The Rate Constant for Fluorescence Quenching[1]

Purpose

This experiment utilizes fluorescence intensity measurements to determine the rate constant for the fluorescence quenching of anthracene or perylene by CCl_4 in an ethanol solvent.

Introduction

The fluorescence intensity of a substance depends on the natural lifetime of its first excited singlet state and on the rate that the first excited state is deactivated by nonradiative processes. Much of the nonradiative deactivation comes about because of collisions of the excited molecule with solvent molecules. However, some substances are particularly efficient at deactivating, or quenching, excited states. These tend to be substances with heavy atoms (for example halogens) or paramagnetic compounds. Oxygen is a particularly good quencher, therefore it is often necessary to remove the oxygen from solution before measuring fluorescence spectra. Fluorescent probes, such as dansyl chloride, are used in biochemistry to study the various binding sites in large macromolecules through the difference of the quenching rates of the bound verses free probe.

Theory

Aromatic hydrocarbons generally show intense fluorescence but no phosphorescence in fluid solution at room temperature. The fluorescence of most of these compounds is efficiently quenched by halogenated alkanes such as carbon tetrachloride and chloroform (2-5). To a certain extent these quenching events result in photochemical reactions between the aromatic hydrocarbon and the quencher. The object of this experiment is to determine the rate constant for this quenching reaction through measurements of the fluorescence intensity of the aromatic hydrocarbon in ethanol with various amounts of the quencher. The theory will be discussed with reference to the quenching of anthracene fluorescene with CCl_4 in ethanol. The method employs the Stern-Volmer mechanism (6,7).

When ultraviolet light is shone on a solution of anthracene in ethanol, a given anthracene molecule may absorb the light and in the process get converted to one of its excited singlet states. If a state other than the lowest excited singlet state becomes populated, the molecule will rapidly ($\sim 10^{-12}$-10^{-13}s) relax to the lowest excited singlet state. The anthracene molecule in this state is denoted by A* and can now do one of several things. It can emit a photon and in the process get converted back to the ground state. This is fluorescence and the fluorescence decay process is a first-order rate process with a rate constant denoted by k_f.

$$A^* \rightarrow A + h\upsilon$$

A second possibility is for the excited anthracene molecule to lose its energy in the form of heat rather than light. In this nonradiative process, the anthracene either gets converted back to ground state (internal conversion) or to the lowest triplet state (intersystem crossing) and then to the ground state. No emission from the triplet state (phosphorescence) has been observed for anthracene in ethanol at room temperature. Both routes result in the disappearance of anthracene in its excited singlet state without the emission of light and can be lumped together in a first-order rate process with rate constant denoted by k_{nr}.

$$A^* \rightarrow A + heat$$

The only other first-order process would be a photochemical reaction, but this does not occur for anthracene in pure ethanol to a significant extent.

The quantum yield of fluorescence, Φ^o, for anthracene in pure ethanol is the rate at which excited anthracene decays via fluorescence divided by the rate at which it decays via both fluorescence and nonradiative decay.

$$\Phi = \frac{k_f[A^*]}{k_f[A^*] + k_{nr}[A^*]} = \frac{k_f}{k_f + k_{nr}} \tag{1}$$

If CCl_4 is present in the solution, a third mechanism for the excited anthracene molecules to become depleted is available. This is the quenching of anthracene by CCl_4 and is assumed to be second-order with rate constant k_q.

$$A^* + CCl_4 \rightarrow \text{Quenching}$$
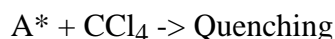
This quenching process occurs through several different paths. Quenching may result in ground state anthracene and heat or in triplet state anthracene and heat. In the latter case the triplet state anthracene will usually relax back to the ground state with the release of heat. It is also possible for the triplet anthracene to react with CCl_4 to a certain extent. In addition, the quenching of the excited singlet state will also occur via a photochemical path to a certain extent.[1] There are about eight different products for this reaction. Two of the products have been identified as 9-chloro- and 8, 10-dichloroanthracene. The chief photochemical product, however, appears to be 9-chloro, 10-trichloro-methyl, 9, 10-dihydroanthracene. All these quenching processes of anthracene fluorescence by CCl_4 are lumped together in the second-order rate constant k_q. The net effect of the presence of CCl_4 is a reduction in the quantum yield of fluorescence of anthracene to the following value[2]

$$\Phi = \frac{k_f[A^*]}{k_f[A^*] + k_{nr}[A^*] + k_q[A^*][CCl_4]} = \frac{k_f}{k_f + k_{nr} + k_q[CCl_4]} \tag{2}$$

