Teaching tomorrow's scientists today:

Lesson plans and resources for UV-Visible spectroscopic techniques

Introduction

This compendium of lesson plans for biology, chemistry, and kinetics laboratory experiments contains ready-made lessons designed to show the capabilities and benefits of ultraviolet-visible (UV-Vis) spectrophotometry. These lesson plans can ease an instructor's workload by providing pre-scripted, step-by-step guidelines for using spectrophotometers for a variety of applications.

UV-Vis spectrophotometry plays an important role in a wide range of fields, from industrial materials to life sciences. The experimental write-ups provided here will engage the next generation of scientists with hands-on access to real-world instrumentation.

In the **Biology** section, students will see first-hand how UV-Vis spectrophotometry can provide fast and easy evaluation of bacterial growth or how it can quickly measure enzyme activity. The analytic technique can even quantify DNA, as shown in an experiment that involves extracting genetic material from a strawberry.

The **Chemistry** section showcases the advantages of UV-Vis over other forms of analysis when measuring the levels of metals—in this specific case, iron—in a solution. The included lesson plans also teach students the use of Beer's Law through the analysis of dyes used in commercial food colorings, and the determination of copper content in a sample of brass.

The section on **Kinetics** highlights the ease and rapidity of UV-Vis analysis, showing how the technique can be used to track the progress of a reaction over time. Lesson plans cover the oxidation of a dye by bleach, the hydrolysis of crystal violet, and the reduction of permanganate ions in the presence of sugar. Instructions walk students through the graphing of relevant data and the calculation of reaction orders.

In addition to the lesson plans in this compendium, Thermo Fisher Scientific offers a broad array of online resources. We have webinars that offer support for teaching these spectroscopic techniques in your classroom, videos that demonstrate how best to use our instruments, application notes that highlight specific uses of UV-Vis, and more. Be sure to contact a Thermo Fisher representative to learn about the ways we can further support your teaching goals.

Contents

Biology

Bacterial Growth Curves: Analysis through OD_{600} measurements

Introduction

Bacteria are single cell organisms often characterized by their size, shape, vulnerability to specific antibiotics, and pathogenicity, among other properties.[1] Under the appropriate conditions (e.g., temperature, nutrients), bacteria can reproduce through binary fission, producing more bacteria over time.[1,2] Microbiologists will often study bacterial growth to better understand the different growth phases for a specific strain, such as the time it takes to double the population; this "doubling time" is a characteristic specific to the species and strain analyzed.[1] Additionally, analysis of bacterial growth can aid in determining the best experimental conditions for growing bacterial colonies (aggregates of clustered bacteria) according to the needs of the scientist.

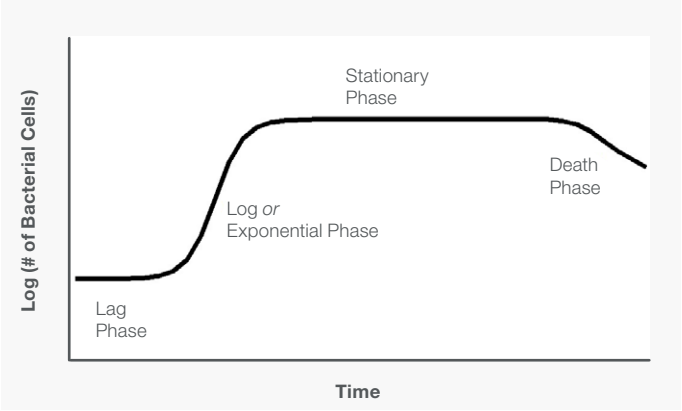

Figure 1. Example diagram for a typical bacterial growth curve.

