

Reaction Kinetics--Spectrophotometric Determination of a Rate Law

Objective: Investigate the effect of reactant concentrations on the rate of reaction; to use kinetics data to derive a rate law for the decomposition of crystal violet; to calculate the rate constant for the reaction

Materials: Stock solutions of crystal violet (1.0×10^{-4} M) and sodium hydroxide (0.10 M NaOH)

Equipment: Two 10.0 mL graduated cylinders; one 50-mL beaker; two 100-mL beakers; one 250-mL beaker (waste); one 100-mL volumetric flask; three 10.00-mL pipettes; spectrophotometer and cuvettes; stopwatch or timepiece with a second hand

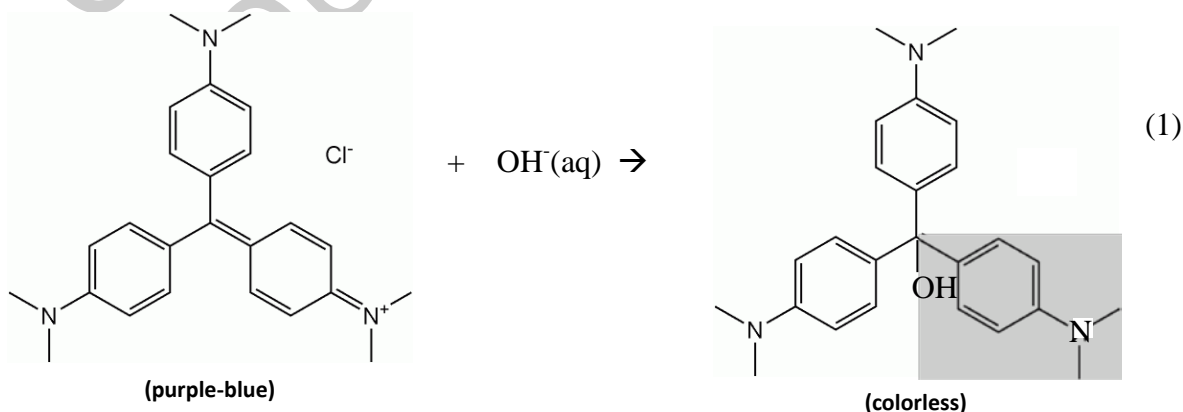
Safety: Sodium hydroxide solutions are caustic; wash thoroughly if contact is made with skin. Safety goggles should be worn at all times in the lab.

Waste Disposal: Excess reagents/reaction solutions may be flushed down the sink with water.

INTRODUCTION

Chemical kinetics is the study of **reaction rate**, or how fast a reaction proceeds. Knowing the factors that control the rate of reactions has tremendous implications in both industry and the environment. Manipulating these factors to increase the rate of a reaction can increase the yield of desirable products of industrial processes, or decrease the rate of undesirable reactions to minimize negative environmental impacts.

A reaction that readily lends itself to kinetic investigations is the reaction between crystal violet and hydroxide ion. Crystal violet (CV), also known as Gentian violet or aniline violet, has many applications, including use as a textile dye, as a histological stain for classifying bacteria, and as a DNA stain in gel electrophoresis. In alkaline solutions, crystal violet reacts with hydroxide according to reaction (1).



We can write a generic **rate law** relating the reaction rate to the concentration of reactants:

$$\text{Rate} = \frac{\Delta[\text{CV}]}{\Delta\text{time}} = k[\text{CV}]^x [\text{OH}^-]^z \quad (2)$$

The rate of the reaction is defined as the change in concentration of CV as a function of time, and x and z represent the **order** of the reaction with respect to the reactants. The values of x and z cannot be determined simply by examination of the balanced equation, but must be determined experimentally. To simplify the experiment, the concentration of one reactant is typically held constant while the other reactant concentration is varied. Any changes in the rate of the reaction must be due to the change in the concentration of the varying reactant. By varying the concentrations of reactants in Reaction (1), and observing the effect of the change in concentration on the rate of the reaction, we can mathematically determine the values of x and z in the rate law. Once the rate law is known, we can use the rate law and experimental data (rates, concentrations) to calculate the value of the rate constant, k , at a given temperature.

