11B. KINETICS OF CRYSTAL VIOLET FADING (SPECTROMETER)

Introduction

Crystal violet is an intensely purple dye commonly used as a biological tissue stain and in the classification of bacteria based on the physical and chemical properties of their cell walls. In the presence of a strong base, the color of the dye fades from purple to colorless. The kinetics of the fading process can be analyzed using colorimetry where the color intensity of the dye solution is plotted against time to determine the rate law.

Concepts

- Kinetics
- Rate law
- Reaction rate
- Reaction order
- Spectrometry
- Beer's law

Background

Kinetics is the area of chemistry that deals with how quickly or how slowly reactions take place. By studying the rate of a reaction, valuable information can be gained about how the reaction proceeds – the reaction mechanism. In general, the rate of a reaction depends on the concentration of the reactants and can be expressed mathematically as the *rate law*. The rate law for a chemical reaction is an equation that relates the rate of the disappearance of reactants or the rate of appearance of products to the concentration of the reactants. Exactly how much the rate changes as the reactant concentration is varied depends on the rate law for the reaction. In this activity, the goal is to determine the rate law for the reaction of a dye (crystal violet) with a bleaching agent (sodium hydroxide). The law summarizes the experimental information in a concise manner. Once the rate law is determined, it is possible to predict the rate of the reaction for a wide range of experimental conditions. The rate law has the form:

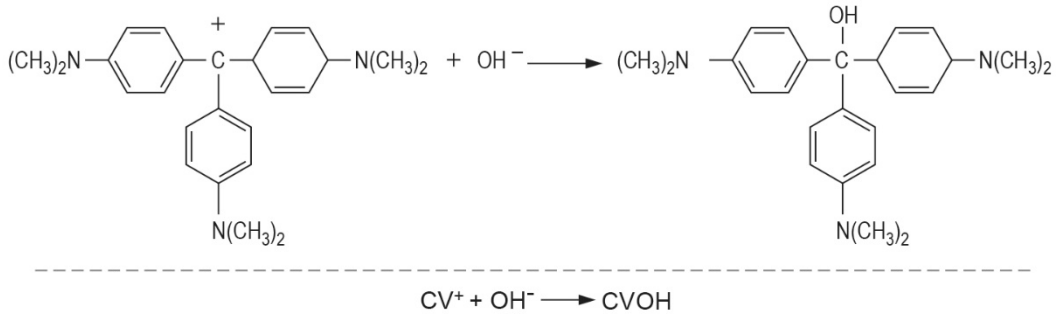
$$\text{Rate} = -\frac{\Delta[dye]}{\Delta t} = k[\text{dye}]^m[\text{bleach}]^n$$

and contains two types of information:

The order of the reaction, m and n, with respect to the concentrations of the reactants. Rate is proportional to concentration raised to the order. Thus, for a first-order reaction, the rate is proportional to concentration raised to the first power, so doubling the concentration doubles the rate. For a second-order reaction, the rate is proportional to the concentration raised to the second power, so doubling the concentration increases the rate by a factor of four.

The rate constant, k above, is the proportionality constant. It is necessary in order to calculate the rate instead of just how the rate changes when concentration is changed.

For the reaction of crystal violet (CV) and sodium hydroxide:



where CV+ is the monovalent purple form of crystal violet and CVOH is the colorless neutral form of the dye after reaction with a strong base, the rate law has the general form:

$$\text{Rate} = k\,[CV+]^m\,[OH-]^n$$

where k = the rate constant, m and n = the individual reaction orders for each reactant. The values of the individual reaction orders m and n must be determined experimentally because they are not based on the mole ratio in the balanced equation. The sum of the individual orders is the overall order of the reaction.

The order of a reaction is commonly zero order, first order or second order. A zero-order reaction is one in which the rate is independent of the concentration of reactants resulting in a straight line with slope *-k*. A first-order reaction is one where the rate depends only on the concentration of one reactant, and in which m = 1. The rate decreases as the reaction proceeds and [dye] decreases. A second-order reaction can be one in which the reaction rate depends on the concentration of two different reactants (where m = 1 and n =1), or where the reaction rate depends on the concentration of one reactant (where m = 2). For a second-order reaction, rate is proportional to $[dye]^2$, and the decrease in rate as the reaction proceeds is more rapid than for the first order reaction.

