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

Cameron Incognito, Ryan Bella, Cassandra Smith, Brandon Alexander

Department of Chemistry, The Pennsylvania State University, University Park, PA 16802

Submitted: February 28, 2013 (CHEM 457, Section 005)

Abstract

In this experiment, fluorescence decay is used to measure the rate constants of the photochemical reaction of pyrene and potassium iodide using a photolysis apparatus. The rate constant of the uni-molecular decay of the pyrene singlet state, \( K_o \), was determined to be \( 3.2 \times 10^6 \pm 2.45 \times 10^5 \) M\(^{-1}\) s\(^{-1}\), and the quenching rate constant for the reaction of I⁻ with excited pyrene, \( K_q \), was determined to be \( 1.7 \times 10^8 \pm 1.0 \times 10^7 \) M\(^{-1}\) s\(^{-1}\). The quenching rate constant for this reaction is large indicating that I⁻ is a very powerful quencher in the reaction with excited pyrene. This experiment successfully demonstrated the measuring of rate constants using photochemical processes.

Introduction

Molecules in their excited states often possess significantly different physical and chemical properties than their ground state counterparts. These changes are able to be measured using a process called fluorescence spectroscopy, which is a type of electromagnetic spectroscopy in which a beam of light (often ultraviolet) excites electrons in a sample and causes the sample to emit light.\(^2\)
Pyrene, seen in Figure 1 above, is a polycyclic aromatic ring system consisting of four fused benzene rings. Pyrene, when excited, possesses a different reduction potential when in its excited state as compared to its ground state. This transition to the excited state can be caused by a source of light or the presence of another chemical with a lower or higher reduction potential than pyrene. One such compound that can cause this transition is potassium iodide which dissociates to the iodide anion and the potassium cation in solution. The iodide anion quenches the reaction of the excited state pyrene and stabilizes the reaction increasing the relaxation rate (time it takes the excited state to fall to the ground state). In this experiment an ultraviolet light of wavelength 337.1 nm was targeted at a pyrene (Py) sample and was absorbed causing the transition to the excited state pyrene (*Py) due to the promotion of an electron into the excited state. There are then two different forms of decay which can occur after excitation: fluorescence of a photon or radiation-less decay. These reactions are shown below:

\[ Py + h\nu_1 \rightarrow *Py \]

\[ *Py \rightarrow Py + h\nu_2 \text{ (fluorescence of a photon)} \]

or

\[ *Py \rightarrow Py + heat \text{ (radiation-less decay)} \]
The time delay between the initial excitation of the molecule and the photon emission is on “the
order of hundreds of nanoseconds,” and can be used to determine several different reaction rate
constants, namely \( k_o \) and \( k_q \).²

When the iodide anion (an electron donor) is added to a solution of pyrene (a strong
electron acceptor due to conjugation), the fall from the excited state to the ground state goes
through a transition state in which \( \text{Py}^- \) and \( \text{I}^- \) are formed.² This reaction is known as a photo
induced electron transfer reaction (single electron transfer from \( \text{I}^- \) to \( \text{*Py} \)) and is shown below:

\[
\text{Py} + \text{I}^- + h\nu \rightarrow \text{*Py} + \text{I}^- \\
\text{*Py} + \text{I}^- \rightarrow ^3\text{L}_b \text{Py}^- + \text{I}^- 
\]

The excited state of this reaction can be regarded as the products of a chemical reaction
and the initial state can be regarded as the reactants. When viewed in this kinetic manner, the
number of excited state molecules relaxing to the ground state can be set equal to a constant, \( k_q \),
multiplied by the total molecules currently in the excited state.² This relationship is shown in the
equation below:

\[
-d[\text{*Py}]/dt = k_o[\text{*Py}] 
\]

Assuming the excited state concentration of pyrene is proportional to intensity, \( I \), the separable
differential equation (1) can be solved to yield the following equation:

\[
I = I_o e^{-k ot} 
\]

