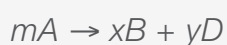


Following reaction kinetics through UV-Visible absorption techniques: hydrolysis of crystal violet

Introduction

In chemistry, there is often the need to understand how fast or slow a reaction proceeds, as well as what factors, like temperature or the presence of a catalyst, can change the total reaction time. By studying the rates of these reactions and how they change under different reaction conditions, a better understanding of the underlying mechanism can be understood.

The rate of a chemical reaction is dependent on the amounts of starting materials (reactants) present which are able to react. If the chemical equation for the reaction is known, a rate law can be predicted based on this equation, relating the dependence of the overall rate by the initial reactant concentration.



Equation 1.

Using the generic chemical equation described in equation 1, the rate law can be expressed as follows in equation 2,

$$Rate = k[A]^m$$

Equation 2.

where k is the rate constant, $[A]$ is the concentration of the reactant involved in the chemical reaction and m is the stoichiometric coefficient of the reactant from the balanced equation (equation 1). The exponent for each respective reactant is referred to as the order for that reactant, while the sum of all the exponents for the reactants involved in the rate law determines the overall reaction order. This order of a reaction defines how the concentration of the reactants affects the overall kinetics. The most common rate orders include zeroth, first, and second order (Figure 1).

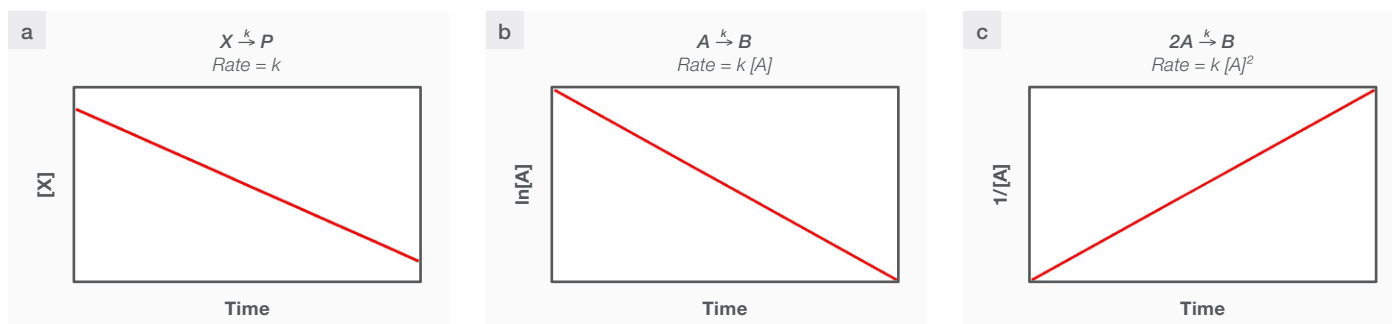


Figure 1. Concentration dependence as a function of time for (a) zeroth order, (b) first order, and (c) second order reactions.

Reaction order	Rate law	Integrated rate law	Slope	Y-intercept
Zeroth order $X \xrightarrow{k} P$	$Rate = k$	$[X] = [X_0] - kt$	$-k$	$[X_0]$
First order $A \xrightarrow{k} B$	$Rate = k[A]$	$\ln[A] = \ln[A_0] - kt$	$-k$	$\ln[A_0]$
Second order $2A \xrightarrow{k} B$	$Rate = k[A]^2$	$\frac{1}{[A]} = \frac{1}{[A_0]} + kt$	k	$\frac{1}{[A_0]}$

Table 1. Comparison of zeroth, first, and second order rate reactions.

The rate of a zeroth order reaction is independent of concentration and is identified as a linear change in the concentration of the reactant over time (Figure 1a). This can occur when the reactant reacts in such low quantities that changes to the concentration are considered negligible or if the reactant is replenished as it is consumed. The chemical equation for first order reactions can be similar to zeroth order, but the behavior of these reactions can be very different. The rate of a first order reaction is dependent on the concentration of the reactant, unlike zeroth order reactions. As the reaction progresses over time, the natural log of the reactant concentration will vary linearly with time; this can be visualized through experimentation and is distinct from zeroth order reactions (Figure 1b).

