Time Resolved Pulsed Laser Photolysis Study of Pyrene Fluorescence Quenching by I⁻ Anion

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Abstract

Fluorescence quenching has been studied as a fundamental chemical phenomenon and as a process in biochemical systems. In biological membrane studies, quenching is often used to reveal the accessibility of fluorophores to quenchers.¹ For example, if a fluorophore is bound to a protein or membrane which is impermeable to the quencher, quenching cannot occur. In this experiment quenching is studied as a chemical phenomenon where different concentrations of an iodine quencher were used to measure the fluorescing and quenching rate constants of a pyrene (Figure 1) iodine reaction using photolysis. The second-order reaction was studied under pseudo-first order assumptions. By observing the trend in fluorescence with different concentrations, the rate constants could be determined. The spontaneous fluorescence decay constant was determined to be 2.83 ± 0.062 E+6 s⁻¹ and the quenching constant was found to be 1.99 ± 0.333 E+04 (mM s)⁻¹. From the results it could be concluded that for a reaction following pseudo-first order conditions, the rate of fluorescence was directly proportional to the quencher concentration.

Introduction

Figure 1. Pyrene Structure²
Fluorescence is the emittance of photons by an excited molecule returning to its ground state. Measured by fluorescence spectroscopy, a type of electromagnetic spectroscopy, it reveals information regarding the excited state of molecules by exciting the electrons in a molecule using light. Ultraviolet light is usually used. Excited state molecules have very different properties than molecules in the ground state. Excited electrons return to their ground states via fluorescence or quenching. Quenching refers to decreasing the fluorescence of the excited molecule by the presence of another molecule willing to accept the electrons. Quenched molecules return to their ground state without emitting any photons. One type of pathway is via a reductive transition that results in the formation of an anion and radical.

The rate constant for an excited molecule returning to its ground state contains two components—the fluorescence rate and the quenching rate. The fluorescence rate ($k_0$) is determined by measuring the rate for a one-component system (without a quenching agent). Equation (1) shows that $k_0$ is determined by plotting the fluorescence intensity versus time where $k_0$ is the exponential constant.\(^5\)

\[
I = I_0 e^{-k_0 t} \quad \text{(Equation 1)}
\]

In order to determine $k_q$, the quenching rate constant, the concentration of the quenching agent and quenched molecules must be considered. In this case, the rate equation would look like equation (2).

\[
\frac{-d[B]}{dt} = k[A][B] \quad \text{(Equation 2)}
\]

There are certain cases where if the concentration of one substance is much greater than the other, the reaction can be considered a pseudo-first order reaction.\(^4\) In this case the lower concentration is ignored in the rate equation and the constant is only dependent on the higher concentration component, creating a rate equation similar to Equation 4.
\[-\frac{d[B]}{dt} = k'[B] \quad \text{(Equation 3)}\]

\[-\frac{d[B]}{dt} = k'[B] \quad \text{(Equation 4)}\]

In order to determine the value of the quenching rate constant, the fluorescing constant found from a single component system in Equation 1 is used. For the same pyrene system with the addition of iodine quencher, the observed rate constant, k_{obs}, is equivalent to k_0 plus k_q where k_q is equal to k' seen in Equation 4 for a pseudo-first order reaction.\(^5\) This yields Equation 5, and differentiating produces Equation 6. From these values, a graph of k_{obs} vs iodine concentration can be plotted which will produce a line with slope k_q and k_0.

\[-\frac{d[*Py]}{dt} = (k_0 + k_q[I^-][*Py]) \quad \text{(Equation 5)}\]

\[\ln I = \ln I_0 - (k_0 + k_q[I^-])t \quad \text{(Equation 6)}\]

The objective of this experiment was to understand how fluorescence decay could be used to measure the rate constants of photochemical reactions using laser photolysis by exploring the excited state of pyrene, seen in Figure 1. This lab will determine the quenching rate constant for iodine and fluorescing rate constant of pyrene.