The value of \( k_o \) can be found as the negative of the slope of the plot of LN(intensity) versus
time.² The quenching rate constant, \( k_q \), for the reaction of pyrene and the iodide anion can be
found via a second order kinetic rate equation of the form below:
\[-d[B]/dt = k[A][B] \quad (3)\]

This rate equation can be simplified if the concentration of one of the reactants \((A)\) is significantly larger (several orders of magnitude difference) than the other \((B)\). If this is the case it can be assumed that the rate equation only depends on the concentration of \(A\) as \([A]\) will not change drastically when \([B]\) diminishes. Therefore a pseudo-first order rate law of the form below can be written:\(^2\)

\[-d[B]/dt = k'[B] \quad (4)\]

\[k' = k[A] \quad (5)\]

In this chemical reaction, the fluorescence rate constant \((k_o)\) can be determined from examining a single component system. The quenching rate equation, however, depends on two variables and must be determined via the observed rate constant, \(k_{obs}\). The constant \(k_{obs}\) is equal to \(k_o\) plus \(k_q\), the “constant of \(I\) quenching \(k'\).” The value of \(k'\) can be obtained from eq. (4) or eq. (5) if utilizing the pseudo-first order approximation.\(^2\) Substituting the equality \(k' = k_q[I]\) into eq. (4) and (5) yields the following equation:

\[-d[*Py]/dt = (k_o + k_q[I])*Py \quad (6)\]

Assuming \([*Py]\) is proportional to intensity, the differential equation (6) can be solved to yield:

\[LN(I) = LN(I_o) - (k_o + k_q[I])t \quad (7)\]

After determining \(k_{obs}\) for each independent \(I\) concentration, a plot of \(k_{obs}\) versus \([I]\) will yield a linear relationship with a slope equal to \(k_q\) and a y-intercept of \(k_o\).\(^2\)
Experimental

This experimental is fully detailed in the Chem 457 laboratory manual. First, the laser photolysis apparatus is assembled as shown in Figure 2. The laser is that of a pulsed nitrogen laser set to emit an ultraviolet wavelength of 337.1 nm at a quartz sample cell. The wavelength is then passed through an optical filter (set perpendicular to the cell) which absorbs light below 375 nm, causing scattered light to be obstructed. The photons are then collected in the photodiode where they are converted into an electrical charge by a semiconductor. These charges are then read and recorded by the oscilloscope.

![Photolysis apparatus setup](image)

**FIGURE 2.** Photolysis apparatus setup

Five different 10 mL solutions were examined in this experiment containing 10 μM pyrene concentration and KI concentrations of 0, 10, 20, 30, and 40 mM. A mass of 0.1661 g of KI was placed into the 40 mM 10 mL volumetric flask and was diluted with approximately 5 mL of 50% ethanol-water solution. The solution was stirred until homogeneous and was then further diluted up to the mark with 50% ethanol-water solution. From the 40 mM volumetric flask, 1 mL of solution was pipeted into the 10 mM volumetric flask, 2 mL of solution into the 20 mM volumetric flask, and 3 mL of solution into the 30 mM volumetric flask. Next, 2 mL of 100 μM
pyrene in ethanol was added to each of the 5 volumetric flasks to produce the intended 10 μM pyrene concentration. Each volumetric flask was then diluted with 50% ethanol-water solution up to the mark.

A quartz cell was 2/3 filled with the 0 mM sample solution and was clamped to a ring stand with a nitrogen gas line placed inside touching the bottom of the cell. A lid was placed on the cell and nitrogen was turned on to a gentle flow for 5 minutes to remove all oxygen in the sample. Oxygen was removed from the sample as it acts as a strong quencher and therefore can behave similarly to the I⁻ anion and obscure the experimental results. After 5 minutes, the nitrogen line was removed while keeping the lid on the cell to minimize oxygen entry. The cell was placed in the photolysis apparatus and the fluorescence intensity of the sample was recorded on the oscilloscope. This experimental was repeated twice (two trials per sample) for all of the sample solutions (0, 10, 20, 30, and 40 mM).