Dividing Φ^o by Φ we get the relative quantum yield, which yields the Stern-Volmer expression:

$$\Phi^o/\Phi = 1 + \frac{k_q}{k_f + k_{nr}}[CCl_4] \tag{3}$$

The relative quantum yield in this expression can be replaced by the relative fluorescence intensity I^o/I which is easily measured. A plot of I^o/I versus $[CCl_4]$ should yield a straight line with a slope $k_q/(k_f + k_{nr})$.

In order to determine the quenching rate constant k_q, the $(k_f + k_{nr})$ must be determined by an independent experiment. It will be shown below that the measurement of the time dependence of the anthracene fluorescence decay in pure ethanol can be used to obtain this. In the absence of CCl_4 the rate of disappearance of excited anthracene is given by
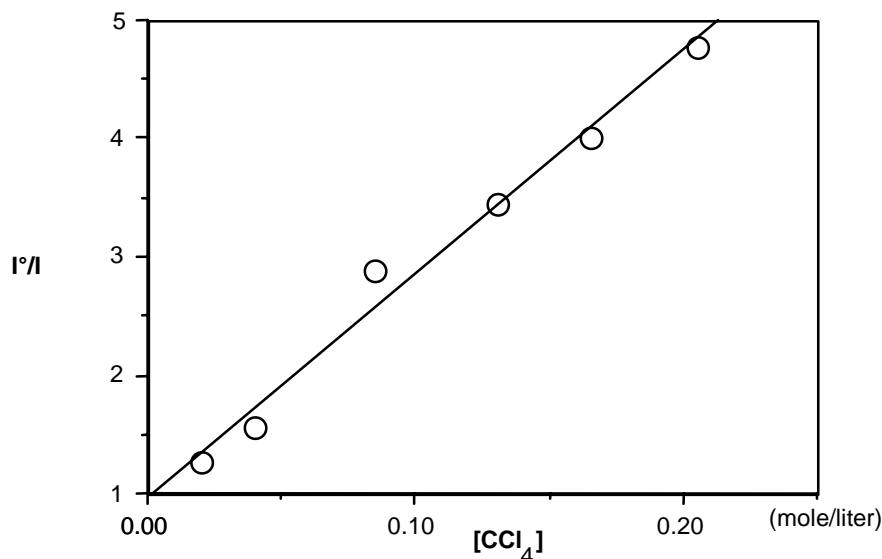
$$\frac{d[A^*]}{dt} = -k_f[A^*] - k_{nr}[A^*] = -(k_f + k_{nr})[A^*] \tag{4}$$

Therefore

$$\frac{d[A^*]}{[A^*]} = -(k_f + k_{nr})dt \tag{5}$$

Integrating between the limits $[A_o*]$ and $[A_t*]$ on the left and 0 and t on the right we obtain

$$\ln[A_t*] \ = \ \ln[A_0*] \ - (k_f + k_{nr})t \qquad\qquad (6)$$

Stern-Volmer plot for the fluorescence quenching of anthracene by CCl$_4$ in ethanol solution.