In this lab, CV^+ will be reacted with concentrations of OH- that are orders of magnitude greater. Because the bleaching agent is in large excess, it is reasonable to assume that as $[CV^+]$ decreases over time, $[OH^-]$ remains constant throughout the experiment. This creates a special condition that reduces the rate equation to:

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

$$\text{where } k' = k[OH^-]^n$$

Thus, the rate equation above is referred to as the *pseudo-rate law*.The constant k' is a *pseudo-rate constant* that takes into account the *real* rate constant k and the $[OH-]^n$ term.

The concentration of CV^+ will be determined using a spectroscopic method and applying Beer's law:

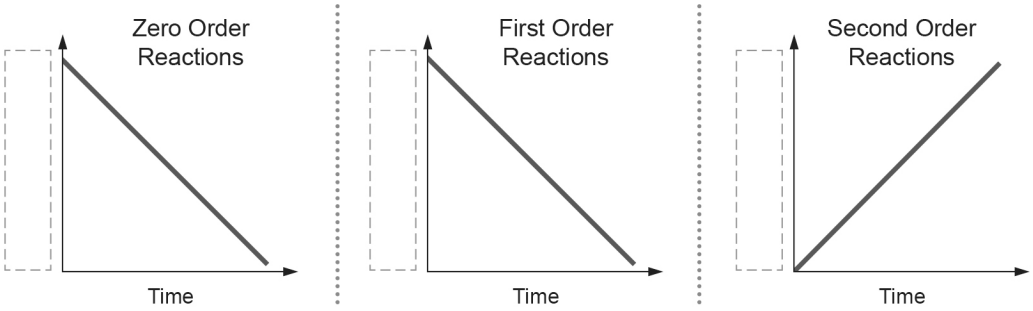
$$A = \boldsymbol{\varepsilon \ell c}$$

where absorbance (A) is the product of the molar absorptivity (ε) and concentration (c) of an absorbing species multiplied by the path length (ℓ) the wavelength of light passes through. Beer's law allows for quantifying concentration of a solution as a function of its absorbance while keeping both molar absorptivity coefficient (ε) and path length (ℓ) constant. In the chemical reaction between CV+ and OH- that produces a colorless neutral product (CVOH), absorbance can therefore be attributed completely to the reactant present in the solution.

In this lab, you will use spectroscopy and Beer's law to generate a calibration curve of crystal violet and sodium hydroxide. The data generated will be used to create three graphs to determine reaction order with respect to $[CV^+]$. If the reaction is zero-order, a plot of concentration vs. time will result in a straight line with the slope = -k. If the reaction is first-order, a straight-line plot will result from a graph of the natural log of concentration vs. time, with the slope equal to -k. If the reaction is second-order, a plot of 1/concentration vs. time will result in a straight line with the slope equal to +k.

Pre-Lab Questions

1. Fill in the *y*-axis label that will result in a linear relationship for each order of reaction.



2. The compound *crystal violet* has a purple color in an aqueous solution. When it reacts with sodium hydroxide, crystal violet fades to colorless.

$$\underset{\text{(Purple)}}{C_{25}H_{30}N_3Cl(aq)} + OH^-(aq) \rightarrow \underset{\text{(Colorless)}}{(C_{25}H_{30}N_3Cl)OH^-(aq)}$$

   a. What sensor could we use to measure the concentration of the crystal violet over time?

   b. The reaction requires much more sodium hydroxide than crystal violet to react in a reasonable amount of time. The sodium hydroxide solution to be used is about 50,000 times more concentrated than the crystal violet solution. Even if all of the crystal violet is consumed, will the sodium hydroxide concentration change much?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

- Data collection system
- PASCO spectrometry software
- Wireless spectrometer
- Cuvettes (10)
- Test tubes, 15 cm x 2 cm (8)
- Test tube rack
- Disposable pipettes (4)
- 0.1 M Sodium hydroxide (NaOH), 12 mL
- 2.5 x 10^{-5} M Crystal violet, 30 mL
- Water, deionized, 20 mL
- Marking pen
- Kimwipes®

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times
- Sodium hydroxide is caustic and should be handled with special care.
- In case of contact with your skin, wash off acid and base solutions with a large amount of water.
- Crystal violet is a dye that stains skin and clothing.
- Wash hands thoroughly with soap and water before leaving laboratory.
- Review chemical handling and disposal instructions as directed by Material Safety Data Sheet.

