\} \quad (1)$$

static quenching

$$[F^*] = [F^*]_i \exp(-k_f t) \quad (2)$$

where k_f is the fluorescence decay rate (also including the non-radiative decay rate), k_q is the collisional deactivation rate constant, [Q] is the quencher concentration, and $[F^*]_i$ is the excited fluorophore concentration when the laser pulse ends. $[F^*]_i$ decreases with increasing [Q]. Both are first-order exponential decays, but in the dynamic case the observed decay rate is linearly dependent upon the quencher concentration. Since the fluorescence intensity is proportional to $[F^*]$, students fit the fluorescence decays for each solution to single exponentials (Figure 2). Note that the faster decays do not fit an exponential well. This is because the instrument cannot respond quickly enough. This can be corrected for by fitting the decays to an exponential convoluted with

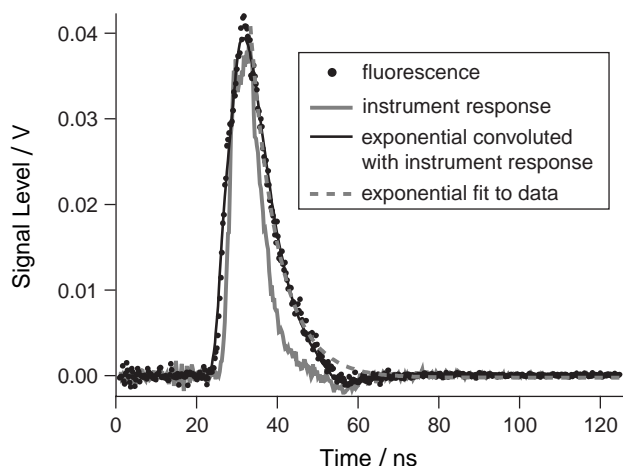
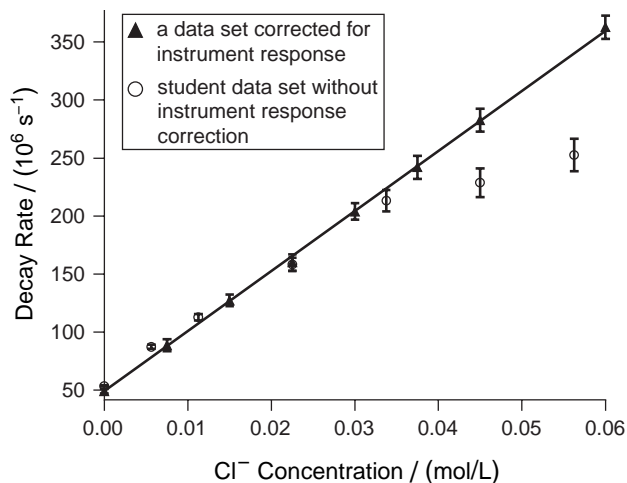


Figure 3. Comparison of a fit to a single exponential decay and an exponential decay convoluted with the instrument response function. Notice that it is even possible to fit the signal rise quite well when the instrument response is taken into account. The instrument response was collected as fluorescence at 480 nm from a sample quenched with enough Cl⁻ to reduce the lifetime to less than 200 ps ([Cl⁻]⁻¹ M).

the instrument response function (Figure 3), see the instructor notes in the Supplemental Material¹¹ and ref 5 for more discussion of convolution. A plot of the fluorescence decay rate versus the quencher concentration suggests the quenching is dynamic, since the lifetime decreases with increasing [Q].

Plots of the decay rate versus chloride ion concentration for a student data set that was fit to single exponentials and a data set that has been corrected for the instrument response are shown in Figure 4. Except for the two data points at the highest chloride concentration the uncorrected data fall almost on the line given by the corrected data. The “roll off”



of the last two data points reflects that the decay rate is approaching the response time of the instrument (~2.5 ns). The unquenched lifetime predicted from the corrected data set (the inverse of the *y* intercept) is 20.4 ± 0.5 ns (95% confidence limit). From the uncorrected data set the value is 18.5 ± 0.4 ns (95% confidence limit). The literature value is: 19.3 ± 0.6 ns (95% confidence limit) (10–12).

The slope of the line in Figure 4 corresponds to k_q and is equal to $(5.2 \pm 0.1) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. This is a little lower than the theoretical range, $5.8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ to $6.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, expected if all collisions lead to quenching. Theoretical values were calculated from

$$k_D = \frac{8RT}{3\eta(T)} \quad (3)$$

where k_D is the rate constant for diffusion, R is the gas constant, T is the absolute temperature, and $\eta(T)$ is the solvent viscosity of 0.5 M H₂SO₄, which in general drops as temperature increases (13, 14). The measured value is still close enough to suggest that quenching is quite efficient. Once corrected for the higher viscosity in this experiment these results are essentially the same as the literature value of $6.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ determined in 0.05 M H₂SO₄ (10).

Summary

The halide ion quenched fluorescence of quinine in acidic solutions has proven to be an ideal experiment for use in physical chemistry laboratory. The experiment is popular with the students. The samples are easy to handle. It provides the students experience with modern equipment (lasers, fast data collection, and computer data analysis), while also requiring the students to review and use what they know about the kinetics of competing processes and electronic spectroscopy. All students get a chance to use the equipment in a three-hour laboratory. A second three-hour period is used to assist the students while they perform the computer data

Figure 4. Plot of decay rate versus chloride ion concentration. The line is a linear least-squares fit to the convoluted data with the points weighted based on the errors in the decay rates. Error bars on the data from single exponential fits are ± 1 s. Error bars on the convoluted data set are estimated as the maximum range of decay rates that give residuals showing only noise.

analysis. The results are good, yielding unquenched lifetimes and collisional quenching rates close to literature values.

Acknowledgments

The author wishes to thank all University of Wisconsin–Oshkosh physical chemistry students who have done this experiment; William Wacholtz for helping set up the apparatus and providing useful commentary; Jennifer Mihalick for useful commentary and for testing this experiment with her physical chemistry students. The student data set presented in this summary was collected by Matthew Dudley, Matthew Lowther, and Scott Omachinski. The chemistry department at the University of Wisconsin–Milwaukee also helped by allowing use of their laser equipment for preliminary development of this experiment.

^WSupplemental Material

Detailed instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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STUDENT HANDOUT:

Laser Induced Fluorescence of Quinine Sulfate and the Kinetics of Cl⁻ Induced Quenching of the Fluorescence

by J. Gutow 1/95; revised 1/96-JG, 4/97-JG, 4/00-JG, 5/03-JG, 12/03-JG

Prelab Reading Assignment

Shoemaker: analog-to-digital conversion (pp. 621-624), oscilloscopes (pp. 722-725), spectroscopic instruments (pp. 754-758), wavelength selection (pp. 743-747), radiation detectors/PMTs (pp. 747-750).

Introduction

In this experiment you will be concerned with the decay kinetics of the fluorescence of quinine sulfate in water solutions and the physical processes that effect these. You may be familiar with this system from analytical chemistry. Quinine is used as a fluorescence standard because the fluorescence is not quenched (decreased) by oxygen but can be quenched in a controlled manner by adding atomic anions (F⁻, Cl⁻, I⁻ and Br⁻) to the solution.¹

Many molecules re-emit some of the light energy they have absorbed as light, but delayed in time and at a different energy than the absorbed light. This is usually referred to as fluorescence or phosphorescence. As shown in figure 1, fluorescence is the emission of light by a molecule in the same electronic state it was initially photo-excited into (usually S₁), whereas phosphorescence is the emission of light from a molecule in a different electronic state (usually T₁). Phosphorescence usually has a longer average time between photon absorption and emission than fluorescence. For a more detailed discussion of these phenomena see any standard p-chem text.^{2,3}

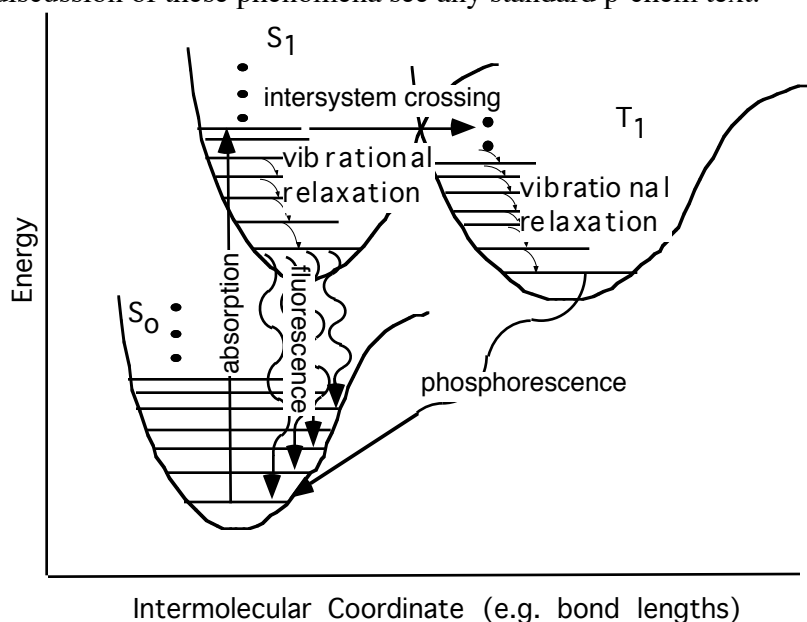


Figure 1: Electronic transitions of fluorescence and phosphorescence.