We can modify the rate law in equation (2) by taking the log of both sides to yield:

$$\ln(\text{rate}) = \frac{\Delta[\text{CV}]}{\Delta\text{time}} = \ln(k[\text{CV}]^x [\text{OH}^-]^z) = \ln(k) + x\ln[\text{CV}] + z\ln[\text{OH}^-] \quad (3)$$

This equation takes the form of a straight line: $y = mx + b$. In one set of experiments, we hold the concentration of crystal violet constant while varying the concentration of OH^- . Plotting the $\ln(\text{rate})$ vs the $\ln[\text{OH}^-]$, we obtain a straight line with a slope $m = z$ and an intercept $b = (\ln(k) + x \ln[\text{CV}])$. Similarly, we can hold the concentration of hydroxide constant while varying the concentration of CV. A plot of $\ln(\text{rate})$ vs the $\ln[\text{CV}]$ produces a straight line with a slope $m = x$ and an intercept $b = (\ln(k) + z \ln[\text{OH}^-])$. Alternatively, we can take advantage of the integrated forms of the rate law in which the rate law expression is integrated with respect to time:

$$\text{1st Order:} \quad \text{rate} = k[\text{A}] \rightarrow \ln [\text{A}]_t = -kt + \ln[\text{A}]_o \quad (4)$$

$$\text{2nd Order:} \quad \text{rate} = k[\text{A}]^2 \rightarrow 1/[\text{A}]_t = kt + 1/[\text{A}]_o \quad (5)$$

Both equations (4) and (5) take the form of a straight line: $y = mx + b$. If the reaction is 1st order in A, a plot of $\ln[\text{A}]$ vs. time should yield a straight line with a slope $m = -k$. If the reaction is 2nd order in A, a plot of $1/[\text{A}]$ vs. time should yield a straight line with a slope $m = k$.

But how can we measure the rate of this reaction? Crystal violet is a highly colored compound and absorbs light in the visible region of the electromagnetic spectrum. Therefore, we can monitor the rate of the reaction by monitoring the change in the concentration of CV over time using spectrophotometric analysis.

Spectrophotometric Analysis

Molecules will absorb light of a given wavelength if the energy of the light matches an electronic transition within the molecule. The amount of light absorbed is defined by Beer's Law, shown in Equation 6:

$$A = \epsilon bc \quad (6)$$

where A is the amount of light absorbed, and c is the concentration of the absorbing species. The amount of light absorbed will also depend on the efficiency of the absorption process, represented by the **molar absorptivity coefficient** (ϵ), and the path length of light traveling through the solution (b).

A spectrophotometer is an instrument consisting of a light source, a sample cell, and a photodetector. Light from the source is directed through the sample cell to the detector. A spectrophotometer can display data using two different scales. **Transmittance** is defined as the ratio of light reaching the detector when an absorbing species is in the sample cell compared to the amount of light reaching the detector when a "blank" or non-absorbing solution is in the sample cell. Transmittance is reported as %T,

$$\%T = \frac{(I)_a}{(I)_o} \times 100 \quad (7)$$

where $(I)_a$, $(I)_o$ represent the intensity of light reaching the detector for the sample and blank solutions, respectively. The other scale is **absorbance**, related to %T as shown in Equation 7.

$$A = 2.000 - \log(\%T) \quad (8)$$

Absorbance is the preferred scale because it is linearly related to concentration, as shown in Eq. 8. The concentrations of unknown solutions can be determined using absorbance data and a calibration plot known as a **Beer's Law plot**, as shown in Figure 1. In this lab we will use spectrophotometry to determine the rate law for the reaction shown in Reaction 1. Since the absorbance of the reaction solution is directly related to the concentration of CV, the change in absorbance over time is directly related to the change in CV concentration:

$$\text{Rate} = \frac{\Delta[\text{CV}]}{\Delta\text{time}} = \frac{\Delta[\text{Abs}]}{\Delta\text{time}} \quad (9)$$