**Experimental**

A 10 mL solution of 0.1 M KI was prepared with 0.1680 g ± 0.0004 g KI measured using a Mettler AE100 balance and a 50% ethanol-water solution. Using serial dilution, five 10 mL ± 0.04 mL samples with concentrations 0, 10, 20, 30, and 40 mM KI were made. Pyrene was then added to each sample to produce 10 µM solutions. To avoid oxygen quenching the pyrene fluorescence, each cell was purged using a stream of nitrogen gas. The cells were then capped to avoid any exposure to ambient oxygen.
Figure 2. Photolysis apparatus setup

A photolysis apparatus as seen in Figure 2 was used to record data. The laser produced UV light at a constant wavelength of 337.1nm and used to excite each sample while an oscilloscope interpreted the data collected for the fluorescent lifetime of each pyrene sample. The photodiode uses a semiconductor that produces a charge difference when detecting a photon. The current is then converted to the oscilloscope’s signal which is proportional to the number of fluoresced photons. Because the laser emits radially, the sample cuvettes were placed perpendicularly to prevent backscattered and incident light which could interfere with the photodiode readings.

Results and Discussion

The five samples with various [KI] concentrations were excited using a 337.1nm wavelength in order to determine the $k_{obs}$ values. The results of the experiment can be seen in Figure 3 and Table 1. Figure 3 is a graph of the fluorescence intensity detected by the photodiode versus time. Since the concentration of iodine in the solution is so much greater than that of pyrene, this reaction can be considered pseudo-first order. This means the rate constant
will be primarily influenced by the concentration of iodine in the sample therefore allowing us to ignore the pyrene concentration in determining the rate constant value (Equation 4). The greater the concentration of [KI] in the solution means there is more quencher and should consequently yield a higher $k_{obs}$ value. This trend can be seen in Table 1, where the $k_{obs}$ was determined from the exponential constant in the exponential fit from Microsoft Excel (Equation 1).

![Graph of fluorescence intensity vs. time to determine $K_{obs}$ values.](image)

**Table 1. $K_{obs}$ values for different I⁻ Concentrations**

<table>
<thead>
<tr>
<th>[KI] (mM)</th>
<th>$R^2$</th>
<th>$K_{obs}$ (1/s)</th>
<th>Uncertainty (1/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>.98</td>
<td>2.869E+06</td>
<td>±1.32E+4</td>
</tr>
<tr>
<td>10</td>
<td>.97</td>
<td>2.942E+06</td>
<td>±1.69E+4</td>
</tr>
<tr>
<td>20</td>
<td>.99</td>
<td>3.273E+06</td>
<td>±6.98E+3</td>
</tr>
<tr>
<td>30</td>
<td>1.0</td>
<td>3.423E+06</td>
<td>±5.31E+3</td>
</tr>
<tr>
<td>40</td>
<td>.94</td>
<td>11.02E+06</td>
<td>±1.29E+5</td>
</tr>
</tbody>
</table>

The initial baseline of each data set was removed in order to achieve a proper fit. For the 40mM sample, points near the end were also removed because they created too much noise that
produced a very low $R^2$ value. The other sample’s $R^2$ values were all very close to one (>0.90) indicating that the fit was good. With increasing quencher concentration, the exponential decay becomes steeper. The trend in the values shown in Figure 4 indicates a steady increase in $K_{obs}$ values until the 40mM concentration where a large increase is observed. A possible explanation for this data outlier could be that the cell was contaminated with ambient oxygen. Oxygen is more electronegative than iodine and will compete for electrons by opening another pathway (act as another quencher) and increase the rate at which excited electrons return to their ground state. There was also room for error when preparing the serial dilutions which would result in nonlinear values.

To better visualize the data, the natural logarithm of the intensity was plotted versus time. This is represented as Figure 4. The data was truncated in order to eliminate the noise effects that appeared after longer times. Plotting the data in this way allows for linear regression statistics to
be performed which gives the uncertainty for each $k_{\text{obs}}$ value. These can be seen in more detail in Figures 6-11 in the Appendix. Table 1 summarizes these values.

The reaction follows pseudo-first order conditions, meaning that the concentration of iodine is so much greater than pyrene. This implies that the pyrene concentration can be ignored because of its small value it has such a small effect on the reaction compared to iodine. These $k_{\text{obs}}$ concentrations were plotted against the [KI] values in Figure 5 to determine the quenching rate constant, $k_q$, and the fluorescing rate constant, $k_0$. These are represented by the slope and intercept of the best fit line, respectively. The $k_{\text{obs}}$ for the 40mM sample was not included in the plot because it is an outlier.