Results and Discussion

The rate constant of the uni-molecular decay of the pyrene singlet state, \( K_o \), was determined to be \( 3.2 \times 10^6 \pm 2.45 \times 10^5 \text{ M}^{-1} \text{s}^{-1} \) from the y-intercept of the plot of \( K_{obs} \) versus [I]. The quenching rate constant for the reaction of I⁻ with excited pyrene, \( K_q \), was determined to be \( 1.7 \times 10^8 \pm 1.0 \times 10^7 \text{ M}^{-1} \text{s}^{-1} \) from the slope of the plot of \( K_{obs} \) versus [I]. The large quenching rate constant indicates that I⁻ is a strong quencher in the reaction with excited pyrene.

To determine the observed rate constant, \( k_{obs} \), for each sample of KI solution (0, 10 ± 0.252, 20 ± 0.244, 30 ± 0.243, 40 ± 0.243 mM), the fluorescence intensity was compared to the respective relaxation times. The two trials for each KI solution were combined to provide more
data points that were representative of the true intensity values of the solution. The data sets also contained a significant amount of “zeroes” which were removed prior to creating plots as these values were not relevant to the data set or the calculations of $k_o$ and $k_q$. These values were obtained before exposure to the ultraviolet light (as there was no excitation present and therefore no fluorescence intensity) and after the sample had completed its transition back down to the ground state. These plots of fluorescence intensity versus time produced exponential trend lines and were therefore difficult to analyze. The plots were each linearized by plotting the natural logarithm of the fluorescence intensity versus time as shown in Figures 4 through 8 below:

**FIGURE 4.** Plot of the natural logarithm of the fluorescence intensity versus relaxation time for the 0 mM KI solution (control) at 337.1 nm. The negative of the slope, $3 \times 10^6 \pm 11087.12 \text{ M}^{-1} \text{s}^{-1}$, is equal to $K_{obs}$. 
**FIGURE 5.** Plot of the natural logarithm of the fluorescence intensity versus relaxation time for the 10 ± 0.252 mM KI solution at 337.1 nm. The negative of the slope, $5 \times 10^6 \pm 11379.08 \text{ M}^{-1} \text{ s}^{-1}$, is equal to $K_{obs}$.

**FIGURE 6.** Plot of the natural logarithm of the fluorescence intensity versus relaxation time for the 20 ± 0.244 mM solution at 337.1 nm. The negative of the slope, $7 \times 10^6 \pm 15512.08 \text{ M}^{-1} \text{ s}^{-1}$, is equal to $K_{obs}$. 

\[ y = -5E+06x - 2.2124 \]
\[ R^2 = 0.9847 \]

\[ y = -7E+06x - 2.1144 \]
\[ R^2 = 0.9863 \]
**FIGURE 7.** Plot of the natural logarithm of the fluorescence intensity versus relaxation time for the 30 ± 0.243 mM KI solution at 337.1 nm. The negative of the slope, $8 \times 10^6 \pm 23925.92$ M$^{-1}$ s$^{-1}$, is equal to $K_{obs}$.