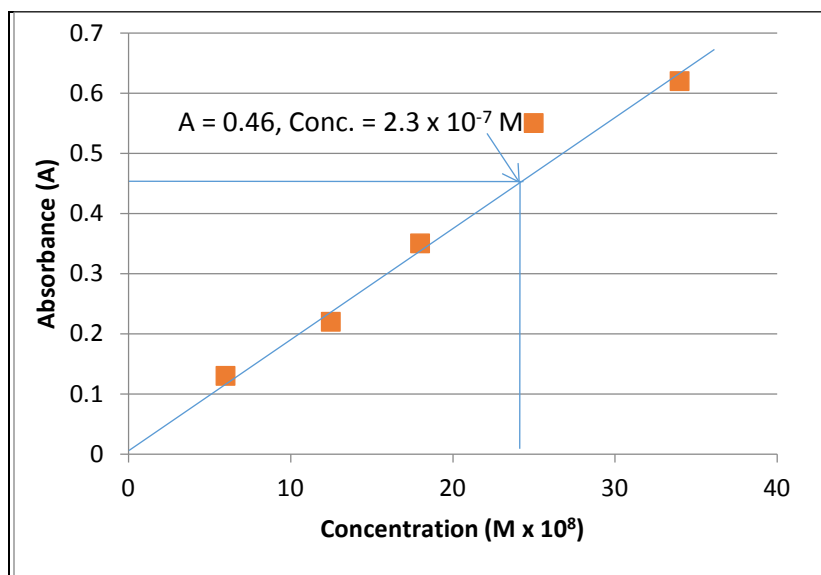


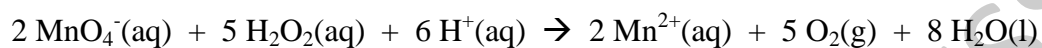
Figure 1. A typical Beer's Law plot.

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Pre-Lab Questions

1. Why is the concentration of crystal violet held constant in these experiments, while the concentration of sodium hydroxide is held varied?

2. Consider the following balanced chemical reaction:



- a) A student wrote the following rate law for this reaction:

$$\text{Rate} = k [\text{MnO}_4^-]^2 [\text{H}_2\text{O}_2]^5 [\text{H}^+]^6$$

Is this correct? Explain.

- b) Briefly describe what must be done to obtain the correct rate law.

3. Define the following terms:

- a) *Rate* :

- b) *Rate law* :

- c) *Rate constant, k* :

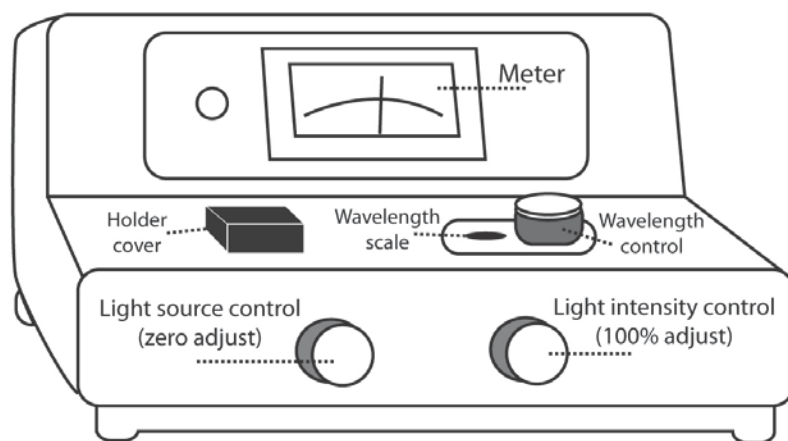
4. What would you expect to happen to the rate of the reaction between crystal violet and hydroxide as temperature increases? Explain your answer in terms of kinetic theory. (refer to the appropriate chapter/section of textbook).

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PROCEDURE

Part A. Beer's Law Plot.