A liner fit executed on the remaining data yielded a $k_q$ value of 1.99E+04 mM$^{-1}$ s$^{-1}$ and a $k_0$ value of 2.83E+6 s$^{-1}$. Regression statistics found in the Appendix (Figure 11) reveal the uncertainty of $k_q$ as 1.99 ± 0.333 E+04 (mM s)$^{-1}$. 

**Figure 5: $K_{\text{obs}}$ versus I$^-$ concentration to determine $K_q$**
Conclusion

Experimental results revealed that as [KI] increased, the pseudo-first order reaction rate, $k_{obs}$ increased linearly. The only error seemed to occur with the 40mM sample which gave a much higher $k_{obs}$ value than expected which was most likely due to oxygen contamination. The concentration was graphed against the $k_{obs}$ rates to determine $k_q$ and $k_0$. The experiment successfully produced results for the quenching rate constant of the reaction of $\Gamma$ with excited pyrene as well as the spontaneous fluorescence rate constant for pyrene. The results are also a testament to the advantage of pseudo-first order reaction conditions. Assuming these conditions made the quenching reaction easily studied as would be compared to a second order reaction.

Acknowledgements

I would like to acknowledge my lab group members, Bashayer Aldakkan, Jason Guth, and Jiaheng Yu for their efforts and skills in helping complete the experiment as well as the laboratory TAs Bradley Rogers and Codey Henderson and Dr. Milosavlejvic for their guidance and direction during the experimental process and data analysis.

References


Appendix

**Figure 6:** Regression Statistics for 0mM \( k_{\text{obs}} \)

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-4.261167299</td>
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<tr>
<td>X Variable 1</td>
<td>-2918427.094</td>
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**Figure 7:** Regression Statistics for 10mM \( k_{\text{obs}} \)

<table>
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**Figure 8:** Regression Statistics for 20mM \( k_{\text{obs}} \)

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**Figure 9:** Regression Statistics for 30mM \( k_{\text{obs}} \)

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**Figure 10:** Regression Statistics for 40mM \( k_{\text{obs}} \)

<table>
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**Figure 11:** Regression Statistics for \( k_q \) and \( k_0 \)

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<td>X Variable 1</td>
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Calculations and Uncertainty Analysis:
**Uncertainty Values**

Mettler Balance: ± 0.4 mg

10 mL Class B flask: ± 0.04 mL

1 mL transfer pipette: ± 0.012 mL

1.) Mass of KI

\[
KI = \frac{0.1 \text{ mol}}{1000 \text{ mL}} \times 10 \text{ mL soln} \times \frac{166 \text{ g}}{1 \text{ mol } K} = 0.166 \text{ g } KI
\]

2.) Uncertainty for [KI] for 40 mM dilution:

\[
C = \frac{M}{V} = \frac{0.168 \text{ g} \times \text{mol}}{10 \text{ mL} \times \frac{1 \text{ mL}}{166 \text{ g}}} = 101 \text{ mM}
\]

Relative Error\(^2\) = \(\left(\frac{0.0004 \text{ g}}{0.168 \text{ g}}\right)^2 + \left(\frac{0.0004 \text{ mL}}{0.01 \text{ mL}}\right)^2 = (6.38 \times 10^{-3})(101 \text{ mM}) = 0.644 \text{ mM}\)

101 ± 0.644 mM

3.) Uncertainty for [KI] for 30 mM dilution

\[
C = \frac{M}{V} = \frac{3 \text{ mL} \times 101 \text{ mM}}{10 \text{ mL}} = 30.3 \text{ mM}
\]

Relative Error\(^2\) = \(\left(\frac{0.00012 \text{ mL}}{0.001 \text{ mL}}\right)^2 \times 3 + \left(\frac{0.0004 \text{ mL}}{0.01 \text{ mL}}\right)^2 = (0.04)(101 \text{ mM}) = 4.04 \text{ mM}\)

30.3 ± 4.04 mM

4.) Uncertainty for [KI] for 20 mM dilution:

Same process as in calculations 2 and 3:

20.2 ± 2.82 mM

5.) Uncertainty for [KI] for 10 mM dilution:

Same process as in calculations 2 and 3:

10.1 ± 1.62 mM