In this experiment you will use an N₂ gas laser system to excite the quinine molecules. An advantage of lasers over typical arc-lamp monochromator combinations is that lasers produce much more intense light, allowing you to excite more molecules thus increasing the fluorescence and making it easier to detect. Also the N₂ laser produces very short pulses of light, <10⁻⁹ sec in

duration, which means the excitation light is on for a short time compared to the fluorescence lifetime of many molecules, $\sim 20.0 \times 10^{-9}$ sec for quinine in water solutions at room temperature. Because of these advantages lasers have made it possible to use fluorescence for remote analysis of gases. One system, called LIDAR, focuses a powerful pulsed laser through a telescope at a remote point, such as the top of a smoke stack, and monitors the concentrations of gas at that point through the telescope by measuring the laser induced fluorescence (LIF) spectrum.⁴

Kinetics of Fluorescence Quenching

Basic Kinetics Observed

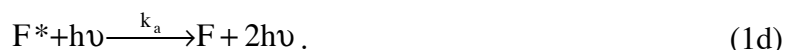
The sample consists of a solution of quinine in water, with a little acid and salt impurities. The acid keeps the quinine protonated so that it fluoresces more strongly. Your primary task is to decide whether the Cl^- ions from the salt quench the quinine fluorescence by collisionally deactivating the excited fluorophore (commonly referred to as “dynamic” quenching) or by forming a nonfluorescing complex with the ground state quinine (commonly referred to as “static” quenching). This is not a question that can be answered using simple fluorescence intensity measurements.⁵ Thus it is necessary to collect the time resolved decays of the fluorescence.

Because they are adequate to describe the observed results we will only consider the simplest kinetic models possible for dynamic and static quenching. For simplicity we will represent the unexcited quinine by the capital letter ‘F’, the excited quinine by ‘F*’, the quenching anion by a capital ‘Q’, the exciting light photon by ‘ $h\nu$ ’ and the fluorescence photon by ‘ $h\nu_f$ ’. Their concentrations will be represented by the symbol in square brackets.

In the case of dynamic quenching, the quinine is excited at some rate by the light and then either relaxes by giving off a photon or by colliding with the quencher and relaxing without fluorescing. This simple kinetic mechanism is shown below:



The nonradiative decay rate (transition to the ground state S_0 without emitting a photon) is missing from this mechanism. It has been absorbed into k_f because the experiment cannot differentiate between the two. Another thing ignored in this simple scheme is stimulated emission. This becomes important when the light intensity is so high that the rate of excitation, $k_a[h\nu]$, is large compared to k_f . Then the process of stimulated emission becomes an important route for returning F to its ground state. This is represented in equation 1d below, but will be assumed unimportant for the analysis that follows.



You should be able to show that at high light intensity the combination of equations 1a and 1d, leads to the conclusion that the transition can be saturated. What is the maximum fraction of F that can be in the excited state if the light intensity is high? For more details look up Einstein coefficients in any p-chem^{2,3} or spectroscopy text.

From these equations we can now write the differential rate laws. Although many people write the rate law for equation (1a) by absorbing the number of photons available into the rate constant k_a , we will not do this to keep the intensity dependence explicit. This way the photons can

be treated as just another reagent. To avoid confusion with the use of “I” representing intensity (power in W/m²) in Beer’s law, we will use [hν] to represent the concentration of photons in moles/L. This convention is commonly used by people studying laser induced chemistry.⁶ At low light intensity and high light intensity the appropriate value for [hν] is directly proportional to the mean average of the light intensity across the sample. In the intermediate range where the intensity is only high enough to cause saturation of the transition part way across the sample the relationship is much more complicated. If you are interested in the details of I versus [hν] consult your instructor.

At any instant one can calculate an effective photon concentration by dividing the number of photons in the cell by the volume of the cell filled with light (often called the excitation volume). In this representation the rate constant $k_a = c\alpha(\nu)$, where c =speed of light, and $\alpha(\nu)$ =the absorptivity (log_e) for absorption at the particular frequency. Notice that k_a has proper second order rate constant units of m³mol⁻¹s⁻¹ (or L mol⁻¹s⁻¹). This treatment is exactly equivalent to using Beer’s law to calculate the number of photons absorbed per second, but allows us to treat the light like any other reagent. The differential rate laws using this notation are:

$$\frac{d[F^*]}{dt} = \frac{-d[F]}{dt} = k_a[F][h\nu] - (k_f + k_q[Q])[F^*], \quad (2a)$$

$$\frac{d[h\nu]}{dt} = f(t) \quad \text{and} \quad \frac{d[Q]}{dt} = 0, \quad (2b)$$

where $f(t)$ varies rapidly during a light pulse. If a constant intensity source were used instead $f(t) = 0$.

If a light pulse is used that is fast compared to the sum of the quenching plus fluorescence decay rates ($k_f + k_q[Q]$), once the light pulse ends some excited molecules will remain. We will call their concentration $[F^*]_i$. With the light off, the first term in the rate equation (2a) is zero, giving us:

$$\frac{d[F^*]}{dt} = -(k_f + k_q[Q])[F^*]. \quad (3)$$

This is just a first order rate equation, and can easily be integrated for the time after the light is off to get:

$$[F^*] = [F^*]_i \exp\left\{- (k_f + k_q[Q])t\right\}. \quad (4)$$

Thus, in the case of dynamic quenching, we expect the fluorescence to decay as a single exponential but with a decay rate that is linearly proportional to [Q].

In the case of the static quenching mechanism, the quencher forms an equilibrium concentration of a complex with the unexcited fluorophore, F. One of two things can happen: either the complex will not absorb an excitation photon, or the excited complex will relax by some route other than fluorescence. The reaction scheme is:



In this mechanism assuming the equilibrium is reached the differential rate laws are:

$$K_{eq} = \frac{[FQ]}{[F][Q]} \quad (6a)$$

$$\frac{d[F^*]}{dt} = \frac{-d[F]}{dt} = k_a[F][h\nu] - k_f[F^*] \quad (6b)$$

$$\frac{d[h\nu]}{dt} = f(t) \quad \text{and} \quad \frac{d[Q]}{dt} = 0. \quad (6c)$$

Equation 6a implies that the concentration of uncomplexed F available when the light turns on will depend on $K_{eq}[Q]$. Recognizing that $[FQ] + [F] = [F]_o$ is a constant one can solve for the concentration of [F] at equilibrium:

$$[F] = \frac{[F]_o}{K_{eq}[Q] + 1}. \quad (7)$$

Substituting for [F] in equation 6b shows us that when the light is on the rate of production of $[F^*]$ decreases with increased [Q]:

$$\frac{d[F^*]}{dt} = \frac{k_a[h\nu][F]_o}{K_{eq}[Q] + 1} - k_f[F^*]. \quad (8)$$

So we see that as the quencher concentration increases fewer molecules reach the excited state during our light pulse. Just as with the dynamic mechanism the first term in this equation is zero when the light is off. The resulting equation is easily integrated to find the time dependence of the excited fluorophore concentration after the light goes off:

$$[F^*] = [F^*]_i \exp\{-k_f t\}, \quad (9)$$

where $[F^*]_i$ decreases with increasing [Q], but the decay rate is independent of the quencher concentration.

Thus to determine if the quenching is static or dynamic, plot the observed fluorescence decay rate versus the quencher concentration. The dynamic case will yield a sloped line. Consider how the slope and intercept are related to k_q and k_f . In the static case the decay rate is constant.