Finally, second order equations involve two reactants, either different compounds or two of the same compound, in which the rate of the reaction is dependent on the concentrations of both reactants (Figure 1c). Reactions which involve two of the same reactant are the simplest case of a second order reaction and lead to a linear dependence on $1/[A]$ over time, as described in Table 1. This correlation between the reactant concentration and reaction time is referred to as the *integrated rate law* and is outlined generally in Table 1 for zeroth, first and second order reactions.

In all of these cases, the slope of the line in the plot of the integrated rate law indicates the rate constant for the reaction, k (Table 1). This value relates the rate of the reaction to the concentration of the reactants through the rate law, as described previously, and can be used to represent how fast or slow a reaction is. The k term can be a useful point of comparison between reactions held under different conditions. The units for the rate constant are dependent on the type of reaction monitored.

Though we can predict the rate law for a reaction, and therefore the order, based on the chemical equation, the true rate law can only be determined through experimental results. To do so, a method for monitoring the reaction as a function of time must be employed. There are multiple techniques which can be used to monitor chemical reactions, including UV-Visible absorption spectroscopy. In this technique, the sample is irradiated with light of a specific wavelength in the UV-Visible range, which promotes electrons from the ground state to the excited state. The phenomenon is referred to as absorption and, through Beer's law (equation 3),

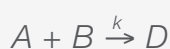
$$A = l c \epsilon$$

Equation 3.

where A is the measured absorbance, l is the path length, c is the concentration and ϵ is the molar extinction coefficient, the absorption of light by a compound is shown to be proportional to the concentration of the reactant. In this way, UV-Visible absorption spectroscopy can be a direct measure of the concentration of a given substance. When the structure of the reactant changes, the ground state and excited state can change, resulting in the loss of absorption. By monitoring the absorption of a compound at the specified wavelength over time, the reaction kinetics can be further analyzed to determine the rate constant.

Experimentally, by graphing the concentration of the reactant, the natural log of the reactant concentration, and the inverse of the reactant concentration (Figure 1) a comparison can be made to determine which plot best fits to a linear function. The graph which best fits to a line determines the overall reaction order and the absolute value of the slope will represent the rate constant for the reaction. For reactions involving more than one reactant, the experiment is repeated with varying concentrations of one reactant involved. In this way, given one substance concentration is the same, a system of equations can be used to solve for the order of each reactant.

For more complicated reactions involving multiple different reactants, analysis of the overall reaction kinetics can be more difficult. For example, let's consider a second order reaction with two different reactants, A and B.



Equation 4.

Unlike the second order reaction described in Table 1, here there are two different reactants dependent on one another, and thus the integrated rate law for this reaction is more complicated to analyze. To make the analysis of these reactions easier, chemists will use a pseudo-first order approximation in which the concentration of one component (A) is so much greater than the other (B) than it is assumed that changes to [A] are so small it can be considered negligible. As a result, the reaction will proceed as if the reaction were first order instead of second order, and the rate law can be written as shown in equation 5

$$Rate = k_{eff} [B]$$

Equation 5.

where the new effective rate constant (k_{eff}) will account for both the starting concentration of B ($[B_0]$) and the true rate constant for the second order reaction (equation 6). From here, the second order rate constant, k , can be determined.

$$k_{eff} = k[B_0]$$

Equation 6.

Herein the reaction between sodium hydroxide and crystal violet, an aromatic organic dye (equation 7), will be studied. Crystal violet is known to absorb in the visible region of the UV-Visible spectrum, with a maximum absorbance at 590 nm. The hydroxide ion reacts with the crystal violet structure, changing the structure and consequently changing the energetics. As a result, the newly formed product does not absorb in the visible range. By monitoring the loss in the absorption of crystal violet through UV-Visible absorption spectroscopy, the change in the concentration can be monitored over time.