**FIGURE 8.** Plot of the natural logarithm of the fluorescence intensity versus relaxation time for the 40 ± 0.243 mM KI solution at 337.1 nm. The negative of the slope, $1 \times 10^7 \pm 28553.44$ M$^{-1}$ s$^{-1}$, is equal to $K_{obs}$. 
Figures 5 through 8 above are all linear plots with $R^2$ values greater than 0.97 indicating that the trend lines can correctly account for more than 97% of the data points contained in the graphs. It can be seen by the graphs above that increasing the concentrations of KI solutions leads to a larger value of $K_{obs}$ (larger absolute value of slope). These plots are of the form of eq. (7) in which the slope of the graph is equal to $-(k_o + k_q[I])$. The quantity $k_q[I]$ can be simplified to $k'$ to yield an equivalent slope of the form $-(k_o + k')$ which is equivalent to $-k_{obs}$. Increasing the concentration of KI in the sample leads to a decrease in the fluorescence intensity and therefore an increase in the absolute value of $k_{obs}$. The decrease in fluorescence intensity indicates that less energy is being emitted from the system meaning that there are an increased number of pyrene molecules in the excited state for an extended period of time. This extended relaxation time is due to stabilization of the excited pyrene molecules from the iodide anion. The higher concentration of iodide anions allows for more pyrene molecules to be stabilized and therefore a longer overall relaxation time for the solution. The values of $k_{obs}$ for the solutions are compiled in Table 1 below. The values of the uncertainty in $k_{obs}$ for each solution (the negative of the slopes of the plots of LN(intensity) versus time) were determined using linear regression analyses.

<table>
<thead>
<tr>
<th>[I] M</th>
<th>Uncertainty in [I] ± M</th>
<th>$K_{obs}$ (M$^{-1}$ s$^{-1}$)</th>
<th>Uncertainty in $K_{obs}$ (± M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>3421803.719</td>
<td>11087.12142</td>
</tr>
<tr>
<td>0.01</td>
<td>0.252</td>
<td>5457710.367</td>
<td>11379.08155</td>
</tr>
<tr>
<td>0.02</td>
<td>0.244</td>
<td>7133959.636</td>
<td>15512.07771</td>
</tr>
<tr>
<td>0.03</td>
<td>0.243</td>
<td>8140571.911</td>
<td>23925.92131</td>
</tr>
<tr>
<td>0.04</td>
<td>0.243</td>
<td>9953895.196</td>
<td>28553.44473</td>
</tr>
</tbody>
</table>

**TABLE 1.** KI solution concentrations and $K_{obs}$, the negative of the slopes of Figures 5 through 8.
Once the values of $k_{obs}$ were determined for each $\Gamma$ solution, a plot of $k_{obs}$ versus $[\Gamma]$ was used to determine the values of $k_o$ and $k_q$ independently. This plot can be seen below in Figure 9:

**FIGURE 9.** Plot of $k_{obs}$ versus iodide anion concentration at 337.1 nm. The plot contains a slope equal to $k_q$ and a y-intercept equal to $k_o$.

From the plot of $k_{obs}$ versus $[\Gamma]$ (Figure 9) it can be seen that the value of the quenching rate constant for the reaction of $\Gamma$ with excited pyrene ($k_q$) is equal to $1.7 \times 10^8 \pm 1.0 \times 10^7 \text{ M}^{-1} \text{s}^{-1}$ and the value of the rate constant for the spontaneous fluorescence decay of pyrene ($k_o$) is equal to $3.2 \times 10^6 \pm 2.45 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$. The values of the quenching rate constant for various reactions are often between the magnitudes of $10^2$ to $10^9 \text{ M}^{-1} \text{s}^{-1}$. The higher quenching rate constants are indicative of highly electron donating molecules which can stabilize excited molecules. For example the quencher Cr(CN)$_3^{3-}$ in $5 \times 10^{-4}$ HClO$_4$ and H$_2$C has a $k_q$ value of $6.2 \times 10^8 \text{ M}^{-1} \text{s}^{-1}$ whereas the quencher nitrobenzene in DMF has a $k_q$ of $2.2 \times 10^2 \text{ M}^{-1} \text{s}^{-1}$. Nitrobenzene is less electron donating than the quencher Cr(CN)$_3^{3-}$ which will ultimately make nitrobenzene a less...
strong quencher with a lower $k_q$ value. The quenching constant for $\Gamma$ is relatively large in magnitude meaning that $\Gamma$ is a strong quencher.