Relationship of Quenching Rate to Collision Rate in Liquids

If the quenching is dynamic k_q can be extracted from the data. It is interesting to compare k_q with the theoretical diffusion limited collisional rate constant (k_D). Since we do not know the hydrodynamic radii of both the quencher and the fluorophore we use the approximate form of k_D where the hydrodynamic radii are assumed to be equal. In this way they cancel out and we get an expression for k_D which depends only upon temperature and the solvent viscosity:

$$k_D = \frac{8RT}{3\eta(T)}, \quad (10)$$

where k_D is the rate constant for diffusion, R is the gas constant, T is the temperature in Kelvin and $\eta(T)$ is the solvent viscosity which in general drops as temperature increases.^{2,3} Since the temperature in the laboratory varies between 20 °C and 25 °C, $\eta(T)$ for the 0.5 M H₂SO₄ (about 5% by weight) solution used is between $1.12 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$ and $1.01 \times 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$.^{7,8} So, if all collisions lead to quenching, k_q will be equal to k_D and will fall in the range $5.8 \times 10^9 \text{ L mole}^{-1} \text{ s}^{-1}$ to

$6.5 \times 10^9 \text{ L mole}^{-1}\text{s}^{-1}$. If some collisions do not lead to quenching, k_q should be somewhat less than the calculated k_D .

What You See if You Do Not Have Time Resolution (Stern-Volmer Analysis)

For continuous excitation the situation is different. Experimentally, we would measure the intensity of fluorescence, which is proportional to $[h\nu_f] \propto [F^*]$, while the light is left on. The differential rate equations are still those of equations 2a and b. Since the light is on all the time we cannot drop the first term from equation 2a for our analysis of the fluorescence observed. By substituting $[F]_o - [F^*]$ for $[F]$, equation 2a can be integrated to find the time dependence of the excited state concentration, $[F^*]$. This shows that there is an initial period after turning on the light where the $[F^*]$ grows and then it reaches a steady state. The initial induction period is very short and it is easier to analyze the mechanism by making measurements after the initial induction period has finished and assuming steady-state conditions. The steady state equilibrium is reached when $[F^*]$ gets large enough, because the first and second terms in equation 2a will cancel. Substituting for $[F]$ and assuming steady-state conditions in equation 2a gives:

$$[F^*] = \frac{k_a [h\nu] [F]_o}{k_f + k_q [Q] + k_a [h\nu]} \quad (11)$$

It turns out that the inverse of this expression is easier to work with:

$$\frac{1}{[F^*]} = \frac{k_f + k_q [Q] + k_a [h\nu]}{k_a [h\nu] [F]_o} = \left(\frac{1}{[F]_o} + \frac{k_f}{k_a [h\nu] [F]_o} \right) + \frac{k_q}{k_a [h\nu] [F]_o} [Q] \quad (12)$$

Note that this is the equation for a line versus the concentration of Q, since $[F]_o$, $[h\nu]$, k_f , k_q and k_a are all constants. The terms in brackets are the y-intercept and the fraction before $[Q]$ is the slope of the line. Plotting $1/(\text{fluorescence intensity})$ versus $[Q]$ yields a straight line with a positive slope, if Q quenches the fluorescence. This sort of an analysis is called a Stern-Volmer analysis and is commonly used to analyze concentration dependent kinetics.^{3,9} This analysis is often carried one step further to generate what is called a reduced Stern-Volmer plot. If the fluorescence intensity (equation 11) is divided by the fluorescence intensity with $[Q]=0$, and then the inverse taken, this yields an equation for a line with a y-intercept of 1:

$$\frac{[F^*]_{[Q]=0}}{[F^*]} = 1 + \frac{k_q}{k_a [h\nu] + k_f} [Q] \quad (13)$$

The slope now depends only upon k_a , the rate of photon absorption, $[h\nu]$, the photon density, k_f , the rate of fluorescence and k_q , the rate constant for quenching. You can calculate $[h\nu]$ from the light intensity, k_a , from the Beer's law absorption coefficients at the excitation frequency and k_q is assumed to equal k_D , which can be estimated based on the diffusion coefficients, as shown above.^{2,3} Thus k_f can be determined. In most formulations of this equation the intensity of the light is assumed to be so small that the $k_a [h\nu]$ term in the denominator can be ignored, making the analysis even easier. Often this equation is written in terms of the quantum yield, $\phi \propto [F^*]$, and the factor in front of $[Q]$ is referred to as K_{SV} , the Stern-Volmer constant:

$$\frac{\phi_o}{\phi} = 1 + K_{sv}[Q]. \quad (14)$$

The problem is that you get the same functional form if static quenching is occurring. If you assume that the equilibrium in equation 5a is fast enough that it can adjust to any change in [F] caused by absorption or fluorescence, you should be able to derive the following reduced Stern-Volmer equation:

$$\frac{[F^*]_{Q=0}}{[F^*]} = 1 + \frac{k_f K_{eq}}{k_f + k_a[h\nu]} [Q]. \quad (15)$$

Notice that the slope of this line is a different function of the constants than in the dynamic case, but you will not be able to tell the difference, since you can only measure the slope.

Experimental

You will use a laser, a monochromator, a photomultiplier tube (PMT) and a storage oscilloscope to collect the time resolved laser-induced fluorescence of quinine sulfate at approximately 10^{-5} M and 1.0 N H_2SO_4 in water. You will observe the effects of Cl^- as a quencher by varying its concentration in the solution.

Use of the N_2 laser

Read the instructions about using the laser in the manual provided. Be careful: the laser uses extremely high voltages, can be damaged if the N_2 gas is not pure enough or flowing rapidly enough, and the UV pulse produced by the laser can easily damage your eyes or burn your hand. Keep extremities and clothing out of the beam and wear plastic glasses that do not transmit the laser light. Also make sure that the dye laser has been moved out of the way. If you have any questions, ask your professor.

Use of monochromator

The laser beam travels through the sample. The line of fluorescence is imaged onto the front slit of the monochromator by mirrors. Keep the slits as narrow as possible to avoid interference from the scattered laser light, but do not force the slits closed. You should set the monochromator to detect 480 nm fluorescence. This is far from the laser frequency of 337.1 nm and is the frequency at which the fluorescence exhibits simple exponential decay. At other wavelengths the emission rate is affected by a number of other processes that we have not considered, and requires a sum of two or more exponential functions to fit the decay.^{1,10,11} If you have time, you might want to collect fluorescence at about 390-400 nm from a sample without quencher to see if you can observe the biexponential decay. See the instruction manual for details on controlling the wavelength and adjusting the slits. If you have any questions, consult with your professor.

Use of PMT

The light that gets through the monochromator is detected by a photomultiplier tube. PMTs are based on the photoelectric effect. After a light photon ejects an electron from the photocathode

the electron is accelerated by a potential voltage into another electrode. The accelerated electron knocks many electrons out of the electrode, which are then accelerated to another electrode. This process is repeated 9-12 times giving a current gain of about 10^6 ! It is possible to see the signal from a single photon; if you have a 350 MHz or greater oscilloscope and the fluorescence intensity is low enough you may be able to see individual spikes (photons) in the decay signal from the PMT. Typically PMTs operate at a negative voltage of between 500 and 1250 V. Be very careful: high voltages are dangerous and a PMT can be easily damaged by exposing it to too much light while voltages are applied. To avoid damaging the PMT do not apply voltage to the tube until the room lights are off and start looking for signal at a low voltage increasing the voltage only enough to get a signal of a few hundred millivolts at its peak. **DO NOT TURN THE VOLTAGE ABOVE 1000 V!** If you have any questions, consult with the professor.

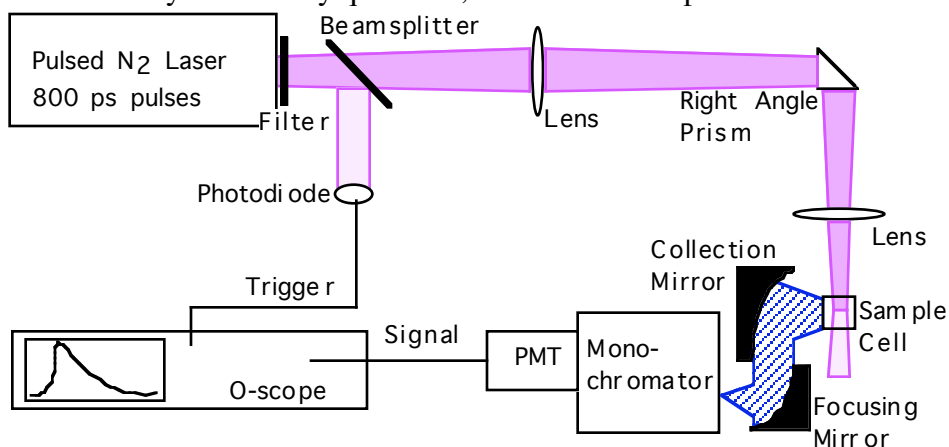


Figure 2: Block diagram of experimental setup (solid lines represent bnc cables)

Use of the Digital Oscilloscope

Explicit instructions for the particular scope you are using will be provided. A digital oscilloscope is different from a standard analog scope. Digital scopes take an input voltage versus time and at regular intervals convert the voltage level to a digital number using standard (usually successive approximation) analog-to-digital conversion techniques. This means that if something happens between samplings you will not see any sign of it (see figure). Using an analog scope, even if something happens faster than the scope can respond it will react in some way; thus your signal looks like a smoothed (averaged) version of the real signal. The advantage of a digital scope for the kind of experiment we are doing is that you can conveniently save and average together multiple traces of a repeated event. There is no way to save and average things on a normal scope. Sometimes you will hear the term transient digitizer, this is just a digital oscilloscope without some of the features usually found in an oscilloscope.