Initial Investigation

Determination of absorbance wavelength for crystal violet

1. Connect the spectrometer to the Spectrometry application using the Bluetooth® or USB connection.

2. Calibrate the spectrometer with a distilled water blank.

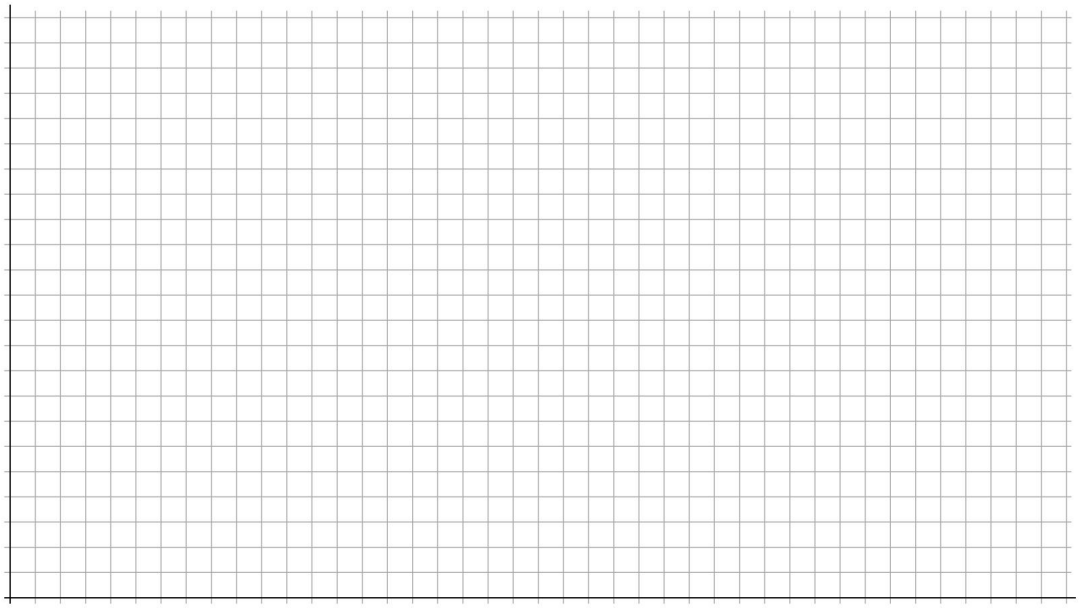
   NOTE: Always wipe cuvettes with a lint- and scratch-free wipe and make sure the cuvette is capped before placing into either the colorimeter or spectrometer. Orient the cuvette in the colorimeter or spectrometer so the light path is not blocked by labels or cuvette ridges. Handle the cuvette only by ridged sides.

3. Label the four pipets as follows: H_2O; CV; NaOH; Mix.

4. Use the CV pipet to add 70 drops of 2.5×10^{-5} M crystal violet solution into a cuvette.

5. Place the cuvette into the spectrometer. Select the RECORD button and determine which wavelength shows the greatest absorbance for crystal violet. Drag the coordinate box to this wavelength and select the check mark next to the box to set the analysis wavelength. A black vertical line will appear on the data display when the wavelength is properly set. Record the analysis wavelength in Table 1.

6. Stop recording data. Graph spectrometry data.



Calibration curve for crystal violet [CV]

7. Use pipets to prepare crystal violet solutions A through D as indicated in Table 1. Add drops directly into each cuvette and label each cap before closing the cuvette. Invert the cuvette 4-5 times to mix. Calculate the final concentration for each solution and enter results in Table 1.

8. Select the Concentration tab in the Spectrometry app. Update the values in the Concentration (mol/L) column to match the concentrations in Table 1.

   NOTE: Use the following format to enter exponents into the table: 2.5e-5

9. Start recording data. Measure the absorbance of each solution and record the results in Table 1. Keep the absorbance reading for each solution by selecting the check mark next to the absorbance reading in the table. If you do not see a check mark, select the cell that shows a live absorbance reading.