Equation 7.

In this experiment, UV-Visible spectroscopy will be used to monitor the loss of crystal violet in the presence of hydroxide ions. By plotting $[C_{25}N_3H_{30}]$ vs time, $\ln[C_{25}N_3H_{30}]$ vs time and $1/[C_{25}N_3H_{30}]$, the rate constant and order for crystal violet can be determined. This reaction will be repeated using various different concentrations of hydroxide in order to set up a system of equations to solve for the order for $[OH^-]$ using the first order approximation method. Additionally, this reaction will be repeated at a lower temperature to determine how temperature can affect the rate, and therefore the rate constant, of the reaction.

References

1. Kazmierczak, N.; Vander Griend, D.A., Improving Student Results in the Crystal Violet Chemical Kinetics Experiments, *J. Chem. Ed.*, **2017**, 94, 61 – 66.
2. Corsaro, G., A Colorimetric Chemical Kinetics Experiment, *J. Chem. Ed.*, **1964**, 41, 48.

Pre-lab questions

1. Determine the volume needed of the provided 15.0 μM crystal violet stock solution, along with the volume of water needed, to prepare crystal violet solutions with the desired concentrations listed in the table below. The total volume of each prepared solution should be 15.0 mL. Include your work:

[Crystal violet] (μM)	Volume of 15.0 μM crystal violet stock (mL)	Volume of water (mL)	Total volume (mL)
15.0			15.0
10.0			15.0
5.5			15.0
3.0			15.0
1.0			15.0

2. Based on chemical equation (1), write the predicted rate law for the reaction.
3. What is the predicted reaction order for OH^- ?
4. What is the predicted reaction order for crystal violet?
5. What would happen to the reaction rate if $[\text{NaOH}] \gg [\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}]$? Write the effective rate law under these conditions.
6. What do you expect to happen if the reaction occurs at a temperature lower than room temperature? What would you expect to happen if the temperature is higher?

Experimental

Materials

- Thermo Scientific™ GENESYS™ G5 Vis or G5 UV-Vis spectrophotometer
- Stopwatch (or smart device with a stopwatch application)
- Cuvette (quartz, glass or plastic/disposable will all work)
- Lint-free lab wipes
- Appropriate glassware for preparing stock and sample solutions
 - Volumetric pipettes, graduated cylinders, beakers, test tubes, etc.
- Ice bath
- Thermometer
- 15 μM Crystal Violet ($\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}$)
- 0.15 M Sodium Hydroxide (NaOH)
- DI or nanopure water

Safety

Eye protection and gloves should be worn at all times when handling the reagents used in this experiment.

Crystal violet and sodium hydroxide are both corrosive substances. Crystal violet is also an environmental hazard. Ensure students and any individual preparing or handling the chemicals described herein are using the correct personal protective equipment and are instructed to follow proper waste disposal procedures. For more details, please refer to the Safety Data Sheet for each material prior to handling.

Instructions

Note: Ensure to calculate dilutions before the lab period to save time!

Work in groups of 2-3 for this experiment.