It can be observed that the value of $k_o$ ($3.2 \times 10^6 \pm 2.45 \times 10^5 \text{ M}^{-1} \text{s}^{-1}$) is less than the value of $k_q$ ($1.7 \times 10^8 \pm 1.0 \times 10^7 \text{ M}^{-1} \text{s}^{-1}$) for the reaction of pyrene and $\Gamma$. This is expected for a reaction which involves an efficient quencher molecule. The iodide anion quencher shifts the reaction so excited state molecules become more stable, ultimately increasing the total population of pyrene molecules which can be found in the excited state. The value of $k_q$ will therefore be larger than the value of $k_o$, the rate constant of an unquenched fluorescence decay. In an unquenched fluorescence decay, the excited state molecules are not stabilized and therefore the molecules remain in the excited state for a more brief period of time. The value of $k_q$ will be larger than $k_o$ for all reactions in which the quencher molecule stabilizes the molecule being excited and the value of $k_o$ will only be greater than $k_q$ if the “quencher” molecule destabilizes the excited state of the other molecule (although in this circumstance the “quencher” molecule would not be preforming the function of a quencher).

In this experiment the presence of oxygen in the sample cell most likely caused the largest errors in calculations of $k_o$ and $k_q$. The process involved the insertion of a needle connected to a nitrogen tank to pump nitrogen into the cell and oxygen out. During the evacuation process, the cell was “capped” using a lid which was partially inserted into the cell. After five minutes of evacuation, the nitrogen line was pulled out of the cell as the lid was pushed further into the cell which created an opportunity for air (and oxygen) to enter back into the cell. Oxygen, like the iodide anion, is a strongly electron donating and is therefore a good quencher with a $k_q$ on the order of $10^9 \text{ M}^{-1} \text{s}^{-1}$. The presence of oxygen would have decreased the fluorescence intensity of a sample and therefore increased $k_{obs}$ and therefore $k_o$ and $k_q$. When
examining results, the observed intensities would be solely attributed to the concentration of iodide anion, but in actuality they should be attributed to both the concentration of I and O2 in the cell. Unfortunately the concentration of O2 in the cell is difficult to measure and therefore was not taken into consideration in this laboratory experiment. If the amount of oxygen in each sample cell was constant the overall slope of the graph of \( k_{obs} \) versus [I] would have remained the same (therefore \( k_q \) would be unaffected) however the y-intercept value would have increased (\( k_o \) would increase). If amount of oxygen in the cell varied from cell to cell, this would have caused the slope and the y-intercept of \( k_{obs} \) versus [I] to increase (affecting both \( k_o \) and \( k_q \)). As the same procedure for the removal of the needle was replicated for each solution, the amount of oxygen in the cell was most likely the same from cell to cell meaning \( k_o \) is slightly larger than its true value. The second largest source of error in this experiment would be the creation of the 0, 10 ± 0.252, 20 ± 0.244, 30 ± 0.243, and 40 ± 0.243 mM KI solutions. As the solutions were created using a dilution technique, an error in the formation of the beginning solution would create errors in the subsequent solutions. If the first solution was more concentrated than originally intended, the following solutions would also be more concentrated. Inversely, if the first solution was less concentrated than intended, the following solutions would also be less concentrated.

