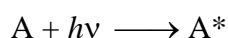


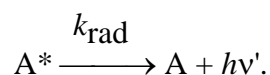
## NAPHTHALENE ELECTRONIC QUENCHING

This experiment is a relatively sophisticated introduction to photochemistry and photophysics. It uses state-of-the-art techniques of research that will be described briefly in this handout. A more complete description of the theory and techniques involved in the experiment can be found in the suggested references. The experiment deals with the measurement of naphthalene fluorescent lifetimes and the shortening or quenching of such lifetimes in the presence of ethyl iodide.

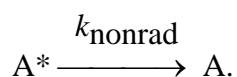
A naphthalene molecule may be raised to an excited state by absorption of an appropriately energetic photon:



Once in the excited state, it may dispose of the excess energy by one of two pathways. First, it can reradiate a photon of lower frequency at a certain rate characterized by some rate constant  $k_{\text{rad}}$ :



If this transition is allowed by selection rules, the phenomenon is known as fluorescence, and the whole process occurs typically on a time scale of several nanoseconds to several hundred nanoseconds. Alternatively, the molecule can lose its energy through some nonradiative pathway such as collisional de-excitation or internal conversion to vibrational energy characterized by some other rate constant  $k_{\text{nonrad}}$ :



Elementary kinetics then indicates that the disappearance or decay of  $A^*$  is given by:

$$\begin{aligned} -d[A^*]/dt &= k_{\text{rad}}[A^*] + k_{\text{nonrad}}[A^*] \\ &= k_d[A^*]. \end{aligned}$$

Separation of variables and integration from  $t = 0$  leads to:

$$[A^*] = [A^*]_{t=0} \exp(-k_d t),$$

a result familiar from first-order kinetics and identical to the form describing radioactive decay.

The excited molecules have an average or mean lifetime,  $\tau$ , which can be shown to be equal to the time required to reduce  $[A^*]$  by a factor of  $1/e$ . This relation reduces to

$$\tau_d k_d = 1$$

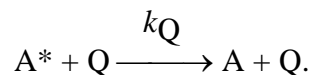
or

$$t_d = (1/k_d) = 1/(k_{\text{rad}} + k_{\text{nonrad}}).$$

Therefore, the lifetime  $\tau$  is a measure of the total rate at which an excited species  $A^*$  transfers its energy to the surroundings, be it in the form of photons or collisions.

Quenching is simply the shortening of the natural lifetime,  $\tau_d$ , of an excited species by addition of chemicals that enhance this transfer of energy. The exact mechanism of quenching is complicated, but (1) it appears to require a collision between quencher and excited molecule and (2) the process is greatly enhanced by either unpaired electrons (such as are found in  $O_2$ ) or by a large nuclear charge in the quenching species. (Iodine,  $Z = 53$ , has the greatest nuclear charge of any element routinely incorporated into organic compounds; hence our choice of ethyl iodide.)

The collisional nature of quenching lends itself to a second-order kinetic description:



Thus, in the presence of quencher, the disappearance of  $A^*$  is given by

$$\begin{aligned} -d[A^*]/dt &= k_d[A^*] + k_Q[Q][A^*] \\ &= (k_d + k_Q[Q])[A^*] \\ &= k[A^*], \end{aligned}$$

where  $k = k_d + k_Q[Q]$  or equivalently:

$$1/\tau = 1/\tau_d + k_Q[Q].$$

By measuring  $\tau$  at various  $[Q]$ , one can construct a linear plot of  $1/\tau$  vs  $[Q]$ . Such a plot is called a Stern-Vollmer plot and has a slope of  $k_Q$  and y intercept of  $1/\tau_d$ .

The first task is measuring the fluorescence lifetime of a given system. Since  $\tau$  is equal to the time required to reduce the initial concentration of  $A^*$  by a factor of  $1/e$ , one must by necessity measure  $[A^*]$  as a function of time. The number of fluorescence photons emitted by the sample at any given instant is proportional to  $[A^*]$ ; thus monitoring these photons with a sensitive photomultiplier could yield information about the time-dependent concentration of  $A^*$ . If there is initially a large number of excited molecules, one would expect that the number of emitted photons would at first be high and then would drop off exponentially. But what if the number of photons that successfully reached the photomultiplier was drastically reduced through the use of filters, until only one photon was detected? Theoretically, this single photon could arrive at any time, since exponential decay never reaches zero, but clearly there would be a greater probability that it would arrive earlier than later, more photons having been emitted by the sample then. If one noted the time at which these single photons arrived in several duplicate experiments, one would eventually have a picture of the relative probability of the emission of a fluorescent photon as a function of time.

The situation would be entirely analogous to the following example. Imagine a very large number of boxes each containing an identical distribution of colored marbles. By removing one marble at random from each box, one would eventually end up with a representative sample from which the color distribution could be determined. The same principle is exploited in single-photon counting, but in this case, repeating the experiment many thousand times, "collecting" one photon at random, and analyzing the distribution yield the time-dependent probability of fluorescence emission, from which one can determine  $\tau$ .