1. Turn on the spectrophotometer if not done already.
2. Make five 15.0 mL crystal violet solutions with the following concentrations:
 - 15.0 μM
 - 10.0 μM
 - 5.5 μM
 - 3.0 μM
 - 1.0 μM
3. Set up the “Fixed” application on the UV-Visible instrument with the following parameters (See Figure 2):
 - Equation: $\text{ABS}(\lambda_1) \times F_1$
 - λ_1 : 590 nm
 - F_1 : 1.000
4. Select continue.
5. Fill the 1.0 cm cuvette with DI or nanopure water.
6. Take a blank measurement of the water.
7. Measure the absorbance of 3.0 mL of each solution made in step 2 as well as 3.0 mL of DI or nanopure water as well. Record the value for each sample in **Table R1** of the results section.
8. Make three 10 mL NaOH stock solutions:
 - NaOH stock solution 1: 0.12 M NaOH
 - NaOH stock solution 2: 0.06 M NaOH
 - NaOH stock solution 3: 0.03 M NaOH
9. Set up the “Live” application on the UV-Visible instrument with the following parameters (See Figure 3):
 - Analysis wavelength: 590 nm
 - Absorbance
10. Record the temperature of the room in **Table R2** in the results section for the appropriate sample.
11. Take a blank measurement using DI water.
12. Make a 3.0 mL solution containing crystal violet and NaOH in a cuvette with the following concentration:
 - **Sample 1** – 10 μM crystal violet, 40 mM NaOH

Calculations:

NOTE: Using a lint-free lab wipe, remove any dust or fingerprints from the outside of the cuvette.

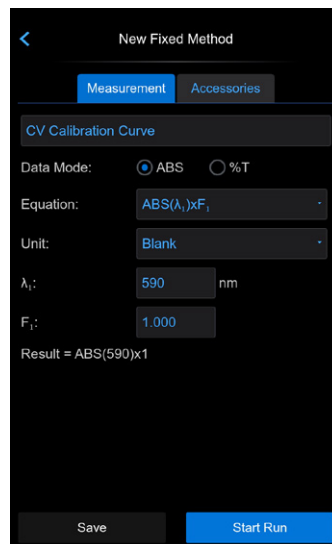


Figure 2. Fixed application parameters for the Genesys instruments.

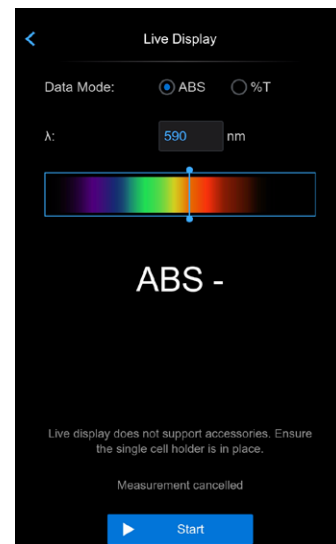


Figure 3. Live display application for the Genesys instruments.

13. Start the stopwatch as soon as the solution is made, transfer the cuvette to the instrument and begin measuring the absorbance of the sample. Be sure to transfer the cuvette to the instrument as quickly as possible to collect the most data.
 - Once the live measurement begins, record the absorbance of the sample every 30 seconds in the appropriate table in the results section (ex: **Table R4** for Sample 1) as well as the time the measurement was determined (round to the nearest second). Measure for 5 minutes from when the reaction was initiated.
14. End the live measurement.
15. Rinse the cuvette with DI or nanopure water.
16. Repeat steps 10-15 for the following samples:
 - **Sample 2** – 10 μM crystal violet, 20 mM NaOH
 - **Sample 3** – 10 μM crystal violet, 10 mM NaOH

Calculations:
17. Take a ~2.0 mL aliquot of 15 mM crystal violet and a ~2.0 mL aliquot of 0.12 M NaOH and add to separate vials.
18. Set up an ice bath. Place each aliquot in the ice bath and allow to equilibrate for a few minutes. Record the temperature of the ice bath.
19. Make a crystal violet + sodium hydroxide sample with the following component concentrations using the chilled stock solutions cooled in the ice bath:
 - **Sample 4** – 10 μM crystal violet, 40 mM NaOH

Calculations:
20. Repeat steps 12-15.

Results

Fill out the following tables according to your observations and instrument measurement. Include observations made during the experiment in the appropriate section. Attach these results with your post-lab questions to be turned in.

$[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}]$ (μM)	Absorbance at 590 nm
15.0	
10.0	
5.5	
3.0	
1.0	
0.0 (only water)	

Table R1. Crystal violet calibration curve data.