**Conclusion**

This experiment successfully calculated the rate constants of the photochemical reaction of pyrene and potassium iodide via a nanosecond laser photolysis technique. The rate constant for the spontaneous fluorescence decay of pyrene, \( k_o \), was calculated as the y-intercept of the plot
of $k_{obs}$ versus [I] to be $3.2 \times 10^6 \pm 2.45 \times 10^5 \text{M}^{-1} \text{s}^{-1}$. The quenching rate constant for the reaction of I with excited state pyrene, $k_q$, was calculated as the slope of the plot of $k_{obs}$ versus [I] to be $1.7 \times 10^8 \pm 1.0 \times 10^7 \text{M}^{-1} \text{s}^{-1}$. In the future, several different reactions can be examined to determine different types of quenching. Examples of reactions include: oxidative reduction transfers from the excited state molecule (*S) to the quencher, a proton transfer from *S to Q or vice versa, and a hydrogen abstraction between *S and Q. As this reaction involving pyrene and KI only examines a transfer of one electron from Q to *S, it would be interesting to examine several other reaction types. To improve this experiment for future students a more effective method of oxygen evacuation can be developed to allow for better calculations of $k_{obs}$ and therefore $k_o$ and $k_q$. A more accurate method would involve a seal in which a vacuum line and a nitrogen line could be inserted. As the nitrogen is pumped into the system, oxygen and nitrogen can be pulled out by the vacuum to fully evacuate the cell.

Acknowledgements

The author would like to thank Stephen Aro, James Hodges, and Dr. Milosavljevic for their availability and help in providing advice and guidance in the laboratory.
References


(4) Sigma Aldrich. 57125 Aldrich Pyrene.

Sample Calculations

1. Concentrations of flasks:

10 mM KI solution

\[
(0.1661 \text{ g KI}) / (166.00 \text{ g/mol KI}) = 0.00100 \text{ mol KI}
\]

1.00 mmol KI / 10 mL = 0.100 M KI solution

\[
(0.100 \text{ M KI})*(1 \text{ mL})*(1 \text{ L} / 1000 \text{ mL}) = 0.0001 \text{ mol KI}
\]

\[
(0.0001 \text{ mol KI}) / (0.01 \text{ L}) = 0.01 \text{ M KI} = 10 \text{ mM KI solution}
\]

20 mM KI solution

\[
(0.100 \text{ M KI})*(2 \text{ mL})*(1 \text{ L} / 1000 \text{ mL}) = 0.0002 \text{ mol KI}
\]

\[
(0.0002 \text{ mol KI}) / (0.01 \text{ L}) = 0.02 \text{ M KI} = 20 \text{ mM KI solution}
\]

30 mM KI solution

\[
(0.100 \text{ M KI})*(3 \text{ mL})*(1 \text{ L} / 1000 \text{ mL}) = 0.0003 \text{ mol KI}
\]

\[
(0.0003 \text{ mol KI}) / (0.01 \text{ L}) = 0.03 \text{ M KI} = 30 \text{ mM KI solution}
\]

40 mM KI solution

\[
(0.100 \text{ M KI})*(4 \text{ mL})*(1 \text{ L} / 1000 \text{ mL}) = 0.0004 \text{ mol KI}
\]

\[
(0.0004 \text{ mol KI}) / (0.01 \text{ L}) = 0.04 \text{ M KI} = 40 \text{ mM KI solution}
\]

Uncertainty Calculations

1. Linear Regression Analysis for the plot of LN(fluorescence intensity) vs. Time: 0 mM
2. Linear Regression Analysis for the plot of LN(fluorescence intensity) vs. Time: 10 mM

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Significance F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>457.13794</td>
<td>457.13794</td>
<td>230941.6896</td>
<td>0</td>
</tr>
<tr>
<td>Residual</td>
<td>3573</td>
<td>70.6275091</td>
<td>0.019797017</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3574</td>
<td>4617.86491</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-5457770.367</td>
<td>11377.08355</td>
<td>-49.720615</td>
<td>0</td>
<td>-5489620.314</td>
<td>-5435400.219</td>
<td>-5435400.219</td>
<td>-5435400.219</td>
</tr>
</tbody>
</table>