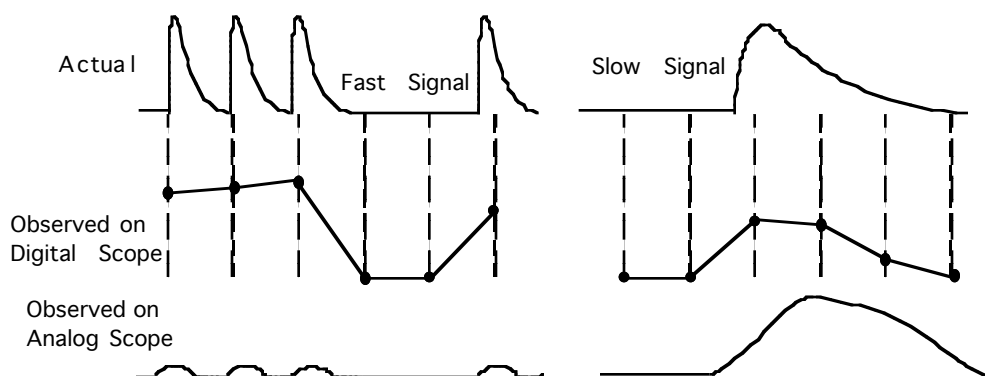


Figure 3: Comparison of a digital scope missing a fast signal and the response of an analog scope. The black dots represent the values actually measured by the digital scope and the dashed lines the time the samples are taken.

The oscilloscope only displays data when told to do so by the occurrence of a trigger. In our experiment we will use a small amount of the light pulse out of the laser to trigger the beginning of data collection. The reason to do this is that the signal we are interested in only occurs over a very brief period of time right after the laser pulse. We would collect mounds of useless data if we saved the signal from the PMT at other times.

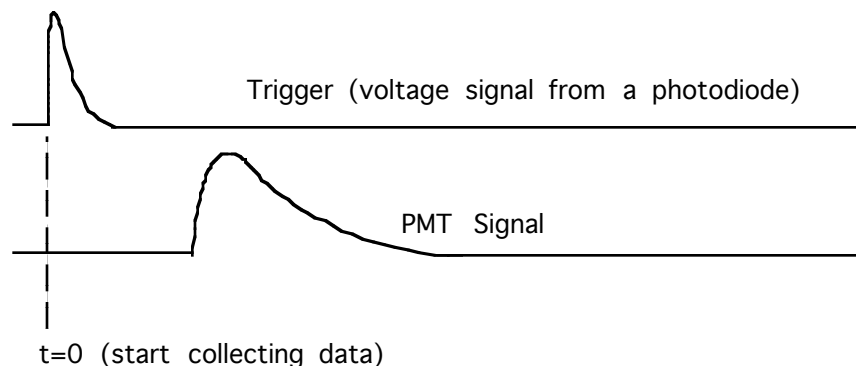


Figure 4: Timing diagram for data collection with a trigger.

Samples

You should make 5 samples with different concentrations of Cl^- ions. The quinine sulphate dihydrate should be about 10^{-5} M in the samples and the same for each sample. The H_2SO_4 in the solution should be 0.5 M (1.0 N). A good range for the quencher concentrations is 0.00-0.08 M. Make sure the cell is very clean before filling it with each sample. Average enough time scans together on each sample that your signal to noise level is $>20:1$. You will need at least 20 scans per sample. Do not forget to collect a background, so that you may subtract out any radio-frequency noise. Collect the background by averaging the signal with the fluorescence blocked by a piece of cardboard.

For each sample you should also take a UV-Vis spectrum to verify that the absorbance does not change with quencher concentration. This will allow you to verify independently of the time-dependent experiment that the quenching occurs in the excited state.

If time allows we will also take a fluorescence spectrum of the quinine sulfate.

Analysis

Depending upon how you collect your data you may be able to measure the dependence of the fluorescence lifetimes on quencher concentration and perform a Stern-Volmer analysis. The data should be saved in a format appropriate to be transferred to a spreadsheet or a data analysis program for processing.

Before doing any analysis you must subtract out the background, which consists mostly of radio-frequency interference from the spark gaps in the N₂ laser. To get the observed rate constant for fluorescence decay, $(k_f + k_q[Q])$ or k_f , fit the decaying part of the signal to a single exponential. To find k_q plot the observed decay rate versus $[Q]$ and fit it to a line. To perform the Stern-Volmer analysis you must get the data into a form that is equivalent to the cw fluorescence intensity. You will only be able to do this if the conditions were exactly the same for all the samples. This means the laser power must stay the same, the slits must not be adjusted, etc. Just integrate the total area under each decay curve to measure the total fluorescence.

Questions

- 1) Based on your observations is the quinine fluorescence quenched dynamically, by complex formation or a combination of both? Explain why.
- 2) The rise time of the PMT is about 2.5 ns, the laser pulse is < 1ns and the sampling rate of the oscilloscope is 2 gigasamples/sec. What is the shortest fluorescence decay time you will be able to measure directly? Is there any way you can do better than this?
- 3) In analytical lab students often do not get very good results for the quinine concentration by measuring the fluorescence intensity. Based on what you have observed in this lab suggest some possible sources of error. Usually, but not always, students underestimate the quinine concentration.
- 4) Report your k_q and k_f . If you performed a Stern-Volmer analysis compare the results from the two methods. You should also compare k_q with k_D . If they are different explain the significance.
- 5) The literature value for the lifetime $(1/k_f) = 19.3 \pm 0.6$ ns (95% confidence limits).^{1,10,12} Compare this with your results and discuss any discrepancies.
- 6) The best available literature value of $k_q = 6.2 \times 10^9$ M⁻¹s⁻¹ determined from lifetime measurements by Barrow and Lentz.¹⁰ This was determined in 0.05 M H₂SO₄ whereas the your results presented were in 0.5 M H₂SO₄. The viscosity of 0.05 M H₂SO₄ is very close to that of pure water (not the same as 0.5 M). Compare your results with these and discuss any discrepancies.

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Instructor Notes:

by J. H. Gutow, December 2003

A Note on Notation (Why use $[h\nu]$ instead of intensity?)

The primary reasons for doing calculations in terms of photon concentration are pedagogical. Using this notation allows chemistry students to do the kinetics calculations in familiar notation using concentration units, without losing any accuracy. Since the light intensity is not actually measured in this experiment I am trying to avoid the confusion usually caused among students by including terms of the form $(I_{\text{abs}})/(\text{path length})$. The final results are the same (see page 3 of the student handout).

The excited population is proportional to effective photon concentration, $[h\nu]$, which is proportional to the intensity in the regime where Beer's law holds (low intensity). In saturation (very high intensity), the excited population is approximately $[F]_0/2$ and does not depend on light intensity. However, between these two extremes where the absorption is saturated for only part of the light path in the cell a weighted average must be used to calculate $[h\nu]$. The complication of the weighted average has been ignored for the students since we do not actually measure intensity in this experiment. See the appendix A on $[h\nu]$ versus intensity for a detailed discussion of excited state population versus position in a sample.

As noted on page 6 of the student handout, most formulations of fluorescence quenching kinetics assume the light intensity is so small that an insignificant fraction of the available ground-state molecules are excited. With short pulse lasers as the excitation source this is often not true. The form of the equations derived in the handout more accurately reflect this. Since one of the topics I cover in physical chemistry lecture is lasers and the idea of saturating a transition, I explicitly include the dependence on light intensity to connect back to the lecture discussions.