Sample number	Volume of crystal violet stock solution (mL)	Volume of NaOH stock solution (mL)	Volume of water (mL)	Concentration of crystal violet (μM)	Concentration of NaOH (μM)	T ($^{\circ}\text{C}$)
1						
2						
3						
4						

Table R2. Reaction sample information.

Write down your observations from the experiment below:

Time (s)	$A_{590\text{ nm}}$	$[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}]$ (μM)	$\ln([\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}])$	$1/[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}]$ (μM^{-1})

Table R3. Sample 1 reaction data, room temperature, 40 mM NaOH.

Time (s)	$A_{590 \text{ nm}}$	$[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}] \text{ (}\mu\text{M)}$	$\ln([\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}])$	$1/[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}] \text{ (}\mu\text{M}^{-1}\text{)}$

Table R4. Sample 2 reaction data, room temperature, 20 mM NaOH.

Time (s)	$A_{590 \text{ nm}}$	$[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}] \text{ (}\mu\text{M)}$	$\ln([\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}])$	$1/[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}] \text{ (}\mu\text{M}^{-1}\text{)}$

Table R5. Sample 3 reaction data, room temperature, 10 mM NaOH.

Time (s)	$A_{590 \text{ nm}}$	$[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}] \text{ (}\mu\text{M)}$	$\ln([\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}])$	$1/[\text{C}_{25}\text{N}_3\text{H}_{30}\text{Cl}] \text{ (}\mu\text{M}^{-1}\text{)}$

Table R6. Sample 4 reaction data, ice bath, 40 mM NaOH.

Post-lab data analysis and questions

- Using a spreadsheet program, plot the following graph using the data from **Table R1** in the results section and attach to your post-lab questions:

a. Absorbance vs $[C_{25}N_3H_{30}Cl]$

NOTE: Include appropriate axes labels and units. Include a linear trendline for the data and R^2 .

- Using the trendline calculated in question 1, convert the recorded absorbance to crystal violet concentration. Use this value to fill out the remaining table columns in the results section (**Tables R3-R6**).

- Using a spreadsheet program, plot the following graphs using the data from **Tables R3-R6** and attach to your post-lab questions:

a. Time (s) vs $[C_{25}N_3H_{30}Cl]$

b. Time (s) vs $\ln[C_{25}N_3H_{30}Cl]$

c. Time (s) vs $1/[C_{25}N_3H_{30}Cl]$

NOTE: Include appropriate axes labels and units for all graphs. Include the data from reaction samples 1, 2, 3 and 4 as separate data sets and use a legend to identify which data set corresponds to which sample. Include linear trendlines for each sample set included in graphs c and d as well as R^2 .

- Using the graph with the best linear fit (R^2 closest to 1), determine the effective rate constant (k_{eff}) for each reaction with appropriate units:

Sample 1: $k_{eff} =$ _____ units: _____

Sample 2: $k_{eff} =$ _____ units: _____

Sample 3: $k_{eff} =$ _____ units: _____

Sample 4: $k_{eff} =$ _____ units: _____

Sample 5: $k_{eff} =$ _____ units: _____

- Using the data from samples 1, 2 and 3, determine the reaction order for OH^- (Include all calculations). Round to the nearest whole number.
- Were the reactions' orders determined in questions 3 and 4 whole numbers? What sources of error could have led to a fractional number?
- Calculate the true rate constant (k) for each reaction with appropriate units:

Sample 1: $k =$ _____ units: _____

Sample 2: $k =$ _____ units: _____

Sample 3: $k =$ _____ units: _____

Sample 4: $k =$ _____ units: _____

Sample 5: $k =$ _____ units: _____
- Compare the calculated rate constant for Samples 1 and 4. What affect did changing the temperature of the reaction have? Why?
- What would you expect to happen if the concentration of NaOH was decreased to $10 \mu M$?