<u>The Determination of $k_f + k_{nr}$:</u> You will determine $k_f + k_{nr}$ as a dry lab, in other words you will use the data in Table 1, instead of measurments from the lab. Since the fluorescence intensity is proportional to the excited anthracene concentration, the fluorescence intensity can replace the excited anthracene concentration in Equation 6. Lifetime data for both anthracene and perylene are presented in Table 1. Plot the ln of intensity versus time and from the slope determine ($k_f + k_{nr}$). Once ($k_f + k_{nr}$) is determined, the value of the quenching rate constant can be determined from the Stern-Volmer plot. The fluorescene lifetime data is simulated from data taken by Dr. Arthur Halpern at Northeastern University using the single photon counting technique.

Equipment: Supplies

       Spectrofluorimeter, Perkin Elmer 650s (see the video tape)
       Fluorescence cuvette
       5 x 25 ml volumetric flasks
       1 x 50 ml volumetric (for anthracene stock solution)
       2 x 100 ml volumetric (for anthracene and CCl$_4$ stock solutions)
       pipets: 2, 3, 5, 10, 15 ml
       anthracene
       CCl$_4$
       Ethanol, 95%

Procedure

       The anthracene concentrations used in this experiment depend on the wavelength of the exciting light. It is important that the absorbance of the solution is not more than about 0.2 so that the fluorescence intensity is uniform throughout the solution. If 366 nm light is used to excite the anthracene its concentration should be 1-2 x10^{-5}M. If perylene is used as the

fluorescing molecule, the same concentration should be used. The CCl_4 concentrations should range from 0 to about 0.20 M for the anthracene quenching and from 0-1.0 M for the perylene quenching. Four different dilutions should be sufficient. It is important to keep these solutions out of bright light so that the fluorescence intensity measurements take place before any of the fluorescing molecule disappears through the photochemical reaction.

Since the concentration of anthracene is so small, the stock solution should be made up in two steps. For example, first make a 5.0×10^{-4}M solution of anthracene by diluting about 9 mg of anthracene to the mark with ethanol in a 100 ml volumetric flask. Now dilute this by 5 by transferring 10 ml of this solution to a 50 ml volumetric and diluting to the mark, giving a 1.0×10^{-4}M stock solution. Then, to make your final solutions pipet 5 ml of the anthracene stock solution and the appropriate amount of CCl_4 stock solution into each of the 25 ml volumetric flasks provided. To make up the CCl_4 stock solution use the volumetric glassware as listed above. Plan out your dilutions before coming to lab. Remember that you also need to determine the fluorescence intensity of anthracene with **NO** CCl_4 present, that is, I^O.

Make sure to rinse the fluorescence cuvette several times with the new solution before taking its spectrum. Follow the cell washing instructions in the spectrophotometer manual.

Discussion

It is interesting to compare the value of k_q with the diffusion controlled rate constant k_d of $6 \times 10^9 1$ mole^{-1} s^{-1} (8). One might conclude from this that roughly k_q/k_d of the collisions between excited anthracene and CCl_4 result in quenching. Determine the fraction of collisions that result in quenching (this is about one half). Determine from k_q the average time it takes for an excited anthracene molecule to become quenched by a CCl_4 molecule at a given concentration of CCl_4. The value obtained can be considered to be a lower limit on the time it takes for a given anthracene molecule to collide with a CCl_4 at the concentration used in the calculation. For example, at a concentration of 1.0 M CCl_4 the average time it takes for a given excited anthracene molecule to become quenched by a CCl_4 molecule is equal to the reciprocal of the quenching constant.

Literature Cited

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    New York, 1966, pp. 663-70.
(8) Reference (7), pp. 626-7.

Table 1: Fluorescence Lifetime Data for Anthracene and Perylene

Time (ns)	Intensity (photons/units time) Anthracene	Perylene
0	62620	66503
1	50408	57647
2	41250	49972
3	32472	43327
4	27218	37587
5	21556	32565
6	17708	28237
7	14247	24467
8	11352	21210
9	9193	18385
10	7560	15950

Endnotes

[1]Only photochemistry coming from the excited singlet state will cause fluorescence quenching. Photochemistry from the triplet state can also occur but will not cause fluorescence quenching.

[2]The derivation of equation (2) assumes that the presence of CCl_4 has no effect on the first-order rate constants k_f and k_{nr}.