The formulation in the handout is incomplete because the stimulated emission is ignored (equation 1d in the handout). If this is included then the proper limiting excited state population of one-half the total absorber population is found. Including this converts equation 2a of the handout to:

$$\frac{d[F^*]}{dt} = \frac{-d[F]}{dt} = k_a[F][h\nu] - (k_f + k_q[Q] + k_a[h\nu])[F^*]. \quad (3)$$

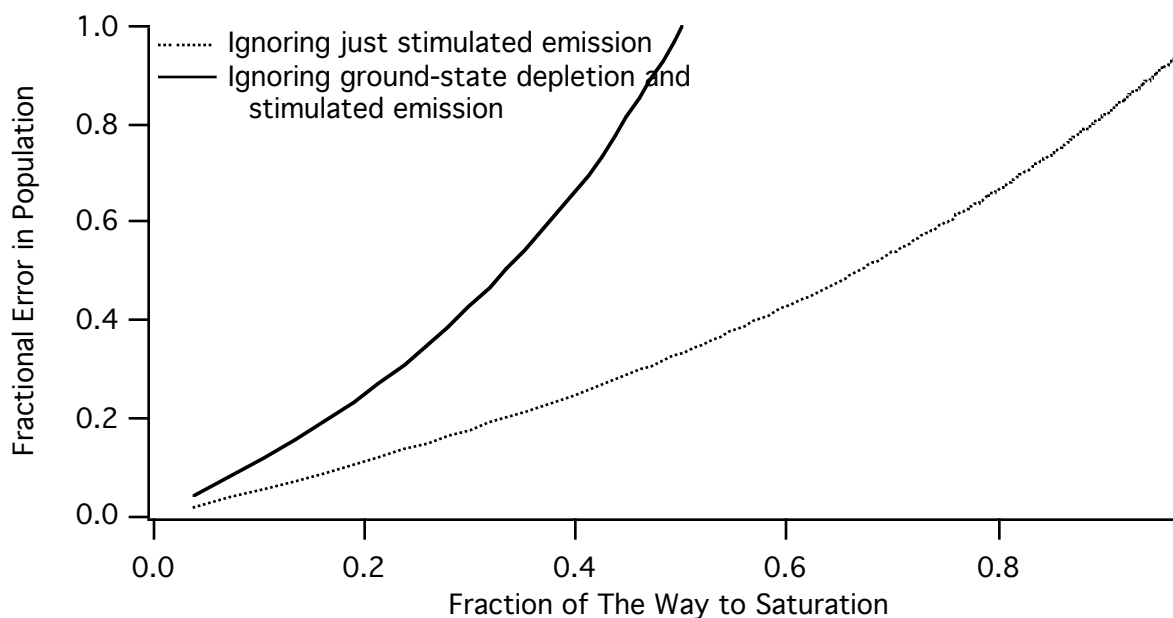
Substituting $[F]=[F]_o-[F^*]$ to properly account for depletion of the ground state we get:

$$\frac{d[F^*]}{dt} = \frac{-d[F]}{dt} = k_a[F]_o[h\nu] - (k_f + k_q[Q] + 2k_a[h\nu])[F^*]. \quad (4)$$

If the light is left on long enough to reach equilibrium (often only a few picoseconds with a laser), we can solve this equation for the steady state population of $[F^*]$ in terms of $[F]_o$:

$$[F^*]_{ss} = \frac{k_a[F]_o[h\nu]}{k_f + k_q[Q] + 2k_a[h\nu]}. \quad (5)$$

Notice that if $[h\nu]$ is large this goes to $[F]_o/2$ as expected. The formulation used in the handout is missing the factor of 2 on the last term in the denominator and has a limit of 100% excitation. I expect the students to work out equation 5 as an exercise. Although the form in the handout is not completely correct it is much better than ignoring ground state depletion even for relatively low intensities. Below is a graph comparing the fractional error in the excited state population ignoring just stimulated emission and both stimulated emission and ground-state depletion.



Notice that by 50% of the way to saturation the error when ignoring both is 100% (the predicted population is 2 times larger than the actual). Although not good, ignoring just stimulated emission reduces the fractional error.

Implementation in a Class Setting

To expedite the laboratory I provide three stock solutions: 1) 2.00×10^{-5} M quinine sulfate dihydrate and ~ 1.0 M H_2SO_4 in H_2O ; 2) 0.150 M KCl in water; 3) deionized water. Other concentrations of sulfuric acid could be used. I have chosen this concentration for two reasons: 1) it matches the concentration for which I found the best lifetime data¹; and 2) it is not the same concentration as was used when the literature values for k_D were determined, which forces the students to show that they understand how viscosity affects k_D . I tell the students ahead of time what concentration solutions they must make and expect them to come to class having figured out how to make all the solutions. A total volume of 10 mL for each concentration is adequate. All solutions should be 1.00×10^{-5} M in quinine sulfate. A good range for the concentrations of KCl is 0 to 8×10^{-2} M. If you do not want the students to see the data curve due to the instrument response time, limit the maximum salt concentration so that the lifetime is 2-3 times the instrument time constant. In our labs with an instrument time constant of about 2.5 ns, this corresponds to keeping the KCl concentration less than 4×10^{-2} M. Each student makes one or two solutions in this range. The whole class is instructed in the use of our time-resolved fluorescence apparatus and then they rotate through collecting the fluorescence decays and the UV-Vis spectra of their solutions. As a class we collect the fluorescence spectrum of an unquenched sample and given time a fluorescence excitation spectrum as well (scan excitation across absorbance band and collect fluorescence at 480 nm). Usually the previous student on the apparatus helps the next one collect their data. In addition to making efficient use of the equipment this rotation strategy forces the students to work together and they learn more because they also teach each other. A class of 6-9 students can collect their data during one three hour laboratory period in this manner. *The fluorescence decays must be collected at 480 ± 10 nm to observe single exponential decays.* Barrow and Lentz's work indicates that the useful window for observing single exponential behavior may be as large as 480 ± 25 nm.¹ If there is time students are encouraged to collect decays at other wavelengths to observe multiexponential decays.

Nonexponential Decays (far from 480 nm)

A full discussion of the fluorescence behavior far from 480 nm is beyond the scope of this work. However, it is worth mentioning some of the proposed explanations for the nonexponential behavior, because it is possible that the effects observed at other wavelengths fortuitously cancel out near 480 nm.^{1,2} Although many people have been able to fit the decays with biexponentials, the fluorescence is believed to occur from more than two excited states. The fluorescing states are believed to be a series of charge transfer states that shift through a number of conformations and exhibit strong solvent relaxation effects. A more complete description of the possibilities may be found in the work of Pant *et al.*^{2,3} Thus this experiment is a good example of how we use models in science that work to describe the observed phenomenon, but may not be complete.

Data Analysis

The data analysis is usually performed during a second three hour class period. Each student analyzes their own fluorescence decays by first subtracting the background and then fitting the decaying part of the curve to a single exponential. The data is then pooled to determine that the quenching is dynamic and estimate the unquenched lifetime and quenching rate constant.

The data is transferred to personal computers for analysis. The software should be able to perform nonlinear least squares fitting of exponential functions. Many software packages do not handle fitting noisy exponential decays very well, because they first take the natural logarithm of the data and then perform a linear fit. These programs often balk because they cannot take logarithms of negative numbers. Additionally the software should comfortably handle data sets of 500 points or more. It also helps if the software allows fitting to arbitrary ranges of your data set and can properly estimate the errors in the coefficients of the fit.⁴ My classes have used the data analysis and plotting software IGOR (Wavemetrics Corp.) running on MacOS and Windows computers. Other software worth investigating are Sigma Plot (Jandel Scientific), Origin (Microcal) and the plotting packages by Fortner Research. Avoid Microsoft Excel which, as of the submission date of this paper, does not do statistics properly.⁵

In addition to the kinetic analysis, the students compare the spectra they have collected. A comparison of the fluorescence spectrum with the absorption spectra illustrates the typical shift of fluorescence to lower energy because of vibrational relaxation in the upper state. By comparing the absorption spectra for the different concentrations they get further evidence that no complex is affecting the absorbance. Time has not been available with only one three hour laboratory period for data collection, but taking fluorescence excitation spectra at different quencher concentrations would also help to verify that no complex is formed.