Bacterial growth is characterized through four different phases: lag, log/exponential, stationary, and death phases (Figure 1). The lag phase is the first phase of bacterial growth and outlines the time it takes for bacteria to begin multiplying. In this phase, the bacteria begin to form the enzymes required to initiate growth. As described previously, the bacterial growth arises from binary fission, where one bacterium divides into two bacteria, which in turn produces two more bacteria each, and so on. This behavior will result in an exponential growth in the number of bacteria formed as a function of time and outlines the second growth phase: exponential or log phase.[2] During this phase, nutrients within the growth medium (*e.g.*, broth, agar) are being consumed. At some point, enough nutrients have been consumed such that the bacteria can no longer keep reproducing at an exponential rate, reaching a point of diminishing returns where the number of living bacteria no longer changes (stationary phase). Once the nutrients are diminished, and in the presence of waste byproducts produced during growth, the bacteria cannot survive indefinitely and will begin to die, leading to the death phase.

There are several different methods that can be employed to monitor the growth of bacterial colonies, including detecting nucleic acid sequences specific to the bacteria being grown and detecting of the structure of the bacteria through antibodies, among others.[1] One commonly employed method involves growing cultures on solid growth media from aliquots of the original growth culture collected at different time intervals during the experiment. The number of viable (living) colonies are then counted and reported for each time point. This method allows for the direct monitoring of the number of bacteria grown at a given time and is specific to living bacteria as dead colonies are unable to grow. Unfortunately, this technique is time-consuming as the bacteria must grow overnight before it can be counted.

Another frequently used technique to monitor bacterial growth involves UV-Visible spectroscopy. In principle, this technique uses light across the UV-Visible range of the electromagnetic spectrum to probe electronic transitions within a given analyte (molecule or biomolecule). Typically, data is reported as an absorption spectrum which describes these transitions. However, in the context of bacteria this technique is different. As the bacterial colonies grow, they become large enough to scatter light, which appears as an absorption feature across the entirety of the UV-Visible spectrum. With an increase in the number of colonies, the measured "absorbance" will increase as well. By measuring the "absorbance" at long wavelengths (600 nm is typically used by convention – OD_{600}), the growth of the bacteria can be monitored much more quickly than using the viable colony counting method, giving an almost instantaneous observation of growth.

It is important to keep in mind that this is not a true measurement of the absorbance of a sample, but an indirect measurement of the scattering of light off the newly formed bacterial colonies. Additionally, this method will measure scatter off any material capable of scattering light indiscriminately, including both living and dead bacteria. While this method can provide a faster method of monitoring bacterial growth, it will inherently have error in the analysis compared with counting viable colonies.

In this experiment, you will be monitoring the growth of *Escherichia coli (E. coli)* K12 cultures as a function of time through two different methods: viable colony counting from cultures grown on plates and OD_{600} measurements. Using the data from both methods, you will construct a growth curve for the formation of *E. coli* as a function of time. The experiment described herein is loosely based on the experiment outlined by McKernan.[3]

References

1. McGoverin, C.; Steed, C.; Esan, A.; Robertson, J.; Swift, S.; Vanholsbeeck, F., Optical Methods for Bacterial Detection and Characterization, *APL Photonics*, **2021**, 6.
2. Harvey, R. A.; Champe, P. C.; Fisher, B. D., *Microbiology Second Edition*; Lippincott Williams & Wilkins, **2006**.
3. McKernan, L. N., Using a Simple Escherichia coli Growth Curve Model to Teach the Scientific Method, *Am. Biol. Teach.*, **2015**, 77, 357-362.

Experimental

Materials

- Thermo Scientific™ GENESYS™ 40 or 50 UV-Visible Spectrophotometer
- Disposable cuvettes (plastic cuvettes are appropriate)
- Lint-free lab wipes
- *Escherichia coli (E. coli)*, Strain: K-12
- Luria Bertani (LB) Broth (1X)
- 50 mg/mL streptomycin
- 10% Bleach solution
- Metal or disposable inoculating loops (10 µL)
- Metal spreader
- LB Agar plates (with 50 µg/mL streptomycin)
- 250 mL baffled flask
- 2 L baffled flask
- Incubator
- Shaker
- Bunsen burner or alcohol burner (optional)
- Igniter (e.g. matches, lighter, spark igniter)
- Pipettor with appropriate pipette tips
- Permanent marker (preferably with a fine tip)

Safety

Note, in this lab students will be using a source of heat to sterilize inoculating loops. Care should be taken to ensure students and instructors do not burn themselves or allow the flame to become uncontrolled. Instruct students on proper fire safety, as well as applicable fire extinguishing methods. Additionally, students should be wearing proper personal protective equipment, including gloves, when performing this experiment or disposing of materials.