X Variable 1 | 457.56883 | 457.56883 | 113500.347 | 0 |

3. Linear Regression Analysis for the plot of LN(fluorescence intensity) vs. Time: 20 mM

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Significance F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>457.56883</td>
<td>457.56883</td>
<td>230941.6896</td>
<td>0</td>
</tr>
<tr>
<td>Residual</td>
<td>2940</td>
<td>60.1716757</td>
<td>0.020602641</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2941</td>
<td>4617.86491</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-7133959.636</td>
<td>15512.07771</td>
<td>-459.8971054</td>
<td>0</td>
<td>-716475.272</td>
<td>-7103544.001</td>
<td>-7103544.001</td>
<td>-7103544.001</td>
</tr>
</tbody>
</table>

X Variable 1 | 457.56883 | 457.56883 | 113500.347 | 0 |

4. Linear Regression Analysis for the plot of LN(fluorescence intensity) vs. Time: 30 mM

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Significance F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>3800.17716</td>
<td>3800.17716</td>
<td>230941.6896</td>
<td>0</td>
</tr>
<tr>
<td>Residual</td>
<td>3578</td>
<td>84.6006161</td>
<td>0.018227876</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3579</td>
<td>3844.99472</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-235208913</td>
<td>500771848</td>
<td>-454.332964</td>
<td>0</td>
<td>-2356941752</td>
<td>-2351814515</td>
<td>-2351814515</td>
<td>-2351814515</td>
</tr>
</tbody>
</table>

X Variable 1 | 457.56883 | 457.56883 | 113500.347 | 0 |
5. Linear Regression Analysis for the plot of $\ln$ (fluorescence intensity) vs. Time: 40 mM

<table>
<thead>
<tr>
<th>Regression Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R: 0.991418136</td>
</tr>
<tr>
<td>R Square: 0.98290992</td>
</tr>
<tr>
<td>Adjusted R Square: 0.982901831</td>
</tr>
<tr>
<td>Standard Error: 0.16118615</td>
</tr>
<tr>
<td>Observations: 2115</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2113</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

6. Linear Regression Analysis for the plot of $k_{\text{obs}}$ vs. $[\Gamma]$

<table>
<thead>
<tr>
<th>Regression Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R: 0.994649731</td>
</tr>
<tr>
<td>R Square: 0.985728027</td>
</tr>
<tr>
<td>Adjusted R Square: 0.985300171</td>
</tr>
<tr>
<td>Standard Error: 366227.766</td>
</tr>
<tr>
<td>Observations: 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
</tr>
<tr>
<td>-----</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Calculation of the Uncertainty of the [KI]:

- Uncertainty of a 1 mL class A glass pipet: ± 0.007 mL
- Uncertainty of a 10 mL volumetric flask: ± 0.02 mL
- Uncertainty of balance: ± 4 mg = ± 0.004 g

\[
\Delta m^2 / m^2 = (0.004 \text{ g})^2 / (0.1661 \text{ g})^2 = 0.0005799 \\
\Delta V_{\text{flask}}^2 / V_{\text{flask}}^2 = (0.02 \text{ mL})^2 / (10 \text{ mL})^2 = 0.000004
\]

For the 10 mM solution:

\[
\Delta V_{\text{pipet}}^2 / V_{\text{pipet}}^2 = (0.007 \text{ mL})^2 / (1 \text{ mL})^2 = 0.000049 \\
\Delta M_{\text{initial}}^2 / M_{\text{initial}}^2 = \Delta V_{\text{pipet}}^2 / V_{\text{pipet}}^2 + \Delta m^2 / m^2 = 0.0005799 + 0.000049 = 0.0006289 \\
\Delta M_{\text{initial}} = [0.0006289 \times (0.1661)^2]^{0.5} = 0.00417 \\
\Delta M_{\text{final}}^2 / M_{\text{final}}^2 = \Delta M_{\text{initial}}^2 / M_{\text{initial}}^2 + \Delta V_{\text{flask}}^2 / V_{\text{flask}}^2 = 0.0006289 + 0.000004 = 0.0006329
\]
\[ \Delta M_{\text{final}} = [0.0006329(0.01)^2]^{0.5} = 0.252 \text{ mM} \]