10. Stop recording data. Scale the graph and apply a linear fit. Record the r-value in Table 1.

11. Generate a data graph with absorbance on the y-axis and CV concentration on the x-axis.

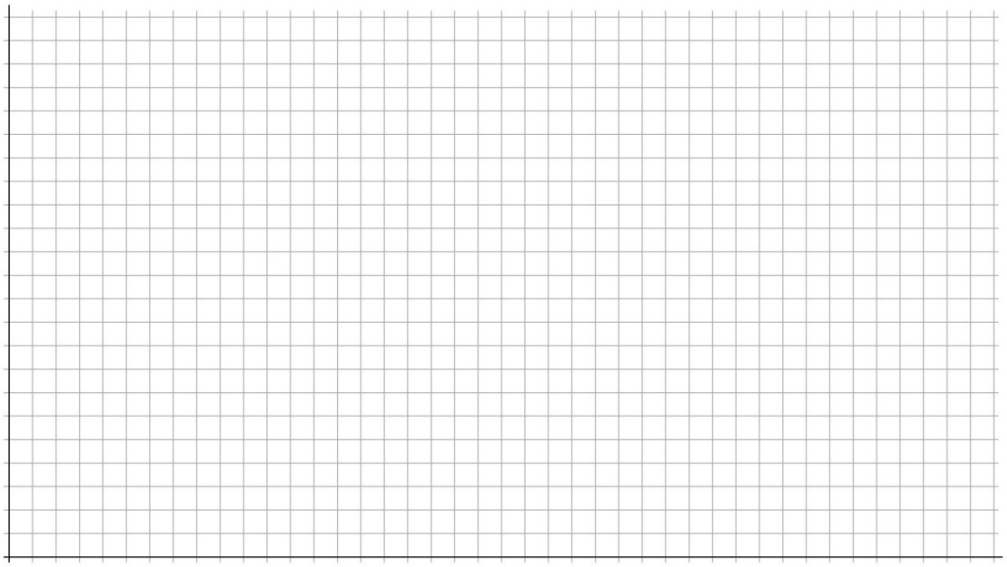


Table 1: Absorbance values for crystal violet solutions

CV Solution	Concentration (mol/L)	Drops of 2.5×10^{-5} M CV	Drops of Distilled Water	Absorbance
Stock		70	0	
A		63	7	
B		56	14	
C		49	21	
D		42	28	
Analysis wavelength:				
r-value from linear fit:				

12. What is the relationship between the molar concentration of a crystal violet solution and its absorbance of a specific wavelength of visible light?

13. How does the testing of a series of standard solutions help describe what will happen during a reaction where the color of a solution is disappearing?

14. Just using the graph you generated, pick an absorbance between your highest and lowest value and estimate the concentration of crystal violet.

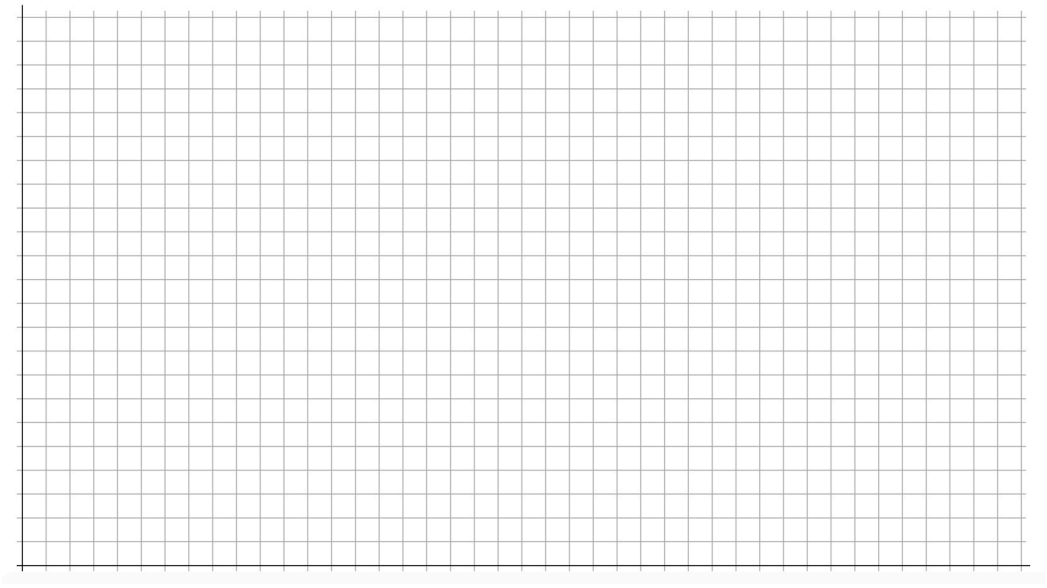
15. What is the linear fit (best fit line) equation for the set of crystal violet standards measured in the Initial Investigation? What does the slope of this line tell you about the standard solutions?