Instructions

Part A – Plating Bacterial Colonies

A1. Clean/disinfect the lab bench.

A2. Obtain (3) agar plates and the *E. coli* strain. Label the bottom of the plates appropriately (e.g. student initials, date, *E. coli* strain)

NOTE: When removing the lid from the agar plate, ensure the lid is placed face up on the lab bench so as to avoid introducing any additional bacteria into the agar plate.

A3. Set up a Bunsen burner or alcohol lamp.

a. Ensure safe lab procedures are followed when handling an open flame.

NOTE: Skip this step if using disposable inoculating loops.

A4. Using a sterile inoculating loop, pick and spread the *E. coli* on an agar plate. See Figure 2a for a visual description for spreading bacteria colonies on a plate for growth.

Instructions for Spreading/Streaking Plates:

a. Using the sterile inoculating loop, pick the *E. coli* colony and spread it in one quadrant of the agar plate. Running the inoculating loop over the flame will sterilize the loop (see Figure 2b).

b. Turn the plate 90˚, obtain a new disposable loop or re-sterilize the metal inoculating loop and wait for the loop to cool.

c. Using the cooled, sterilized loop, spread from the first quadrant into the next quadrant.

d. Turn the plate another 90˚, obtain a new disposable loop or re-sterilize the metal inoculating loop and wait for the loop to cool.

e. Using the cooled, sterilized loop, spread from the second quadrant into the next quadrant.

f. Turn the plate another 90˚, obtain a new disposable loop or re-sterilize the metal inoculating loop and wait for the loop to cool.

g. Using the cooled, sterilized loop, spread from the third quadrant into the next quadrant.

NOTE: If using disposable inoculating loops, sterilization is not necessary. Dispose of the loop after each use, then use a new one each time the plate is turned.

A5. Repeat step A4 on a second agar plate.

A6. Using just a sterile inoculating loop (no *E. coli*), spread on the final agar plate without *E. coli*, repeating the procedure outlined in step A4. This will serve as your negative control.

A7. Extinguish the flame.

A8. Place covers on each agar plate and place the plates in the incubator. The plates must be placed in the incubator upside down.

A9. Set the temperature to 37 ˚C and allow to incubate overnight.

Part B – Overnight Culture

B1. Following incubation, check for colony growth on your agar plate. Check with your instructor to make sure the colonies are acceptable to be used for the overnight culture.

B2. Clean/disinfect the lab bench.

B3. Fill a 250 mL baffled flask with 50 mL of LB broth.

B4. Calculate the volume of 50 mg/mL streptomycin needed to result in a final concentration of 50 µg/mL in 50 mL of solution:

B5. Add the calculated amount of 50 mg/mL streptomycin to the LB broth.

B6. From one of the plates, pick a single bacterial colony using a sterile inoculating loop.

B7. Dip the inoculating loop with the picked colony in the baffled flask and stir it a little.

B8. Remove the loop and properly dispose of it or, if it is not disposable, flame the inoculating loop as shown in Figure 2b to sterilize. Allow to cool before putting away.

B9. Place the flask in the incubator and allow to incubate at 37 ˚C overnight (maximum of 16 hours). Ensure the solution is able to shake during the incubation. The shaker should be set to 300 rpm.