1. A typical spectrophotometer is illustrated in Figure 2. Note the knobs in the figure for wavelength control, zero adjust, and 100% adjust. Using the wavelength control, adjust the wavelength on your spectrophotometer to 590 nm. With no sample in the sample holder, adjust the %T reading on the meter to zero using the zero adjust knob.

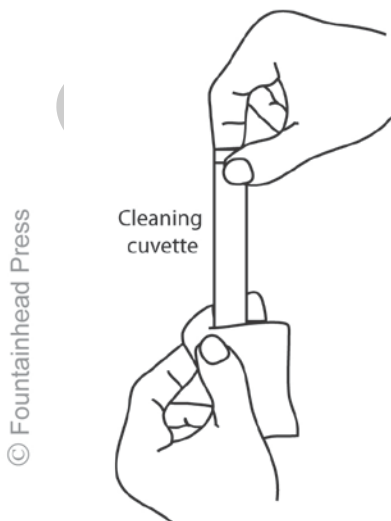


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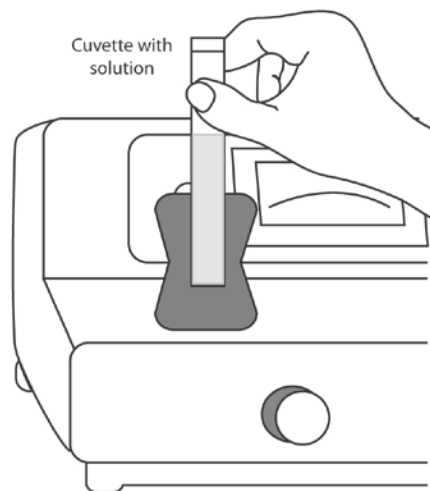
Figure 2. A typical spectrophotometer.

2. Obtain a cuvette and fill it with DI water. Carefully clean the surface of the cuvette as illustrated in Figure 3. Insert the cuvette into the sample holder as shown in Figure 4 so the reference line on the cuvette is aligned with the mark on the sample cell. Close the holder cover and adjust the %T reading to 100% with the 100% adjust knob. Repeat steps 1 and 2 until you obtain stable 0% and 100% readings.

Figure 3. Handling/cleaning of a cuvette. **Figure 4. Inserting cuvette into sample holder.**



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- Prepare a standard solution by pipetting 30.0 mL of the stock crystal violet solution to a 100.0-mL volumetric flask and adding DI water to the mark and mixing well. Pipette 10.0 mL of this standard solution and 10.0 mL of DI water into a 50-mL beaker. When the solution is well mixed, fill a spectrophotometer cuvette with the solution. Clean the surface of the cuvette and place it in the spectrophotometer as illustrated in Figures 3 and 4.
- Calculate the concentration of the solution prepared in step 3 and record this concentration as S1 in Table 1 on the Data Sheet. With the spectrophotometer wavelength set to $\lambda_{\text{max}} = 590 \text{ nm}$, record the absorbance of this solution. Discard the solution in the cuvette to the waste container. Transfer 10.0 mL of the solution prepared in the 50-mL beaker in step 3 to another clean 50-mL beaker, and add an additional 10.0 mL of water and mix well. Calculate the concentration of this new solution and record it as S2 in Table 1 on your Data Sheet. Rinse the cuvette with a few mL of this solution and discard the rinse in the waste container. Fill the cuvette with the new solution, insert it into the spectrophotometer and record the absorbance on your Data Sheet.
- Repeat the dilution procedure in step 4 (serial dilution) until you have obtained absorbance readings for 5 separate solutions. Be sure to calculate and record the concentrations of solutions S3--S5 on you Data Sheet, and to record the absorbance of each solution.

