CRystal violet LAB

Introduction
Crystal violet is a common, beautiful purple dye. In strongly basic solutions, the bright color of the dye slowly fades and the solution becomes colorless. The kinetics of this “fading” reaction can be analyzed by measuring the color intensity or absorbance of the solution versus time to determine the rate law.

Background
Crystal violet belongs to a class of intensely colored organic compounds called triphenylmethane dyes. The structure and color of crystal violet depend on pH, making it a valuable acid–base indicator as well as an excellent dye. The major structural form of crystal violet is the monovalent cation, abbreviated CV⁺, which is shown in Figure 1a. CV⁺ is the predominant form of crystal violet in the solid state and in aqueous solution across a broad range of pH values from pH 1 to 13. The positive charge shown on the central carbon atom in Figure 1a is delocalized via resonance to the three nitrogen atoms. See Figure 1b for one of the three additional resonance forms with the positive charge on a nitrogen atom. Delocalization of the charge across the system of double bonds in the benzene rings stabilizes the carbocation and is responsible for the vibrant purple color of the dye.

In strongly basic solutions the purple CV⁺ cation slowly combines with hydroxide ions to form a neutral product, CVOH, which is colorless (see Figure 2). The rate of this reaction (Equation 1) is slower than typical acid–base proton transfer reactions and depends on the initial concentration of both crystal violet and hydroxide ions.

\[
CV^+ + OH^- \rightarrow CVOH \quad \text{Equation 1}
\]

Purple → Colorless

Exactly how much the rate changes as the reactant concentration is varied depends on the rate law for the reaction. In the case of the reaction of CV⁺ with OH⁻ ion, the rate law has the general form

\[
\text{Rate} = k [CV^+]^x [OH^-]^y \quad \text{Equation 2}
\]

The exponents x and y are defined as the order of reaction for each reactant and k is the rate constant for the reaction at a particular temperature. The values of the exponents x and y must be determined by experiment. If the reaction is carried out under certain conditions, then Equation 2 will reduce to the form

\[
\text{Rate} = k' [CV^+]^x
\]

where \( k' = k [OH^-]^y \) \quad \text{Equation 3}

The constant k’ is a new “pseudo” rate constant incorporating both the “true” rate constant k and the \([OH^-]^y\) term. Equation 3 is referred to as a pseudo-rate law because it is a simplification of the actual rate law, Equation 2.

The pseudo-rate law is valid when the concentration of OH⁻ ions is much greater than the concentration of CV⁺ ions. Under these conditions the \([OH^-]^y\) term in Equation 2 will not change much over the course of the reaction and may be treated as a constant in the rate equation.
Recall that the absorbance for a specific concentration of a solution with a fixed path length varies directly with the absorbivity coefficient of the solution. This relationship is known as Beer’s law.

\[ A = abc \]  

*Equation 5*

where \( A \) is absorbance, \( a \) is the molar absorbivity coefficient, \( b \) is the path length in cm, corresponding to the distance light travels through the solution, and \( c \) is the concentration of the solution. Beer’s law provides the basis of using spectroscopy in quantitative analysis. Using this relationship, concentration and absorbance may be calculated if one variable is known while keeping \( a \) and \( b \) constant. This relationship is also extremely valuable in kinetics experiments, making it possible to follow the rate of disappearance of a colored substance by measuring its absorbance as a function of time.

**Purpose**

1) Construct a Beer’s law calibration curve for 20.0 µM crystal violet.
2) Determine the pseudo-rate law for the color-fading reaction of crystal violet with sodium hydroxide, specifically by determining the order with respect to crystal violet.

**Pre-lab Considerations**

1) Select the optimum wavelength for generating a Beer’s law calibration curve. (Absorbance measurements are most accurate and sensitive in the range of 0.2—1.0)

![Absorption Spectrum for Crystal Violet](image)

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>468 nm – Blue</th>
<th>565 nm – Green</th>
<th>610 nm - Orange</th>
<th>660 nm – Red</th>
</tr>
</thead>
</table>

2) If starting with 20.0 µM CV stock solution, select an appropriate concentration of NaOH to react with the CV in order to determine the pseudo-rate law for the reaction.

3) Determine the volume of stock solution and water required to make the concentrations needed for the Beer’s law calibration curve. The vial can hold 6 mL of solution.

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>0</th>
<th>2.0</th>
<th>4.0</th>
<th>6.0</th>
<th>8.0</th>
<th>10.0</th>
<th>20.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of stock solution (mL)</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td>Volume of water (mL)</td>
<td>6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Safety Precautions**

* Dilute sodium hydroxide solution is irritating to eyes and skin. Crystal violet is a strong dye and will stain clothes and skin. Clean up all spills immediately. Wear chemical splash goggles, chemical-
resistant gloves, and a chemical-resistant apron. Avoid contact of all chemicals with eyes and skin and wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

**Use of the Colorimeter**

1) Use only one vial for the entirety of each procedure. Each vial may have slight inconsistencies that could affect measured absorbances.
2) Be sure to wipe the vial of all fingerprints prior to inserting into the colorimeter.
3) Be sure to place the vial with the arrow on the cap pointing toward the silver screw.
4) The green button on the colorimeter should only be pressed ONCE per procedure when zeroing the colorimeter. DO NOT PRESS IT AGAIN OR ALL OF YOUR DATA WILL BE RENDERED USELESS!
5) Use the computer to read and record individual absorbances for each concentration.