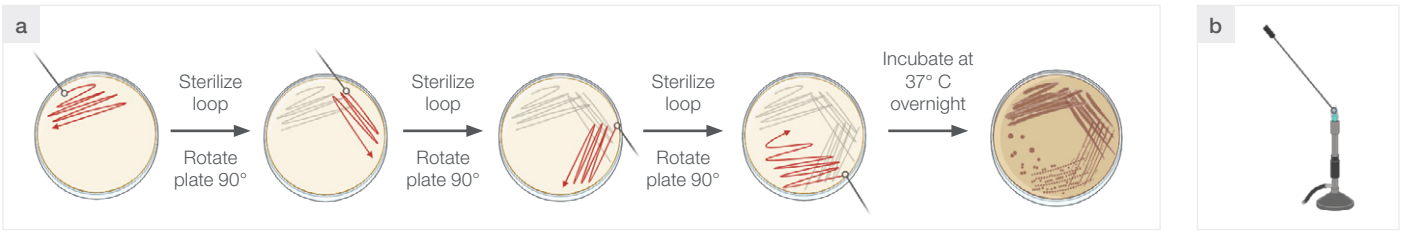

Figure 2 - (a) Scheme demonstrating streaking a plate. (b) Depiction of inoculating loop sterilization. Images created with BioRender.com.

Part C – Bacterial Growth Curve

C1. Clean/disinfect the lab bench.

C2. Turn on the UV-Visible spectrophotometer.

C3. Open the "Fixed" Application and select the following instrument parameters:

a. Wavelength (λ_1): 600 nm

b. Factor (F_1): 1.000

c. ABS mode

C4. Calculate the volume of 50 mg/mL streptomycin needed to result in a final concentration of 50 µg/mL in 612 mL of solution:

C5. Add the calculated amount of 50 mg/mL streptomycin to enough LB broth to result in a final solution volume of 612 mL.

C6. Bring the mixture of LB broth and streptomycin to 37 ˚C.

C7. Measure the blank/baseline for the experiment using the UV-Visible spectrophotometer:

a. Fill a 1.0 cm cuvette with 2 mL of the warmed LB broth.

b. Place the filled cuvette in the spectrophotometer cuvette holder.

c. Close the sample compartment and press the "Blank" button.

d. Remove the cuvette and dispose of the solution and cuvette in the appropriate waste disposal receptacles.

C8. Prepare the batch culture by transferring 12 mL of the overnight culture to the warmed broth. Keep the batch culture held at 37 ˚C and shaking at 300 rpm for the remainder of the experiment.

C9. Remove 5 mL from the batch culture using a sterile pipette.

C10. Using 2.0 mL of the reserved batch culture, fill a 1.0 cm cuvette and place in the UV-Visible spectrophotometer sample holder.

C11. Press "Measure" to measure the absorbance of the sample. Record the measured absorbance and growth time in Table 1 in the Lab report section of this experiment.

C12. Repeat steps C8 – C11 every 20 min. until the end of the lab period.

C13. Add bleach to the batch and overnight cultures and properly dispose of the solution.

C14. Using the aliquots collected at 0, 60, 120, 160 min, serially dilute 1:10 with LB broth for a total of 6 serial dilutions per time point as described below:

a. Dilution 1: Add 1.0 mL of batch culture to 9.0 mL of LB broth. Cap and mix by shaking.

b. Dilution 2: Add 1.0 mL of Dilution 1 to 9.0 mL of LB broth. Cap and mix by inverting.

c. Dilution 3: Add 1.0 mL of Dilution 2 to 9.0 mL of LB broth. Cap and mix by inverting.

d. Dilution 4: Add 1.0 mL of Dilution 3 to 9.0 mL of LB broth. Cap and mix by inverting.

e. Dilution 5: Add 1.0 mL of Dilution 4 to 9.0 mL of LB broth. Cap and mix by inverting.

f. Dilution 6: Add 1.0 mL of Dilution 5 to 9.0 mL of LB broth. Cap and mix by inverting.

C15. Set up a Bunsen burner or alcohol lamp.

C16. For each time point, pipet 100 µL of Dilution 4, 5 and 6 onto separate plates.

C17. Sterilize a metal spreader by running it over a flame. Allow to cool.

C18. Use the sterilized or single-use spreader to spread the solution across the entire surface of the agar plate in a circular motion.

C19. Re-sterilize the spreader, if applicable, and repeat for each plate.

C20. Sterilize the spreader, if applicable, once again and allow to cool before putting away.

C21. Wait a few minutes for the sample to diffuse in the agar, then incubate each plate overnight at 37 ˚C. Like in Part A, the plates should be placed upside down in the incubator.