The advantages of single-photon counting techniques are threefold. First, significantly less stringent demands are placed on the operating speed of the electronics, allowing accurate measurements of very short lifetimes. Second, and more important, fluorescence lifetime measurements on extremely low intensity systems can be routinely made. And finally, the counting techniques employed have a very large dynamic range (almost five orders of magnitude in the configuration used in the present experiment), and thus lifetimes may be determined very accurately by plotting the decay over a time in which the signal decays by many factors of  $1/e$ .

## EXPERIMENTAL

A block diagram of the apparatus is provided at the end of this handout. A brief procedural description will be presented here; your lab instructor can provide more detail.

A spark-gap discharge powered by a high-voltage (6 to 7.5 kV) DC power supply delivers extremely short ( $\sim 4$  ns) pulses of ultraviolet light at a repetition rate of 5 to 10 kHz. This light is passed through a monochromator, in which a particular wavelength (296.3 nm) is selected. This monochromatic pulse then is absorbed in a solution of naphthalene contained in a quartz cell. The naphthalene molecules in turn fluoresce, and a photomultiplier detects these photons and translates them into current pulses. An electronic pulse derived from the current discharge across the spark gap is used to trigger

the START input of the time to pulse height converter (TPHC); the current pulse from the photomultiplier is amplified and used to trigger the STOP input of the TPHC. By means of very fast linear electronics, the TPHC then outputs a voltage that is proportional to the time delay between the START and STOP pulses. A computer interfaced with the apparatus then arranges for the conversion of this voltage into a binary number between 0 and 1023 by means of an analog to digital (A/D) converter. A portion of the computer's memory is divided into 1024 channels, the addresses of which are the same as the possible binary numbers obtainable from the A/D converter. The net result is that these channels represent 1024 time intervals that cover the range of the TPHC, and the time delay associated with each fluorescence photon is stored by incrementing the appropriate channel count.

A computer program controls the operation of the equipment and the recording of the photon counting data. Details for starting and running the computer and a brief description of the program are given in the appendix.

A standard stock solution of ethyl iodide in ethanol is provided in the lab. From this solution and using good quantitative analytical technique, make dilutions of 1, 2, 3, 4, and 5 mL of the stock solution in 25 mL volumetric flasks with ethanol as the solvent. DO NOT pipette from the stock solution flask or the ethanol bottle. Pour some of each into clean containers and pipette from them.

The glassware is reserved for this experiment only and should NOT be rinsed with anything except the stock solution or ethanol as appropriate.

Naphthalene lifetime spectra are taken on each of the solutions by filling the fluorescence cell with each solution in turn and adding ~2 mg of naphthalene to the solution in the cell. Before taking a spectrum, the solution in the cell must be purged of oxygen by bubbling with nitrogen. Have the TA or Lab Coordinator demonstrate the technique of filling, capping, and purging the cell.

Also take a spectrum on a sample of naphthalene in ethanol only (no ethyl iodide quencher). If at all possible make dilutions of 6, 7, and 8 mL and run those also.

## CALCULATIONS

The data will be in the form of 1024 channels, each containing the number of photons ( $N$ ) that arrived during the time interval that the channel represents. Since  $N(t)$  is proportional to  $[A^*]$ , the photon distribution will also be first order, i.e.

$$N(t) = N_0 e^{-kt}$$

or

$$\ln N(t) = \ln N_0 - kt. \quad (1)$$

Thus a graph of the natural logarithm of the number of counts vs. time should give a straight line with slope  $-k$ . Owing to equipment limitations, the first few and last few channels may not lie on the "straight line"; therefore, you must select which channels contain the best data for you to analyze. A least-squares fit of the data to Eq. (1) can be employed to determine the slope of the best fit and, in turn,  $\tau$  for each run. This fitting routine is part of the computer program that runs the experimental apparatus. (See appendix.)

Construct a Stern-Vollmer plot to determine the quenching rate constant  $k_Q$  and the natural lifetime  $\tau_0$ . Be sure to include an appropriate error analysis with your final values.

This experiment also affords an excellent opportunity to verify some fundamental principles of statistics. You will observe on the display plot how the data converges quite nicely to a straight line for channels with large numbers of counts, while there is considerable scatter for channels with low numbers of counts. Elementary statistics predicts that the percentage scatter should decrease as  $1/(\sqrt{N})$  where  $N$  is the number of counts in a given channel. Verify this result for one data set by calculating the root mean square deviation for 10 consecutive channels at three stages in the decay, such that the three regions correspond to large, intermediate, and small numbers of photon counts. (See computer operations appendix for instructions on getting these numbers printed out.) Root mean square deviation can be found by calculating the difference,  $\Delta$ , between the real channel count and the count predicted by your least-squares fit, squaring this difference, summing over the 10 channels, dividing by the number of channels minus one, and taking the square root, i.e.:

$$\text{R.M.S. dev} = \sqrt{\frac{\sum_{i=1}^{10} (\Delta_i)^2}{(10-1)}}$$

Dividing the R.M.S. deviation by the number of counts in the middle channel gives one a close approximation to percentage scatter, which, if statistical theory is in fact applicable, should be close to  $(\sqrt{N})/N$  or  $1/(\sqrt{N})$ .