Stern-Volmer Analysis

If the students complete the data analysis quickly, I also have them treat the data as if they had collected fluorescence intensities versus quenching concentration without any time resolution. For this to work the collection conditions (excitation intensity, monochromator slit widths, collection frequency, etc.) must remain unchanged between samples. To analyze the data as if it were collected with continuous excitation the students integrate the area under the fluorescence decays and use these as the fluorescence intensities for a Stern-Volmer analysis. In a Stern-Volmer analysis the ratio of the unquenched fluorescence intensity to the quenched intensity is plotted versus the quencher concentration.^{6,7} See the student handout for a more detailed discussion of the Stern-Volmer analysis of the dynamic quenching mechanism which yields the following functional form for the observed line :

$$\frac{[F^*]_{[Q]=0}}{[F^*]} = 1 + \frac{k_q}{k_a[h\nu] + k_f} [Q], \quad (6)$$

where $[F^*]_{[Q]=0}$ is the excited molecule concentration in the absence of quencher. The slope of this line is often referred to as K_{SV} , the Stern-Volmer constant. In many formulations the fact that $[F]$ is not rigorously equal to $[F]_0$ is ignored. As long as the light intensity is low this leads to very small errors. In these formulations the light intensity dependence of K_{SV} disappears and $K_{SV} = k_q\tau_0$, where $\tau_0 = k_f^{-1}$ is the observed exponential lifetime at zero quencher concentration.⁷ So,

when this mechanism is valid you can estimate τ_0 by calculating k_q from viscosity measurements and dividing the observed slope of the line by k_q .

The results presented in this paper yield $K_{SV} = 107 \pm 3 \text{ M}^{-1}$ for quinine in 0.5 M H_2SO_4 . In 0.05 M H_2SO_4 $K_{SV} \approx 119 \text{ M}^{-1}$ was observed by Barrow and Lentz.¹ The difference is almost exactly the factor of 1.13 expected from the differences in viscosity. The unquenched lifetime does not appear to change significantly in this range of acid concentrations.²

The problem with not having time resolution is that the static quenching mechanism also yields a linear Stern-Volmer plot,⁷ of the following functional form:

$$\frac{[F^*]_{[Q]=0}}{[F^*]} = 1 + \frac{k_f K_{eq}}{k_f + k_a[h\nu]} [Q]. \quad (7)$$

Since both processes depend linearly upon the quencher concentration, one cannot tell which process is occurring by inspecting the Stern-Volmer plot. If both processes are occurring simultaneously, you will not get a straight line unless the slopes are by chance equal.^a Thus if we were to collect the data in only this manner the students would not be able to determine which process is occurring. It is theoretically possible to determine which is occurring by collecting temperature dependent Stern-Volmer plots. However, it is not possible to calculate k_f without estimating either k_q or K_{eq} .⁷

Correcting for the Instrument Response

Figure 2 in the published summary shows a selection of fluorescence decays for a set of samples. The slower decays fit exponentials quite well, but as the decay rate approaches the instrument response time ($\sim 2.5 \text{ ns}$) the fit becomes worse. The instrument response can be corrected for by fitting an exponential convolved with the instrument response function to the observed data. This works quite well, as can be seen in Figure 3 of the published summary. For

^aIt should be noted that other mechanisms with additional intermediate states can lead to nonlinear Stern-Volmer plots even when only dynamic or static quenching is occurring. One example is the case of a phosphorescence where both the singlet and the triplet states are collisionally quenched. However, these more complicated mechanisms are not necessary to explain the data collected in this experiment.

the data shown the convolution was performed using fast Fourier transform convolution and a very simple nonlinear least squares minimization of χ^2 . For the minimization the next guess for each parameter was made by assuming that χ^2 locally fit a parabola in each dimension. The IGOR routines for performing this procedure under partial manual control are available from the author. Due to time constraints I have not had the students perform this deconvolution on their data. Further discussion of problems associated with instrument response times may be found in the paper of Muentner and Deutsch.⁸

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Instructor Notes Appendix A:

How $[h\nu]$, Effective Photon Concentration, and I , Intensity, are Related

by Jonathan Gutow, December 2000

Updates: May 2003, December 2003-JG

The discussion below describes in detail the excited state population and light intensity as the light penetrates through the sample volume. One of the most important lessons of this discussion is that Beer's Law is the low light level ($I_0 \rightarrow 0$) limiting case which is only approximately correct when $I \neq 0$. One should also note that with high intensity lasers it is not simple to calculate the excited state population in a sample. Note that even measuring the amount of light absorbed will not give you the excited state population in the high intensity case, unless you also know non-radiative relaxation rates and spontaneous emission rates.

Introduction and Notation

We will be calculating the photon concentration assuming the light is propagating in the z direction at a constant velocity. The flux across a plane at position z in moles photons $s^{-1}m^{-2}$ is:

$$j(z) = \frac{I(z)}{N_A h\nu}, \quad (1)$$

where $I(z)$ is the intensity of the light in Wm^{-2} and h is Planck's constant, ν is the excitation

frequency and N_A is Avogadro's number. The instantaneous concentration in an infinitesimal volume near z is just the flux divided by the speed ($c=2.99792 \times 10^8 \text{ m s}^{-1}$) at which the photons are traveling:

$$[h\nu]_z = \frac{j(z)}{c} = \frac{I(z)}{N_A ch\nu}. \quad (2)$$

If scattering is ignored and no absorption occurs then $I(z)$ is a constant and this expression is the photon concentration in the excitation volume. If the intensity depends upon z then this expression

must be integrated from $z=0$ to ℓ , the path length, and then divide by the path length to normalize to a mean average. If there is absorption the photon concentration drops as we cross the sample.

There are three possibilities for the intensity dependence: low light intensity following Beer's Law ($I(z)$ falls off exponentially); high light intensity so that absorption is saturated across the whole sample ($I(z)$ falls off linearly); and an intermediate range where regions near the beam entrance are saturated while near the exit they are not. In the first case since the excited population is just proportional to the intensity it doesn't matter whether you use a mean average or actually calculate the excited population as the sum of the populations at each position. In the second case since the system is saturated and the excited population will not change with intensity a mean average also works. In the third case we are switching between these two regimes and things are more complicate.

Case 1: (Beer's law applies)

The expression for the intensity dependence versus position when Beer's Law applies is:

$$I(z) = I_o \exp(-\alpha(\nu)[F]z), \tag{3}$$

where I_o is the intensity at the entrance to the sample, $\alpha(\nu)$ is the molar absorptivity (base e), and $[F]$ is the molar absorber concentration. Integrating

$$[h\nu]_z = \frac{j(z)}{c} = \frac{I(z)}{N_A c h \nu} \tag{4}$$

and dividing by the path length to give the average concentration yields:

$$[h\nu]_{avg} = \frac{\frac{I_o}{N_A c h \nu} \int_0^\ell \exp(-\alpha(\nu)[F]z) dz}{\int_0^\ell dz} = \frac{I_o}{\ell N_A c h \nu \alpha(\nu)[F]} \{1 - \exp(-\alpha(\nu)[F]\ell)\} \tag{5}$$

To complete the comparison with the calculation in terms of intensity we need an expression for the moles of photons absorbed per second. This is just $k_a[h\nu][F]$. As defined in the student handout

$k_a = \alpha(\nu)c$. This implies:

$$\text{moles photons absorbed } s^{-1}m^{-3} = k_a [h\nu]_{\text{avg}} [F] = \alpha(\nu) c [h\nu]_{\text{avg}} [F] = \frac{I_o}{\ell N_A h\nu} \{1 - \exp(-\alpha(\nu)[F]\ell)\} . \quad (6)$$

If you do the same calculation in terms of the incoming intensity you get the same answer. The difference between using concentration notation and intensity is that using intensity you acknowledge that the excited concentration may not be constant across the excitation volume. The final expression substituted into the differential equation looks very different from what students are used to for kinetics. Many people avoid this complexity by writing I_{abs} in place of the final expression, but this masks the intensity and concentration dependence of the term. Using $k_a[h\nu][F]$ in the rate equations maintains the form the students are familiar with and doesn't mask the parameters the equations depend upon (see equation 1d of the student handout).

The Saturation Cases

The z dependence of the intensity is different than in the Beer's Law case. First consider dI/dz .

$$\begin{aligned} \frac{dI}{dz} &= \text{amount emitted at } \nu - \text{amount absorbed at } \nu \\ &= \alpha(\nu) I(z) [F^*] + \alpha_s(\nu) [F^*] - \alpha(\nu) I(z) [F], \end{aligned} \quad (7)$$

where $\alpha_s(\nu)$ is the spontaneous emission coefficient for emission in the forward direction, with units of $W \text{ mol}^{-1}$. This is at most one half of the spontaneously emitted photons. If your beam diameter is about 2 mm and you are collecting light at about 10 cm from the sample only photons emitted into a solid angle in the forward direction of 0.115° will reach the detector. This is only about 3×10^{-4} of the total emitted light. Thus spontaneous emission must be very strong for it to significantly effect the measured intensity. However, it does effect the photon intensity in the volume immediately next to where it is emitted.