For the 20 mM solution:
\[
\Delta V_{\text{pipet}} = [(\Delta V^2_{\text{pipet}})(V_1)^2 + (\Delta V^2_{\text{pipet}})(V_2)^2]^{0.5} = [0.000098]^{0.5} = 0.0099 \\
\Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} = (0.007 \text{ mL})^2 / (2 \text{ mL})^2 = 0.00001225 \\
\Delta M^2_{\text{initial}} / M^2_{\text{initial}} = \Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} + \Delta m^2 / m^2 = 0.0005799 + 0.00001225 = 0.000592 \\
\Delta M_{\text{initial}} = [0.000592*(0.1661)^2]^{0.5} = 0.00404 \\
\Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} = (0.007 \text{ mL})^2 / (2 \text{ mL})^2 = 0.00001225 \\
\Delta M^2_{\text{final}} / M^2_{\text{final}} = \Delta M^2_{\text{initial}} / M^2_{\text{initial}} + \Delta V^2_{\text{flask}} / V^2_{\text{flask}} = 0.000592 + 0.000004 = 0.000596 \\
\Delta M_{\text{final}} = [0.000596*(0.01)^2]^{0.5} = 0.244 \text{ mM} \\
\]

For the 30 mM solution:
\[
\Delta V_{\text{pipet}} = [(\Delta V^2_{\text{pipet}})(V_1)^2 + (\Delta V^2_{\text{pipet}})(V_2)^2 + (\Delta V^2_{\text{pipet}})(V_3)^2]^{0.5} = [0.000147]^{0.5} = 0.012 \\
\Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} = (0.007 \text{ mL})^2 / (3 \text{ mL})^2 = 0.00000544 \\
\Delta M^2_{\text{initial}} / M^2_{\text{initial}} = \Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} + \Delta m^2 / m^2 = 0.0005799 + 0.00000544 = 0.000585 \\
\Delta M_{\text{initial}} = [0.000585*(0.1661)^2]^{0.5} = 0.00410 \\
\Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} = (0.007 \text{ mL})^2 / (3 \text{ mL})^2 = 0.00000544 \\
\Delta M^2_{\text{final}} / M^2_{\text{final}} = \Delta M^2_{\text{initial}} / M^2_{\text{initial}} + \Delta V^2_{\text{flask}} / V^2_{\text{flask}} = 0.000585 + 0.000004 = 0.000589 \\
\Delta M_{\text{final}} = [0.000589*(0.01)^2]^{0.5} = 0.243 \text{ mM} \\
\]

For the 40 mM solution:
\[
\Delta V_{\text{pipet}} = [(\Delta V^2_{\text{pipet}})(V_1)^2 + (\Delta V^2_{\text{pipet}})(V_2)^2 + (\Delta V^2_{\text{pipet}})(V_3)^2 + (\Delta V^2_{\text{pipet}})(V_4)^2]^{0.5} = [0.000196]^{0.5} = 0.014 \\
\Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} = (0.007 \text{ mL})^2 / (4 \text{ mL})^2 = 0.00000306 \\
\Delta M^2_{\text{initial}} / M^2_{\text{initial}} = \Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} + \Delta m^2 / m^2 = 0.0005799 + 0.00000306 = 0.000583 \\
\Delta M_{\text{initial}} = [0.000583*(0.1661)^2]^{0.5} = 0.00401 \\
\Delta V^2_{\text{pipet}} / V^2_{\text{pipet}} = (0.007 \text{ mL})^2 / (4 \text{ mL})^2 = 0.00000306 \\
\Delta M^2_{\text{final}} / M^2_{\text{final}} = \Delta M^2_{\text{initial}} / M^2_{\text{initial}} + \Delta V^2_{\text{flask}} / V^2_{\text{flask}} = 0.000583 + 0.000004 = 0.000587 \\
\Delta M_{\text{final}} = [0.000587*(0.01)^2]^{0.5} = 0.243 \text{ mM} \\
\]