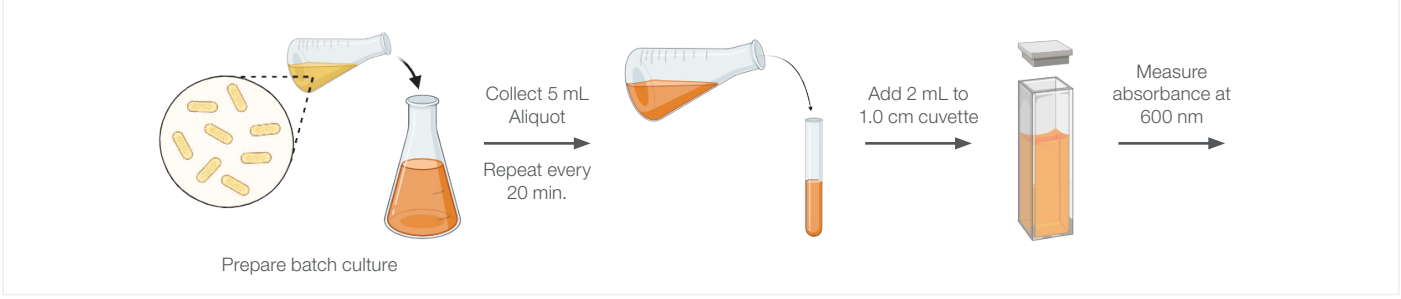

Figure 3. General OD 600 procedure from Part C. Created with BioRender.com.

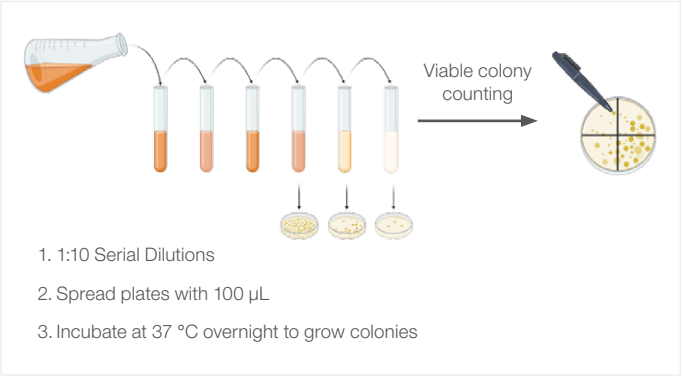

Figure 4. Viable cell counting procedure. Created with BioRender.com.

Part D – Viable Colony Counting

D1. Using the plates spread from Part C, count the number of viable colonies in each plate which can be counted for each time point. It can be helpful to use a marker to count each colony on the bottom of the plate to avoid re-counting errors. Consult your instructor for other helpful methods for keeping track of the colony number (*e.g.*, count in batches, use a handheld counter, etc.)

   a. If the plate has too many colonies, use a marker to divide the plates into quadrants. Count one quadrant and multiply by 4 to estimate the total number of colonies on the plate (See Figure 4).

D2. Back-calculate how many colonies were present in the diluted solution for each countable plate. Record your results in Table 2.

D3. Determine the bacterial cell count for each time point in CFU/mL.

Lab Report

Results Tables

Growth Time (min)	Absorbance at 600 nm (A.U.)	Growth Time (min)	Absorbance at 600 nm (A.U.)

Table 1. OD_{600} Growth curve data.

Growth Time (min.)	Colony Count – Dilution	Colony Count – Stock
0		
60		
120		
180		

Table 2. Viable bacterial colony count data.

Based on the data in Table 1 and 2, construct the growth curve for the samples analyzed herein. Include appropriate units, axes labels and significant figures in the graph. Attach the graph to the lab report questions. Include labels for the bacterial growth phases observed.

Questions

1. From the data collected in this experiment, what phases of growth were you able to observe?

2. What was the doubling time for this strain of *E. coli* calculated using the viable colony counting data, and what was it when calculated using the OD_{600} data? Use the equation below for your analysis:

$$t_D = \frac{ln(2)(t_2 - t_1)}{ln\left(\frac{B_2}{B_1}\right)}$$

NOTE: Only the data collected in the exponential/log phase will be used for this analysis.