To remove the dependence on $[F]$ we next substitute for $[F]$ with $[F]_o - [F^*]$:

$$\begin{aligned}
 \frac{dI}{dz} &= \alpha(\nu)I(z)[F^*] + \alpha_s(\nu)[F^*] - \alpha(\nu)I(z)\{[F]_0 - [F^*]\} \\
 &= 2\alpha(\nu)I(z)[F^*] + \alpha_s(\nu)[F^*] - \alpha(\nu)I(z)[F]_0 \\
 &= \{2\alpha(\nu)I(z) + \alpha_s(\nu)\}[F^*] - \alpha(\nu)I(z)[F]_0.
 \end{aligned} \tag{8}$$

Now we need an expression for $[F^*]$ in terms of the intensity. This is also where we take care of fluorescence and phosphorescence which occur at a different wavelength than the incoming light. We will ignore small effects such as Raman and assume that intensities are still low enough that no other nonlinear phenomena occur. We will also assume that at any point in the solution an equilibrium is reached among: stimulated absorption; stimulated emission; relaxation to the ground state through non-radiative decay; fluorescence; and phosphorescence. Thus:

$$\begin{aligned}
 0 &= \left. \frac{d[F^*]}{dt} \right|_z = \frac{\alpha(\nu)}{N_A h \nu} I(z)[F] - \frac{\alpha(\nu)}{N_A h \nu} I(z)[F^*] - \frac{B\alpha_s(\nu)}{N_A h \nu} [F^*] - k_R [F^*] \\
 \Rightarrow 0 &= \frac{\alpha(\nu)}{N_A h \nu} I(z)\{[F]_0 - [F^*]\} - \frac{\alpha(\nu)}{N_A h \nu} I(z)[F^*] - \frac{B\alpha_s(\nu)}{N_A h \nu} [F^*] - k_R [F^*] \\
 &= \frac{\alpha(\nu)}{N_A h \nu} I(z)[F]_0 - \left\{ 2\frac{\alpha(\nu)}{N_A h \nu} I(z) + \frac{B\alpha_s(\nu)}{N_A h \nu} + k_R \right\} [F^*]
 \end{aligned} \tag{9}$$

where the division by $N_A h \nu$, converts the absorption and emission coefficients into rate constants

(see above), k_R is the relaxation rate constant for all non-radiative paths to the ground state (including quenching, $k_q[Q]$), and B is a geometric factor greater than 1 ($> 10^4$ in example of 2 mm beam given above) that accounts for more spontaneous photons being emitted than reach the detector. Solving for the steady-state concentration of $[F^*]$ we get:

$$[F^*]_{ss}|_z = \frac{\frac{\alpha(\nu)}{N_A h \nu} I(z)[F]_0}{2\frac{\alpha(\nu)}{N_A h \nu} I(z) + \frac{B\alpha_s(\nu)}{N_A h \nu} + k_R} = \frac{\alpha(\nu)I(z)[F]_0}{2\alpha(\nu)I(z) + B\alpha_s(\nu) + k_R N_A h \nu}. \tag{10}$$

We can see what happens to the intensity more clearly by substituting $[F^*]_{ss}|_z$ back into the expression for dI/dz and eliminating the dependence upon $[F^*]$:

$$\begin{aligned}
 \frac{dI}{dz} &= \{2\alpha(v)I(z) + \alpha_s(v)\}[F^*] - \alpha(v)I(z)[F]_o \\
 &= \{2\alpha(v)I(z) + \alpha_s(v)\} \frac{\alpha(v)I(z)[F]_o}{2\alpha(v)I(z) + B\alpha_s(v) + k_R N_A h\nu} - \alpha(v)I(z)[F]_o \\
 &= \frac{\{2\alpha(v)I(z) + \alpha_s(v)\} \alpha(v)I(z)[F]_o - \alpha(v)I(z)[F]_o \{2\alpha(v)I(z) + B\alpha_s(v) + k_R N_A h\nu\}}{2\alpha(v)I(z) + B\alpha_s(v) + k_R N_A h\nu} \\
 &= \frac{-\alpha(v)I(z)[F]_o \{(B-1)\alpha_s(v) + k_R N_A h\nu\}}{2\alpha(v)I(z) + B\alpha_s(v) + k_R N_A h\nu}.
 \end{aligned} \tag{11}$$

In the limit of large k_R and small $\alpha_s(v)$ this reduces to Beers law. For k_R and $\alpha_s(v)$ small you get:

$$\frac{dI}{dz} = \frac{-[F]_o \{(B-1)\alpha_s(v) + k_R N_A h\nu\}}{2}, \tag{12}$$

which shows that the intensity drops off linearly in a saturated situation with the slope determined by the absorber concentration, the rate of spontaneous emission and the rate of relaxation to the ground state.

Notice that this also shows how you get photobleaching. If the concentration or relaxation rates are small enough compared to the intensity then there is little drop off in intensity as you cross the sample and the sample will appear transparent to the light beam. Photobleaching is not exhibited as obviously by the kinetic model which averages over the whole volume. Notice that in this saturation regime the amount of light absorbed does not depend on the intensity of the light (the photon concentration) only the absorber concentration and the volume of the sample illuminated.

Case 2: (Saturation across the whole sample)

In the case where the intensity is high enough that saturation occurs all the way across the sample then $[F^*]_{s|z} = [F]_o/2$. This is what happens when the last two terms in the denominator of equation 10 become negligible. Integrating from 0 to ℓ and dividing by ℓ yields $[F^*]_{ss} = [F]_o/2$, which is the same result arrived at using the photon concentration notation (see instructor notes equation 5 and the paragraph that follows).

Case 3: (Saturation across only part of the sample volume)

In this situation the excited population at any position in the sample is neither independent of light intensity (Case 2) or proportional to light intensity (Case 1). This means the average excited state concentration is not a constant times the mean average photon concentration. This is easiest to see in the following plots of the excited state concentration versus position in the sample. Notice how the functional form of the concentration does not match the light intensity.

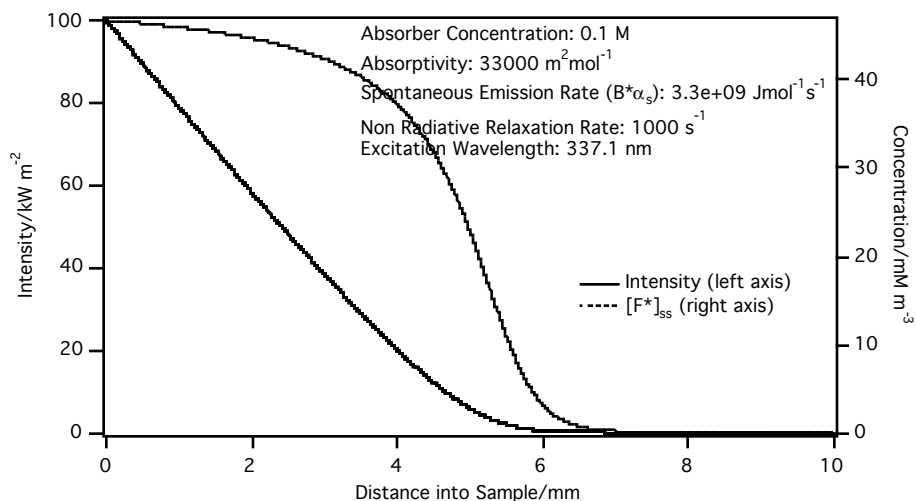


Figure 1: Plot of light intensity and excited state population across a 10 mm sample under conditions where saturation of the transition only occurs at the entrance. The light intensity was calculated numerically using the expression for dI/dz to increment the light intensity in small steps. This light intensity was then used to calculate $[F^*]_{ss|z}$.

The following figures show some other conditions that can occur. Notice that the results approach the Beer's Law as expected at lower absorptivity and higher relaxation rates.

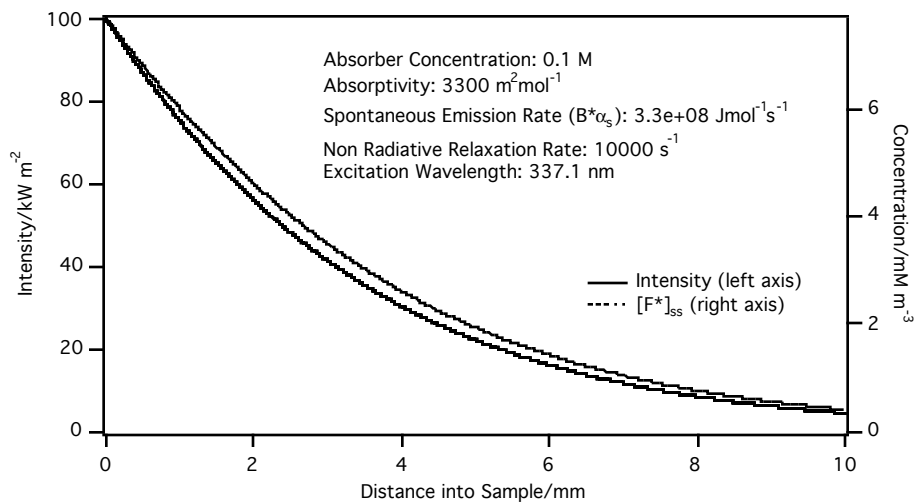


Figure 2: Case where the absorptivity is lower and the relaxation rate is relatively high. Observe that the light intensity falls off almost exponentially and that $[F^*]_{ss|z}$ is almost directly proportional to the intensity. If the relaxation rate were higher or the absorptivity lower we would approach closer to Beer's law behavior.