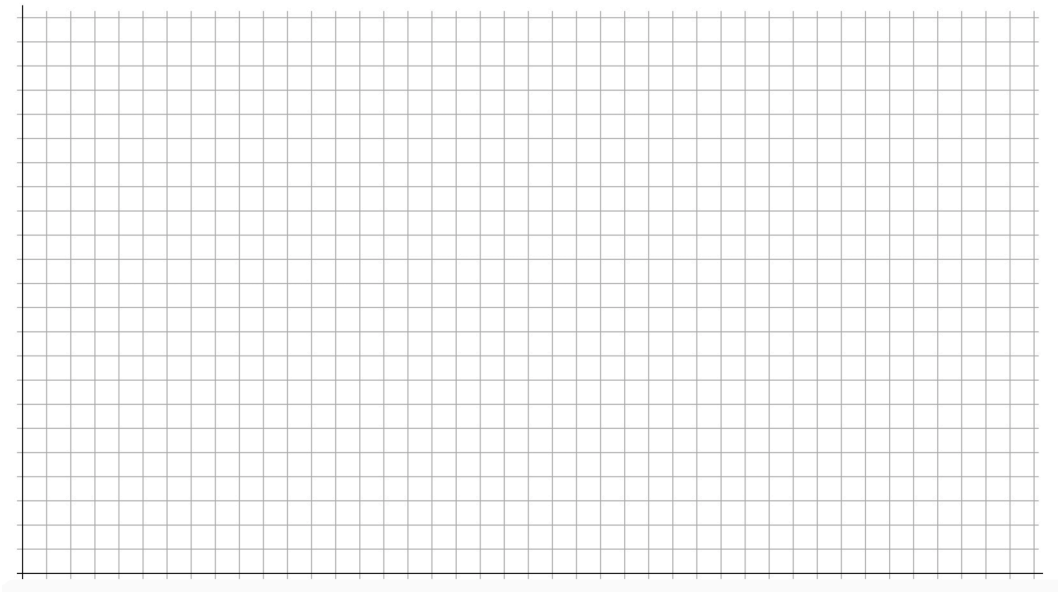
16. What is the r-value for the linear fit, and what does it indicate about the linearity of your absorbance-concentration data?

Advanced Investigation

Rate of reaction of crystal violet with sodium hydroxide

1. Transfer 45 drops of 2.5×10^{-5} M crystal violet to one test tube.
2. Transfer 45 drops of 0.10 M sodium hydroxide to another test tube.
3. Read through steps a-d before performing them in order to complete them as quickly as possible:
   a. Switch to the Time tab in the Spectrometry app.
   b. Decrease the Sample Rate to 2.00 s.
   c. Pour the sodium hydroxide into the crystal violet test tube. Swirl the test tube a few times to mix.
   d. Carefully pour the mixture into a clean cuvette. Fill the cuvette ¾ full. Add the cap to the cuvette and place it in the spectrometer. Set the test tube in the test tube rack.
   e. Start recording data. Allow the reaction to proceed for 200 seconds.
   f. Stop recording data after 200 seconds.
4. Apply a linear fit. Record the slope and r-value in Table 2.
5. Use the calculator icon to toggle the y-axis between Absorbance, ln(Absorbance), and 1/Absorbance. Determine which graph gives the best linear fit.
6. Record the r-value for each data display in Table 2 and on your graphs.