3. From Part A, what did your negative control look like? Why did we need to make a negative control when streaking plates?

4. Why is it important the agar plates and LB broth have streptomycin?

5. What would you expect to happen to the growth curve if you added 24 mL of the overnight culture instead of 12 mL to the LB broth?

6. What would you expect your growth curve to look like if you incubated the batch culture at 25 ˚C instead of 37 ˚C?

Notes for Instructors and Teaching Assistants:

Below includes an example of on OD_{600} measurement coupled with a viable cell count for an *E. coli* (strain K12) growth experiment according to the procedure outlined in this lesson plan with the exception of the intervals at which the aliquots were measured. Note, the experimental procedure requires 5 hours to begin observing the end of the exponential/log phase. If your lab period only lasts 3 hours, consider having students compare their data to the figure below or complete the experiment in your lab for the full 5 hours to produce a data set for students to compare their data against.

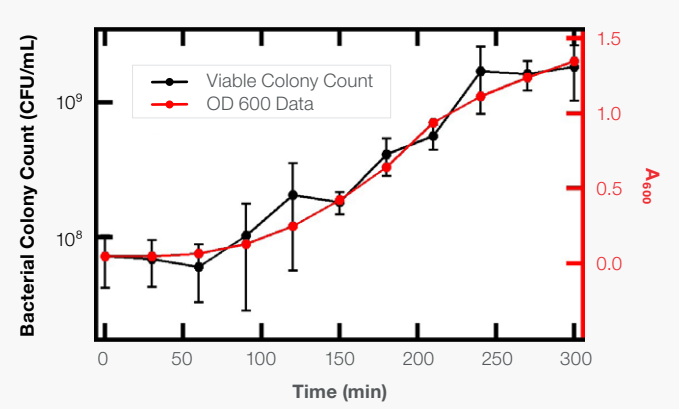

Figure 5. Example OD 600 curve. The left axis correlates to the black data points (bacterial colony count) and is plotted on a logarithmic scale. The right axis represents the measured absorbance at 600 nm and correlates to the red data points. Both right and left axes were scaled to overlay well on one another.

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Lesson plan

Activity of Acid Phosphatase Extracted from Wheat Germ

NanoDrop Ultra/Ultra^{C} Spectrophotometer

Introduction

Enzymes

Essential to living organisms, enzymes are proteins that catalyze chemical reactions required to sustain life. The active site of an enzyme binds with a specific substrate, helping to transform the substrate into a new product with great efficiency (Figure 1).[1,2] Most enzymes are classified by the suffix "-ase" and the substrate they act upon. For example, the enzyme phosphatase is involved in dephosphorylation, or the removal of a phosphate group.[1] Acid phosphatases are widespread enzymes found in plants and mammals that serve to dephosphorylate a substrate to form free phosphate and alcohol products.[3]

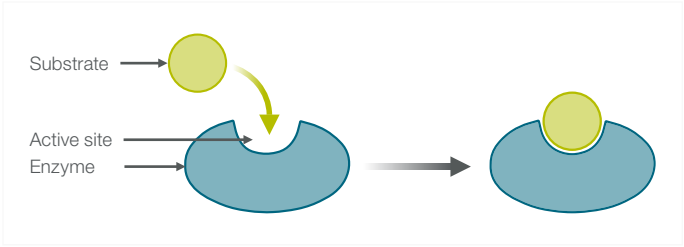


Figure 1. Enzyme binding with a substrate at the enzyme's active site. Image created with BioRender.com.

An enzyme deficiency or surplus can carry clinical significance in the form of diabetes, neurological disorders, or other maladies. This illustrates the importance of classifying enzymes and their activity.[1,4] Enzyme activity describes the amount of product made per unit time, while the specific activity describes the activity per milligram of protein. Specific activity provides a purity assessment of the enzyme because specific activity increases as the enzyme purity increases.[2]

During protein extraction and purification, the plant or mammalian cells are lysed, releasing all the cellular contents including proteins, nucleic acids, carbohydrates, and lipids. The next step in protein purification is called "salting out", which uses salt such as ammonium sulfate to reduce the solubility of protein. This decrease in solubility can also be achieved by using high volumes of organic solvents such as methanol or ethanol. As the protein solubility is reduced either with salts or solvents, the proteins precipitate out of the solution and are pelleted via centrifugation.[5] Confirming an enzyme's purity through specific activity is a key component of protein purification before continuing with additional enzyme studies.