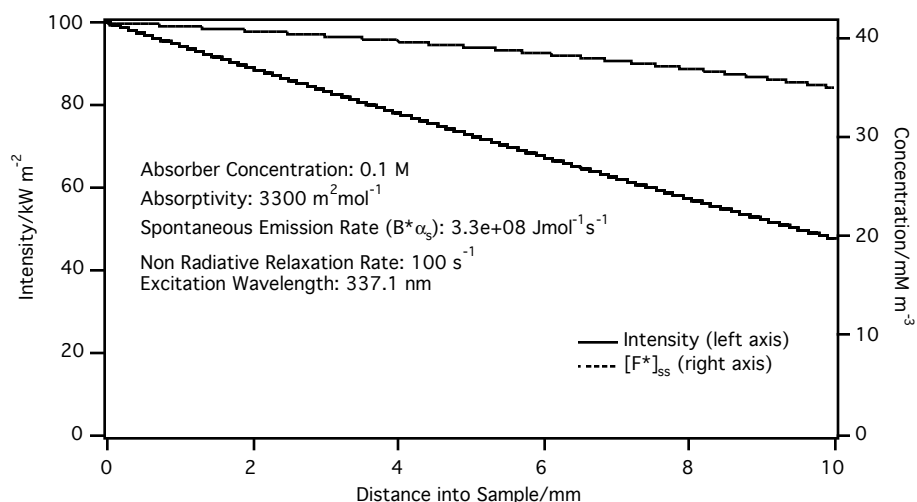


Figure 3: Case where the relaxation rate is low enough that the absorption is nearly saturated across the whole sample. Notice the slight curvature in $[F^*]_{ss}|_z$.

It is possible to find an analytical solution for $I(z)$. One was found using Maple V, Release 4. However, the solution involves LambertW functions of exponential functions and is more difficult to interpret than the numerical results.

If you integrate the intensity across the sample in the three figures above you can then divide this by the path length to get the mean average intensity across the sample (I_{avg}). The mean average photon concentration is: $[h\nu]_{avg} = I_{avg}/cN_A h\nu$. This average photon concentration can be plugged into equation 5 of the instructor notes to calculate $[F^*]_{ss}$. This can be compared with the result got by integrating $[F^*]_{ss}|_z$ in each of the above figures and dividing that by the path length. Using the data in figure Case3a there is a large discrepancy between the two calculations of the average $[F^*]_{ss}$. Using the data in the other two figures which are closer to the extremes of Beer's law and complete saturation the two different calculations of $[F^*]_{ss}$ are very similar. As a reminder the following equivalences become important when converting between normal rate constants and the notation above: $k_a = \alpha(\nu)c$, $k_f = B\alpha_s(\nu)/N_A h\nu$ and $[h\nu]|_z = I(z)/cN_A h\nu$.

Table 1: Comparison of values for $[F^*]_{ss}$ calculated using equation 5 of the instructor's notes and actual $[F^*]_{ss}$ values from the calculations in figures Case3a-Case3c. Notice that using equation 5 overestimates $[F^*]_{ss}$.

Figure Data is from	$[F^*]_{ss}$ from integration of $[F^*]_{ss} _z$	$[F^*]_{ss}$ from $[h\nu]_{avg}$ (eq. 5)
Figure 1	0.0220 M	0.0392 M
Figure 2	0.00262 M	0.00271 M
Figure 3	0.0387 M	0.0390 M

If the average photon concentration is calculated to account for the varying ratio of excited state population to intensity across the sample then it can be used in equation 5 to calculate the actual average excited state concentration in the excited volume. However, this redefines the average from a normal mean to a specially weighted average. The functional form of this special average photon concentration can be derived by setting equation 5 equal to the integral used to calculate $[F^*]_{ss}$ from the actual intensity and solving for $[h\nu]_{sp}$:

$$\frac{k_a[h\nu]_{sp}[F]_o}{2k_a[h\nu]_{sp} + k_f + k_R} = [F^*]_{ss} = \frac{1}{\ell} \int_0^\ell \frac{k_a I(z)[F]_o}{2k_a I(z) + k_f cN_A h\nu + k_R cN_A h\nu} dz. \quad (13)$$

The solution is:

$$[h\nu]_{sp} = \frac{(k_f + k_R) \int_0^\ell \frac{k_a I(z)[F]_o}{2k_a I(z) + k_f cN_A h\nu + k_R cN_A h\nu} dz}{\ell k_a [F]_o - 2k_a \int_0^\ell \frac{k_a I(z)[F]_o}{2k_a I(z) + k_f cN_A h\nu + k_R cN_A h\nu} dz}. \quad (14)$$

Although this solution is valid it is not very convenient. However, since there is a solution that includes both extremes and the middle, using an average photon concentration is a valid way of formulating the expressions.

Instructor Notes Appendix B:

CAS Registry Numbers

by Jonathan Gutow, May 2003

Quinine Sulfate Dihydrate: 6119-70-6

Sulfuric Acid: 7664-93-9

Water: 7732-18-5

Instructor Notes Appendix C:
Answers to Questions in Handout

by Jonathan Gutow, May 2003

- 1) Based on your observations is the quinine fluorescence quenched dynamically, by complex formation or a combination of both? Explain why.

The students should note that the fluorescence decay rate depends upon quencher concentration in a linear manner except in the region where the decay rate is so high that the decay time is similar to the time resolution of the instrument. This implies dynamic quenching as described in the handout.

- 2) The rise time of the PMT is about 2.5 ns, the laser pulse is < 1ns and the sampling rate of the oscilloscope is 2 gigasamples/sec. What is the shortest fluorescence decay time you will be able to measure directly? Is there any way you can do better than this?

I expect an answer along the lines of the best you would expect to be able to do is about 2.5 ns. I usually show them the results of the deconvolution and some students suggest that with very noise free data we might be able to measure decay times of slightly less than 2.5 ns. I don't discuss other techniques such as time correlated single photon counting that could provide higher time resolution, which is one way to get improved time resolution. Other options are to use a Faster PMT. Last time I checked they could get into the 500 ps rise time regime. Of course the students should also note that without changing the laser we will never be able to do better than about 1 ns time resolution.

- 3) In analytical lab students often do not get very good results for the quinine concentration by measuring the fluorescence intensity. Based on what you have observed in this lab suggest some possible sources of error. Usually, but not always, students underestimate the quinine concentration.

I am expecting them to answer that there is halide salt contamination, probably NaCl, from fingers or inadequately cleaned glassware. This is because the salt will reduce the fluorescence intensity below that expected for the actual amount of quinine. If they contaminate their reference they may overestimate the sample concentrations instead.

- 4) Report your k_q and k_f . If you performed a Stern-Volmer analysis compare the results from the two methods. You should also compare k_q with k_D . If they are different explain the significance.

See the discussion of k_q versus k_D in the published summary. The students usually get good results if they perform the calculations correctly. They should comment on the fact

that their results are lower than the calculated k_D which implies that there may be some collisions that do not lead to quenching. However, since the calculation is only an estimate based on a lot of assumptions any disagreement is not surprising and should not be considered very significant.

- 5) The literature value for the lifetime ($1/k_f$) = 19.3 ± 0.6 ns (95% confidence limits). Compare this with your results and discuss any discrepancies.

I expect the students to discuss whether their results are the same within errors or different. If they are different, I expect the students to propose reasons (preferably experimental) why they might be different.

- 6) The best available literature value of $k_q = 6.2 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$ determined from lifetime measurements by Barrow and Lentz.¹⁰ This was determined in 0.05 M H_2SO_4 whereas the your results presented were in 0.5 M H_2SO_4 . The viscosity of 0.05 M H_2SO_4 is very close to that of pure water (not the same as 0.5 M). Compare your results with these and discuss any discrepancies.

Here I want them to go through the calculation described in the published summary. Usually if they properly calculate the ratios of the viscosity constants their results and the literature result are in close agreement.