Part B. Preparation of Reaction Solutions and Reaction Kinetics

I. Determination of Reaction Order for Hydroxide.

- Fill one of the 100-mL beakers with about 50–60 mL of the stock solution of 0.10 M NaOH. Obtain a 250-mL beaker and label it “Waste.”
- Obtain two clean, dry 10-mL graduated cylinders, and label them “A” and “B.” Obtain a clean, dry 50-mL beaker. Using a pipette, transfer 10.0 mL of the crystal violet solution prepared in part A from the 100.0-mL volumetric flask to graduated cylinder A. Using a different pipette, transfer 10.0 mL of the stock NaOH solution obtained in step 6 to graduated cylinder B. These solutions will be used to prepare reaction mixture E1, as indicated in Table 1.

Table 1. Solution Volumes for Reaction Mixture E1--E5

Reaction Mixture	Volume of Crystal Violet ($3.0 \times 10^{-5} \text{ M}$)	Volume of DI Water	Volume of NaOH (0.10 M)
E1	10.0 mL	0.0 mL	10.0 mL
E2	10.0 mL	2.0 mL	8.0 mL
E3	10.0 mL	4.0 mL	6.0 mL
E4	10.0 mL	6.0 mL	4.0 mL
E5	10.0 mL	8.0 mL	2.0 mL

- Transfer the 10.0 mL of crystal violet solution to the 50-mL beaker. While Student 1 adds the contents of graduated cylinder B to the beaker and mixes well, Student 2 should start the stopwatch. After mixing well, Student 1 will transfer some of solution E1 to a clean, dry cuvette and insert the cuvette into the spectrophotometer, following the procedures illustrated in Figures 3 and 4.
- At $t = 10$ seconds, Student 1 will read the % transmittance of the solution, and Student 3 will record this reading on the Data Sheet in Data Table 2 in the column labeled E1. While Student 2 continues to mark the time in 10 second intervals, Student 1 should read out the % transmittance measurement at each time and Student 3 should record the corresponding reading in Data Table 2 in the column for E1. Continue recording % transmittance measurements as a function of time for a total of 250 seconds.
- Discard the cuvette sample and reaction solution from the 50-mL beaker in the waste beaker.
- Clean and dry both 10.0-mL graduated cylinders, the 50-mL beaker, and the cuvette.
- Repeat steps 7–11 using the solution volumes indicated in the table for reaction mixture E2. The NaOH and DI water will both be placed in graduated cylinder B.
- Repeat until % transmittance vs time data have been obtained for all solutions E2--E5.

CALCULATIONS

Part A. Beer's Law

- Plot the absorbance measurement obtained for each of the standard solutions in Data Table 1 vs. the calculated [CV]. You may use either the graph paper provided or you may plot the data using a spreadsheet (e.g., Excel). If you use a spreadsheet program, be sure to select the "scatter plot" option when plotting the data. Based on Beer's Law, this plot should produce a straight line. Calculate the slope of this line, and record your result on the Data Sheet as ϵ . The accepted literature value of $\lambda_{\max} = 87,000 \text{ M}^{-1}\text{cm}^{-1}$. Compare your calculated value with the accepted value, and calculate the percent error as:

$$\text{Percent error} = [(\text{accepted value} - \text{experimental value})/(\text{accepted value})] \times 100$$

Record your % error on the Data Sheet.

Part B. Rate Law

In order to determine the rate law we will need to determine the *rate* (in units of M/sec), the *order* of the reaction with respect to the reactants (m and n), and the value of the *rate constant*, k . To determine the *rate*, we will need to know the original concentration of the reactants and how long it took them to react.

1. **Reaction Rates.** For each of the reactions E1 through E5, calculate the absorbance the absorbance from % transmittance using Eq. 8 and plot the absorbance of the solution vs. time. You may either plot the data on the graph paper provided, or you can enter the data into a spreadsheet (e.g., Excel).

The rate of a given reaction is calculated as the initial slope of the plot of absorbance vs time. If you view the plot, the slope may decrease over time, so it is important to calculate the slope in the initial portion of the graph (i.e., first 100 to 200 seconds) in a region where the plot appears to be linear. If you are using a spreadsheet program, you can take advantage of the “add trend line” option to calculate the best-fit straight line, which will include a calculation of the slope.

