Fluorescence Lifetime Quenching of Ruthenium-Polypyridal Complexes: Development of an Undergraduate Lab Exercise

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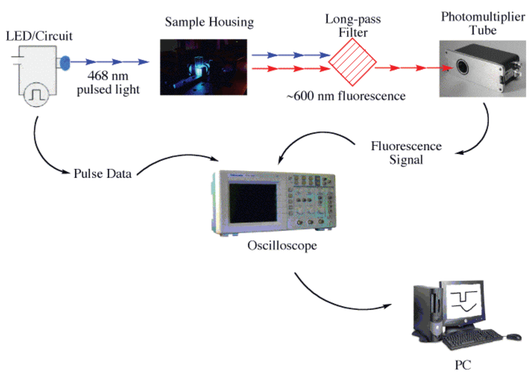
Introduction:

Fluorescence quenching is the dynamic interaction of a fluorescent molecule with a quencher: a molecule which causes de-excitation from the fluorescent state. it is a process which finds applications in fields as diverse as photosynthesis, protein dynamics, and photochemical engineering. Fluorescence lifetime quenching, in which the quenching process is monitored through the reduction of the fluorescence lifetime upon the introduction of quencher, is a useful method for studying the fluorescence kinetics. Briefly, a pulsed light source excites fluorescent molecules; a detector monitors the decay rate as the excited molecules relax back to their ground state. By observing the change in fluorescence decay time as concentration of quencher is increased, the rate constant for the interaction between the two molecules can be determined. This process is valuable as a laboratory experiment in physical chemistry, as it lies at the interface between spectroscopy and kinetics. Intermolecular interactions and photoelectronic properties of molecules can be studied simultaneously and interdependently.

We set out to develop an undergraduate-level experiment in fluorescence lifetime quenching as a project in the Advanced Chemistry Lab curriculum at Carleton College. In order to do this, we needed to design and build an instrument to measure fluorescence lifetime and to identify a set of fluorescent and quenching molecules whose quenching interactions could be measured; an appropriate experimental procedure then had to be developed and tested.

Instrument Design:

We chose to use an instrument based roughly on the design of Rusak et al., who use a pulsed LED as an excitation source for fluorescence. This has several advantages over the more traditional laser-powered setup: it is relatively inexpensive, safe, and simple to set up and use.



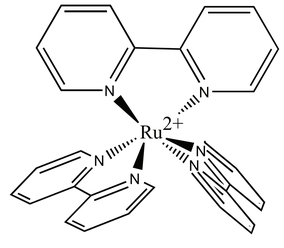
The experimental setup is depicted in Fig. 1. A circuit, designed and built by Tom Baraniak of the Carleton Physics Department, is coupled to an LED outputting at 468 nm; the circuit design controls the pulse rate and duration, with variability in both settings. The LED irradiates a sample cuvette under inert atmosphere, and fluorescent signal is monitored with a photomultiplier tube (Hamamatsu R928) coupled to a benchtop oscilloscope (Tektronix 1002). The oscilloscope outputs data to a PC, which saves data as a spreadsheet file.

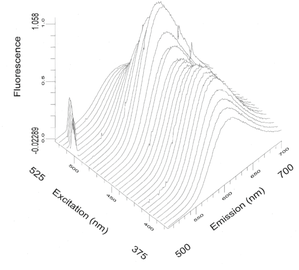
References:

1. Rusak, D.A.; James, W.H.; Ferzola, M.J.; Stefanski, M.J. *J. Chem. Educ.* **2006**, 83, 12.

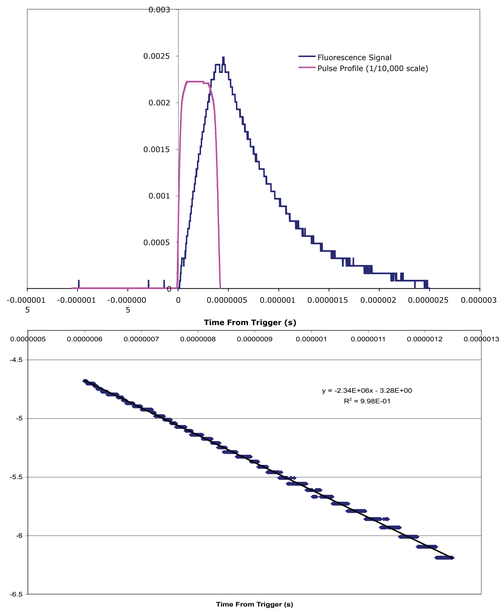
Experiment Design:

Our work was centered around designing an experiment for Chemistry 301, the upper-level kinetics course at Carleton College. Students would be given an introduction to the concepts of fluorescence lifetime quenching and the Stern-Volmer kinetic equations, which describe the effect of the quenching on fluorescence lifetime in terms of an intermolecular rate constant. They would then experimentally obtain the rate constant for a pair of fluorescent and quenching molecules. In designing the experiment, several such molecules were considered. $Ru(bipy)_3^{2+}$ as a fluorophore due to the large difference between its absorption and emission frequencies and its long fluorescence lifetime. For initial lab experiments, Fe(III) was used as a quencher. In the future we hope to try out derivatives of $Ru(bipy)_3^{2+}$ to observe trends in substituent effects on quenching, and to try various quenchers, perhaps to observe between the electron-transfer and energy-transfer based mechanisms of quenching.

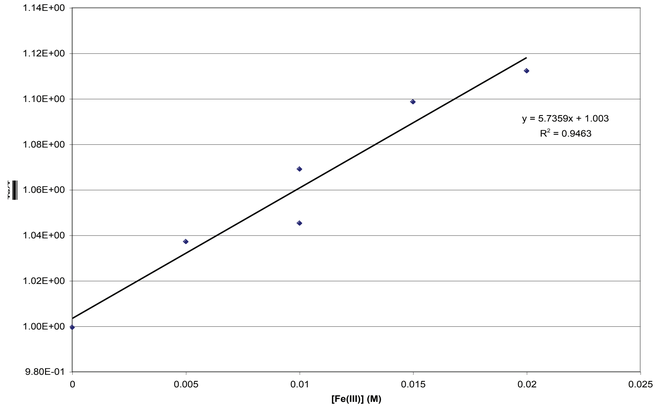




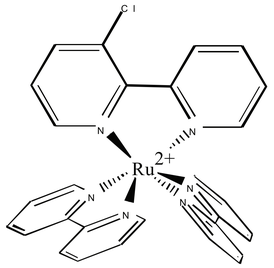
With the instrument built and sample molecules selected, the procedure for carrying out the experiment had to be designed and tested, and _reference_ data had to be obtained. One of the principal obstacles to making the project successful was the elimination of molecular oxygen, a pervasive and efficient quencher, from the reaction environment. To prepare the samples, an inert gas, Ar or N_2 was bubbled through flasks containing the sample and solution; fluorescence cuvettes were fitted with septa and similarly _degassed._ Sample was then syringed into the cuvettes and diluted with a ___amount of___. Several such cuvettes were thus prepared with varying amounts of quencher and a fixed $Ru(bipy)_3^{2+}$ concentration. Data was then taken and __plotted to obtain lifetime and rate constant values__. Typical data sets, from a solution of $Ru(bipy)_3^{2+}$ quenched with 1 mM Fe(III), are shown in Fig. 2.

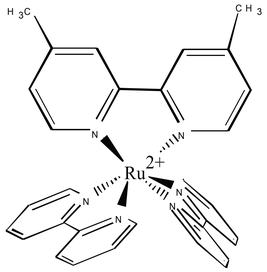


Results:



Future Work:





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