**Procedure 1: Beer’s Law Calibration Curve**

1) Begin with the pure distilled water and zero the colorimeter. Press the green button once. When the green light turns off, the colorimeter should be zeroed and readings from the computer screen should confirm.
2) Rinse the pipettes with each solution prior to the first volume measurement (i.e. water pipette with water and CV pipette with CV).
3) Beginning with the smallest concentration, use the graduated pipettes to mixed the appropriate volumes of stock CV and distilled water.
4) Follow appropriate procedures to place the vial in the colorimeter and use the computer to read the absorbance. Record this value.
5) Empty the contents of the vial into the waste container, but do NOT rinse with water.
6) Repeat steps 3-5 for each subsequently larger concentration, ending with the 20.0 µM CV.
7) Follow the instructor’s directions for clean-up, if needed.

<table>
<thead>
<tr>
<th>Concentration CV (µM)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
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<tr>
<td>8</td>
<td></td>
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<tr>
<td>10</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

**Data Analysis Procedure 1**

Create a Beer’s law calibration curve for 20.0 µM CV. Be sure to include the slope-intercept equation and R² value for the best-fit line on the graph (Chart Layout 9 without the legend).

**Procedure 2: CV reaction with NaOH**

1) Begin with 6.0 mL of NaOH and zero the colorimeter. Press the green button once. When the green light turns off, the colorimeter should be zeroed and readings from the computer screen should confirm.
2) Rinse the pipettes with each solution prior to the first volume measurement (i.e. NaOH pipette with water and CV pipette with CV).
3) Add 3.0 mL of the NaOH (concentration determined earlier) to the vial.
4) Quickly add 3.0 mL of 20.0 µM CV to the vial, cap, wipe, and insert into the colorimeter. Start collecting data as soon as possible after mixing.
5) Let the reaction proceed for 5-10 minutes, or until the absorbances being measured do not appear to be changing anymore.

6) Empty the contents of the vial into the waste container. Rinse the vial.

7) Follow the instructor’s directions for further clean-up.

Data Collection Procedure 2
Data will be exported from the SPARKVue software used to collect it. This data does NOT need to be submitted with the lab report.

Data Analysis Procedure 2
Create a graph that contains data points for [CV] vs time, ln[CV] vs time, and [CV]⁻¹ vs time. Each set of data should have a best-fit line with the slope-intercept equation and R² value displayed (Chart Layout 9). A legend is most definitely appropriate when combining all data sets in one graph.

Discussion
Answer the following questions

1) Match each linear graph shown below with that expected if the reaction is (a) zero order, (b) first order, and (c) second order with respect to [A].

![](Graph1.png)  ![](Graph2.png)  ![](Graph3.png)

2) Explain how the value of the pseudo-rate constant $k'$ can be calculated from the appropriate linear graph shown above for a first-order reaction.

3) Explain additional variables that may affect the reproducibility or accuracy of the experiment and how these variables can be controlled.

4) What is the order of the reaction with respect to CV?

5) What is the pseudo-rate law?

6) What is the value of the pseudo-rate constant?

7) Using collision theory, predict how increasing the temperature should affect the rate of a chemical reaction. State the prediction in the form of a hypothesis and explain your reasoning.

8) Using collision theory, predict how increasing the concentration of a reactant should affect the rate of a chemical reaction. State the prediction in the form of a hypothesis and explain your reasoning.

Consider a classic iodine clock reaction between iodide ions and persulfate ions (Equation 6).

$$2I^−(aq) + S_{2}O_8^{2−}(aq) \rightarrow I_2(aq) + 2SO_4^{2−}(aq) \quad \text{Equation 6}$$

The following rate data was collected for different initial concentrations of iodide and persulfate ions.

<table>
<thead>
<tr>
<th>Trial</th>
<th>[I⁻]</th>
<th>[S₂O₈²⁻]</th>
<th>Initial rate (mol/L·s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.040 M</td>
<td>0.040 M</td>
<td>7.4 x 10⁻⁶</td>
</tr>
<tr>
<td>2</td>
<td>0.080 M</td>
<td>0.040 M</td>
<td>1.5 x 10⁻⁵</td>
</tr>
<tr>
<td>3</td>
<td>0.040 M</td>
<td>0.080 M</td>
<td>1.4 x 10⁻⁵</td>
</tr>
</tbody>
</table>

9) Compare trials 1 and 2: How did the concentration of iodide ions change in these two trials, and how did the rate change accordingly? What is the reaction order for iodide ions?

10) Which two trials should be compared to determine the order of reaction with respect to persulfate ions? What is the reaction order for persulfate?

11) Write the combined rate law for this version of an iodine clock reaction. Could the rate law have been predicted using the coefficients in the balanced chemical equation? Explain.