Record the reaction rates for each reaction mixture on the Calculation Sheet.

2. **Reaction order.** Since the CV concentration was monitored spectrophotometrically, the absorbance of the reaction solution was directly related to the [CV] by Beer’s Law. Therefore, we can take advantage of the integrated rate laws to determine the order of the reaction with respect to CV. Using the absorbance data vs. time for reaction E1, calculate $\ln[\text{Abs}]$ and $1/[\text{Abs}]$ for each data point and record these data in Data Table 3.

Using experimental data for reaction mixture E1, prepare two graphs. For the first order graph, plot $\ln[\text{Abs}]$ vs. time; for the second order graph, plot $1/[\text{Abs}]$ vs. time. You can plot the data using the graph paper provided or you can use a spreadsheet program (e.g., Excel). Based on the graph that produces a straight line, record the value of x on the Data Sheet.

To determine the value of z we take advantage of the fact that only one reactant concentration was changed during the experiment. Note that the [CV] was the same for Reactions E1--E5, while the [OH⁻] varied. The generic rate law can be written as

$$\text{Rate} = k [\text{CV}]^x [\text{OH}^-]^z$$

Taking the natural log of both sides of the equation yields:

$$\ln \text{rate} = \ln k + x \ln [\text{CV}] + z \ln [\text{OH}^-] \quad (3)$$

Since x and [CV] are both constants for E1--E5, we can combine them with $\ln k$ to make a new constant $\ln k'$, which yields:

$$\ln \text{rate} = \ln k' + z \ln [\text{OH}^-] \quad (10)$$

This new equation resembles the equation for a straight line, $y = mx + b$. Plotting $\ln \text{rate}$ vs. $\ln [\text{OH}^-]$ for experiments E1--E5 yields a straight line with a slope = z .

Using the volumes and concentrations for CV and NaOH solutions, calculate the initial concentration of CV and OH⁻ in each of the reactions E1--E5, and record these concentrations under Part B3 in Calculations. Using the rates for E1--E5, plot $\ln \text{rate}$ vs. $\ln [\text{OH}^-]$. Calculate the slope of this plot and record it as z on your Calculations Sheet. You should round your results to the nearest integer.

3. **Rate Constant.** We now know the exact form of the rate law from Part B1 and B2. We can rearrange the rate law expression to solve for the rate constant, k .

$$k = \frac{\text{Rate}}{[\text{CV}]^x [\text{OH}^-]^z} \quad (11)$$

Using Eq. (11), calculate the value of k for each of the reaction mixture E1--E5. For the calculation of reaction orders, we calculated reaction rate as a change in absorbance vs. time. In order to obtain appropriate units for k , we must first calculate the rate in appropriate units, or as a change in concentration of CV vs time. On the Calculations Sheet, record the initial concentration of CV for each reaction mixture E1--E5. Using the measured absorbance at $t = 100$ seconds and the Beer's Law plot from Part A, determine the $[\text{CV}]$ at $t = 100$ sec. Record this concentration for each reaction mixture E1--E5 in Part 3 on the Calculations Sheet. The rate of the reaction for each mixture can now be calculated as

$$\text{Rate (M/s)} = ([\text{CV}]_0 - [\text{CV}]_{t=100}) / 100 \text{ sec.} \quad (12)$$

Record these calculate rates in Part 3 on the Calculations sheet. The value of k for each reaction mixture can now be calculated using Equation (11). Record the calculated values of k for reactions E1--E5, and use these values to calculate and record an average value of k .

Data Sheet: Reaction Kinetics

Part A. Beer's Law Plot

$\lambda_{\max} =$ _____ nm

Data Table 1.

<u>Standard Solution</u>	<u>[Crystal Violet] (M)</u>	<u>Absorbance</u>
S1.	_____	_____
S2.	_____	_____
S3.	_____	_____
S4.	_____	_____
S5.	_____	_____

Calculated ϵ : _____

Percent error in ϵ : _____

Calculations:

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Data Sheet: Reaction Kinetics (cont.)