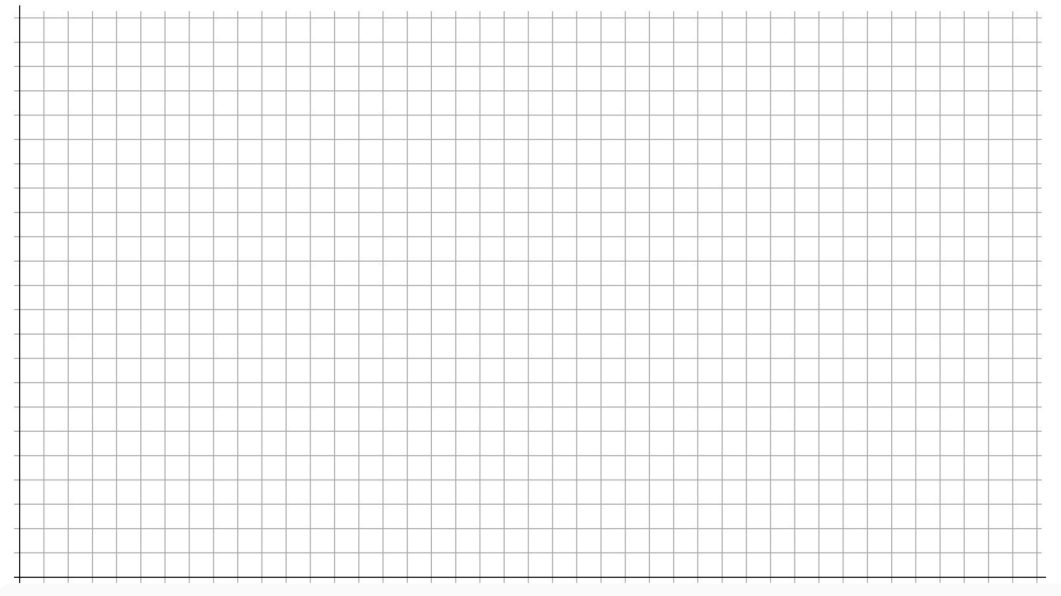


Table 2: Linear fit analysis of CV:NaOH reaction

y-axis value	Slope from linear fit (s^{-1})	r-value from linear fit (s^{-1})
Absorbance		
ln(Absorbance)		
1/Absorbance		

7. What is the observed crystal violet-sodium hydroxide reaction rate?

8. Why is it necessary to work quickly once the reactants are combined?

9. Predict how the reaction rate may change if you increased the concentration of crystal violet.

10. What is the order of the reaction between crystal violet and sodium hydroxide with respect to crystal violet? Support your answer with data.

Reaction order with respect to sodium hydroxide

1. Label 6 test tubes as follows: Run1CV, Run2CV, Run3CV, Run1NaOH, Run2NaOH, Run3NaOH.

2. Dispense the number of drops of reactant and water in each test tube as indicated in Table 3. Calculate the final concentration of each solution in each test tube and enter results in Table 3, then transfer the NaOH concentration values to Table 4.

Table 3: Reactant Concentrations BEFORE Mixing Solutions

Run #	Drops of 2.50×10^{-5} M CV	Drops of Distilled Water	Final CV Concentration (M)	Drops of 0.10 M NaOH	Drops of Distilled Water	Final NaOH Concentration (M)
1	35	35		35	35	
2	35	35		18	52	
3	35	35		70	0	

3. Calibrate the spectrometer with a distilled water blank. Select an appropriate analysis wavelength.

4. Go to the Time tab in the Spectrometry app. Set the sample rate to 2 s.

5. *As quickly as possible:* Add the contents of Run1NaOH to the Run1CV test tube. Swirl to mix and pour the contents into a cuvette. Cap the cuvette, place it in the spectrometer, and start recording data.

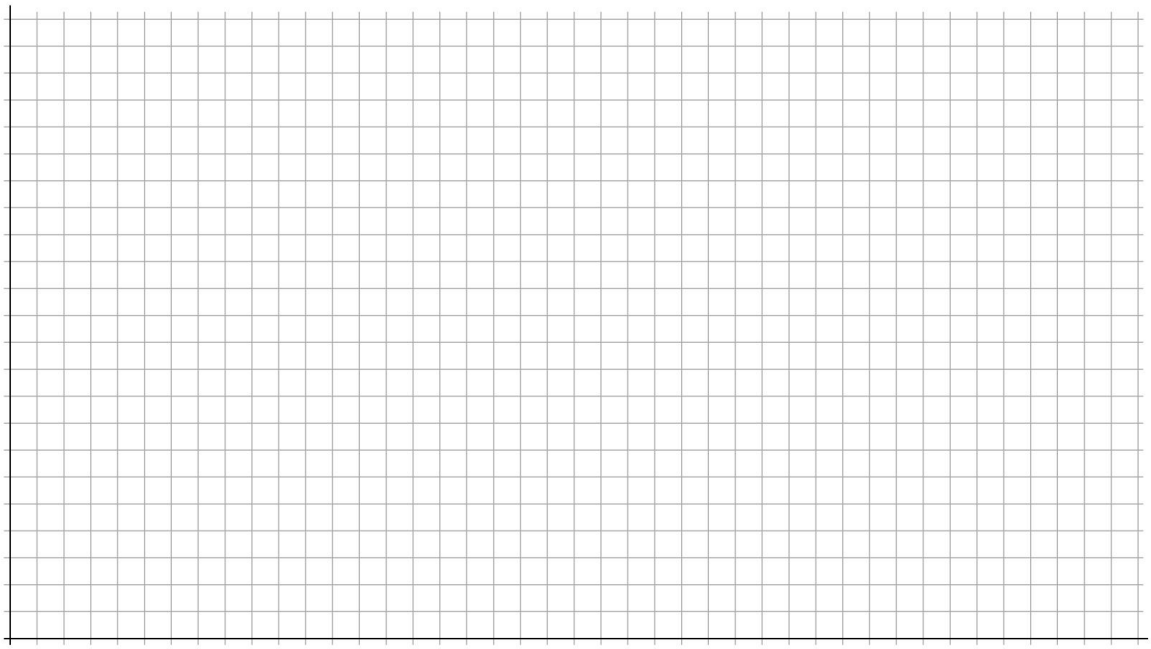
6. Stop data collection after 200 seconds. Use the QuickCalcs button to display ln(Absorbance) on the y-axis.