## DISCUSSION

1. Naphthalene has an extinction coefficient of 300 L/(mol cm) at 296.3 nm. How many milligrams of naphthalene are necessary to absorb 99% of the light in the 1-cm pathlength in a 3-cm<sup>3</sup> fluorescence cell? Why would it be undesirable to make the solution more concentrated than this?

2. Why does  $\tau$  depend upon [Q] but not at all on the concentration of naphthalene?

3. For naphthalene,  $k_{\text{rad}}$  is only 25% as large as  $k_{\text{nonrad}}$ , that is, only one out of every five photons absorbed results in a fluorescence photon. How is it that by observing these photons, we can still only measure  $k_{\text{nat}} = k_{\text{rad}} + k_{\text{nonrad}}$ ?

4. If fluorescence photons are arriving at 1/10 the rate at which the flashlamp fires, then about  $(1/10)^2$  or 1% of the time, two photons would arrive at the photomultiplier from a single flash.

a) The TPHC will respond only to the first photon after each flash. Why would such "two-photon events" distort the overall data?

b) There are 1024 channels to store counts in. Estimate how many photons must be counted before an average channel has enough counts so that scatter is less than 1%?

c) If the flashlamp frequency is 10 kHz, about how long must you wait before scatter is, on the average, less than 1%?

## REFERENCES

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## **APPENDIX 1**

### **COMPUTER INSTRUCTIONS for NAPHTHALENE EXPERIMENT**

Turn on timing module power switch (small switch below the red lamps at right side of timing module rack).

Turn on FLASH LAMP POWER SUPPLY and set variac dial on the front panel so that the RATEMETER reads about 6 kHz. The power supply voltmeter should not read more than 7 kV. Once it is turned on and set, DO NOT change the setting or turn it off until you are done for the day.

Obtain your assigned DATA disk (assignments are on the lab sign-up/schedule sheets) from the Lab Coordinator or TA. Insert the DATA disk in the B: drive. Close the disk drive latch.

Turn on computer. Switch is on a power strip near the computer. Turn on the printer. Switch is on the left side of the printer.

#### **Commands**

At the C:> prompt change to the NEQ subdirectory :  
    cd neq

You should get a new prompt: C:\NEQ>

At this prompt type  
    n11a3-sa

This starts the Fluorescent Lifetime program to take spectra and compute lifetimes.

Note: Steps to this point may already be done by the lab staff.

#### **NEQ PROGRAM DESCRIPTION**

The Fluorescent Lifetime program is written in a combination of MicroSoft QuickBASIC and machine language. It controls the data acquisition and lifetime computation for the experiment.

The program is interactive and operates from a command list. You will be asked for additional information (such as filenames for saving spectra) when the program needs it.

Brief description of the procedure:

1. Prepare the sample in the quartz cuvette and place in the sample compartment. Replace the compartment cover, and cover the area with the blackout cloth. Turn on the PMT power supply.
2. On the computer do CLEAR DATA ARRAY then SAMPLE ID. Do START DATA and allow to run for about 5 minutes. While it is running, you can do DISPLAY DATA LOOP, to watch the data accumulate. Return to command menu a few seconds before you want to stop, then STOP DATA. Do a SAVE DATA. SET CURSORS if necessary and then do CALCULATE LIFETIME. When the calculation is complete answer y (yes) to the "dump screen to print" message. When the message comes up again, answer n (no; unless you want another copy); then return to command menu.

Some Notes:

1. Use the CLEAR DATA ARRAY command before starting a run (but after you have computed and printed the lifetime for the previous run, or at least saved the previous data to disk).
2. The program is not "bullet proof". Incorrect entries in response to command requests can cause the program to abort.
3. No background spectrum is required.
4. To get the data to do the statistics (RMS deviation) calculation use the menu selection "Dump Statistical Data." BEFORE you select this item make sure the cursors are positioned correctly. This routine will print ten channels starting at the left cursor, ten channels immediately to the left of the right cursor, and ten channels from about 1/3 of the way between the cursors. This only needs to be done for ONE data set. One of the higher quencher concentrations is best, because the right hand portion of the spectrum will have low numbers of counts, giving the largest spread of values for channel counts.
5. As soon as you do the STOP DATA command you can turn off the PMT power supply, remove the cuvette from the sample chamber, and start preparing the next sample while your partner does the remaining computer operations (i.e. SAVE, SET CURSORS, etc.)