Part B. Reaction Solutions and Reaction Kinetics

Data Table 2. % Transmittance Readings vs. Time for Reaction Solutions E1--E5

Time	E1	E2	E3	E4	E5
10	_____	_____	_____	_____	_____
20	_____	_____	_____	_____	_____
30	_____	_____	_____	_____	_____
40	_____	_____	_____	_____	_____
50	_____	_____	_____	_____	_____
60	_____	_____	_____	_____	_____
70	_____	_____	_____	_____	_____
80	_____	_____	_____	_____	_____
90	_____	_____	_____	_____	_____
100	_____	_____	_____	_____	_____
110	_____	_____	_____	_____	_____
120	_____	_____	_____	_____	_____
130	_____	_____	_____	_____	_____
140	_____	_____	_____	_____	_____
150	_____	_____	_____	_____	_____
160	_____	_____	_____	_____	_____
170	_____	_____	_____	_____	_____
180	_____	_____	_____	_____	_____
190	_____	_____	_____	_____	_____
200	_____	_____	_____	_____	_____
210	_____	_____	_____	_____	_____
220	_____	_____	_____	_____	_____
230	_____	_____	_____	_____	_____
240	_____	_____	_____	_____	_____
250	_____	_____	_____	_____	_____

Calculations

Data Table 3. Absorbance Readings vs. Time for Reaction Solution E1

Time	Absorbance	ln (Abs)	1/(Abs)
10	_____	_____	_____
20	_____	_____	_____
30	_____	_____	_____
40	_____	_____	_____
50	_____	_____	_____
60	_____	_____	_____
70	_____	_____	_____
80	_____	_____	_____
90	_____	_____	_____
100	_____	_____	_____
110	_____	_____	_____
120	_____	_____	_____
130	_____	_____	_____
140	_____	_____	_____
150	_____	_____	_____
160	_____	_____	_____
170	_____	_____	_____
180	_____	_____	_____
190	_____	_____	_____
200	_____	_____	_____
210	_____	_____	_____
220	_____	_____	_____
230	_____	_____	_____
240	_____	_____	_____
250	_____	_____	_____

Calculations (con't.)

Part B. Reaction Rates

1.	Reaction Mixture	Reaction Rate ($\Delta[\text{Abs}] / \Delta\text{time}$)	$[\text{CV}]_0$	$[\text{OH}]_0$
	E1	_____	_____	_____
	E2	_____	_____	_____
	E3	_____	_____	_____
	E4	_____	_____	_____
	E5	_____	_____	_____

2. **Reaction Orders:** $[\text{CV}], x =$ _____ $[\text{OH}], z =$ _____

3.	Reaction Mixture	$[\text{CV}]_0$	$[\text{CV}]_{t=100}$	Rate	$[\text{OH}]_0$	k
	E1	_____	_____	_____	_____	_____
	E2	_____	_____	_____	_____	_____
	E3	_____	_____	_____	_____	_____
	E4	_____	_____	_____	_____	_____
	E5	_____	_____	_____	_____	_____

Average k (with units!) = _____

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Post-Lab Questions

1. Based on your results, what is the order of the reaction with respect to CV? What is the order of the reaction with respect to hydroxide ion? What is the overall order of the reaction?
2. A student inadvertently added hydroxide solution instead of DI water to the CV solution in graduated cylinder A in Part B(II) of the Procedure. How would this affect the data collected by the student, and how would it affect the reaction rate calculated from the plot of absorbance vs. time?
3. Students in lab section #1 performed this experiment when the room temperature was 20 °C, while students in lab section #2 performed this experiment when the room temperature was 28 °C. How would the reaction rates compare for lab sections #1 and #2? How would the values of k compare?
4. Using procedures similar to this lab exercise, a student used the reaction rates based on Absorbance measurements from Part B1 of the Calculations, rather than the rates calculated from concentrations in Part B3. How would this affect the calculated value of k , and what would be the units associated with this value?