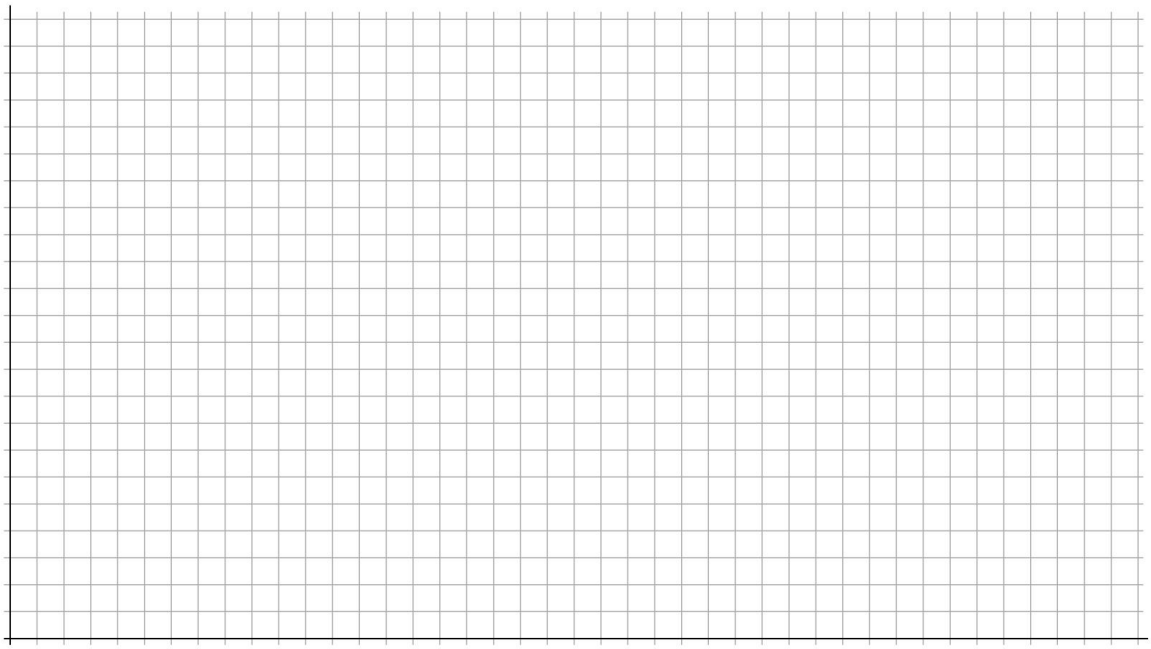
7. Scale the data and apply a linear fit. Record the slope in Table 4. Calculate the NaOH concentration after mixing test tube contents and enter results in Table 4.

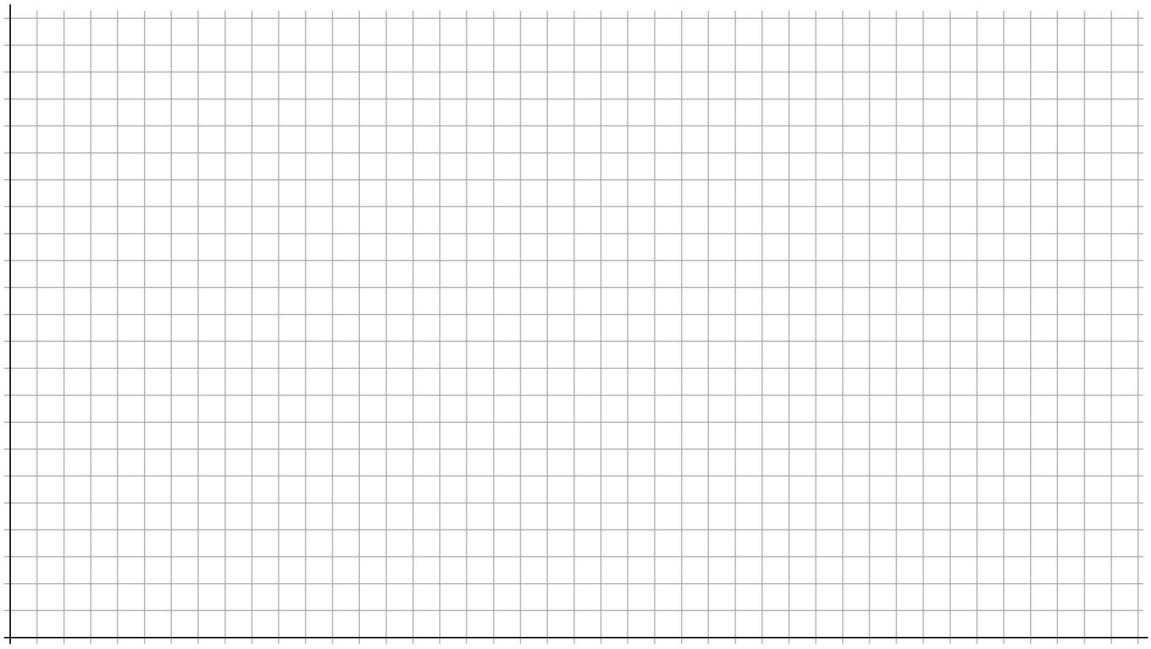
Table 4: Rate constant values (k)

Run #	Slope (s^{-1})	k' (s^{-1})	[NaOH] before mixing (M)	[NaOH] after mixing (M)
1				
2				
3				

8. Repeat Steps 5-7 with appropriate solutions for Run 2 and Run 3.





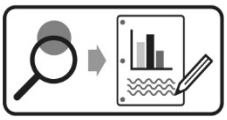


9. Calculate the reaction order of sodium hydroxide using data from Runs 1 and 2.

10. Given the equation: $k = k'/[OH\text{-}]^n$, calculate k based on the slope (reaction rate) and concentration of hydroxide for all runs.

11. What is the overall reaction order?

Extended Inquiry Investigation



Experiment design: Kinetics of iodine fading with acetone

Iodine reacts with acetone to form iodo-acetone according to the following reaction:

$$I_2 + CH_3\text{-}CO\text{-}CH_3 \rightarrow HI + CH_2I\text{-}CO\text{-}CH_3$$

Iodine is brown; the acetone, hydrogen iodide, and iodo-acetone are colorless. The order of both reactants is 1. Propose a method to measure the rate constant for this reaction.

The reaction can be monitored by measuring the absorbance of iodine. From the absorbance, the iodine concentration can be calculated the same way it was calculated in this experiment for the crystal violet.

Synthesis Questions

1. When only one species is absorbing it is easy to follow the reaction. However, if there are two species involved in absorption, the situation is somewhat more complicated. Consider the following hypothetical reaction:

   $$A + B \rightarrow C + D$$

   where A absorbs in the blue range and C absorbs in the yellow range of the visible spectrum. Do you think the reaction can be monitored by monitoring the absorption of the solution? If so, how? Explain!

2. How would your strategy be different from this experiment if you were to monitor the concentration of C (a product) in the previous example?

AP® Chemistry Review Questions

1. The rate of the reaction between CV^+ and OH^- depends:

   a. Only on the concentration of the reactants.
   b. On the concentrations of the reactants and the rate constant, k.
   c. Only on the rate cosntant, k.
   d. On the ratio of the concentrations of the reactants and the rate constant, *k*.

2. In the reaction between CV^+ and OH^-, doubling the concentration of CV^+:

   a. Will not change the reaction rate.
   b. Will double the rate constant.
   c. Will double the reaction rate.
   d. Will have no effect on the reaction at all.

3. Generally, in a chemical reaction, changing the concentration of a reactant:

   a. Will always change the rate of the reaction.
   b. Will always change the rate of the reaction except if the order of that reactant is zero-order.
   c. Will always change the rate of the reaction except if the order of that reactant is first-order.
   d. Will always change the rate of the reaction except if the order of that reactant is second-order.

4. The absorbance is:

   a. Not related to the concentration of the absorbing species.
   b. Proportional to the concentration of the absorbing species and the path length of the solution the light passes through.
   c. Proportional to the concentration of the absorbing species only.
   d. Proportional only to the path length through which the light has to pass.