UV-Visible Spectrophotometry

A simple technique to measure enzyme activity is ultraviolet-visible (UV-Vis) spectrophotometry. A spectrophotometer measures the amount of light absorbed by a sample. To measure absorbance, a beam of light passes through the sample to a detector and as the sample absorbs more light, this equates to more molecules in the sample. Thus, absorbance is directly proportional to concentration. To relate absorbance and concentration, Beer's Law describes the following relationship:

$$A = \varepsilon bc \quad or \quad c = \frac{A}{\varepsilon b}$$

Equation 1.

Through rearranging Beer's Law, concentration (c) is determined by dividing the absorbance (A) by the pathlength (b) and the sample-specific extinction coefficient (ε). The extinction coefficient is a measure of how strongly a sample absorbs light and the pathlength is the length the light travels through the sample. Traditional spectrophotometers typically use a 1.0 cm pathlength cuvette but the Thermo Scientific™ NanoDrop™ Ultra/Ultra^C Microvolume UV-Vis Spectrophotometer uses a range of pathlengths to accommodate higher sample concentrations without the need for dilutions.

Isolating one enzyme of interest requires high-end laboratory equipment, but a substrate called a chromogen can help quantify the activity of an enzyme of interest in a mixture of different proteins. The product of an enzyme acting upon a chromogen produces a color whose absorbance can be determined with visible spectrophotometry.[2] Acid phosphatase acts upon the chromogen p-nitrophenyl phosphate (PNPP) via hydrolysis to produce yellow p-nitrophenol (PNP), which can be measured at 405 nm using the extinction coefficient 18,300 M^{-1} cm^{-1} (Figure 2).[6,7]

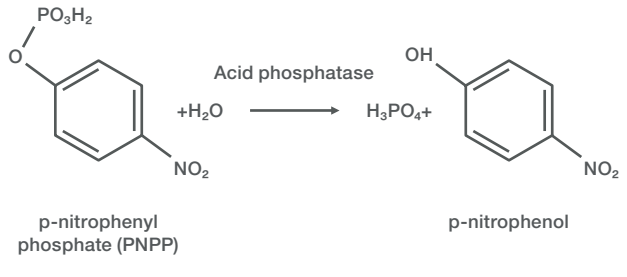


Figure 2. Chemical reaction of p-nitrophenyl phosphate as the chromogen and acid phosphatase as the catalyst to yield p-nitrophenol and inorganic phosphate. Image created with BioRender.com.

Experimental

Purpose

The purpose of this experiment is to determine the enzyme activity and specific activity of acid phosphatase extracted from wheat germ. There are four parts to this experiment:

- Part A: Extraction of acid phosphatase from wheat germ (adapted from Joyce & Grisolia, 1960)
- Part B: Determination of protein concentration with the BCA assay
- Part C: Completion of the phosphatase assay with p-nitrophenyl phosphate (adapted from Sigma-Aldrich, doc. no. SSPNPP02)
- Part D: Calculation of enzyme activity and specific activity

Materials

- Thermo Scientific™ NanoDrop™ Ultra/Ultra^C Spectrophotometer
- Deionized (DI) water
- Acid phosphatase control (Sigma-Aldrich, P3627)
- Store-bought wheat germ
- 1.0 M Magnesium chloride ($MgCl_2$)
- 4.10 M Ammonium sulfate (saturated) $(NH_4)_2SO_4$
- Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Thermo Scientific, 23225)
- p-nitrophenyl phosphate (PNPP) (Sigma-Aldrich, 20-106)
- 0.1 M Sodium hydroxide (NaOH)
- 0.09 M Citrate buffer solution, pH 4.8 at 37°C (Sigma-Aldrich, C2488)

Before beginning: write your experimental hypothesis in #1 of the Lab Report.

Part A: Extracting acid phosphatase from wheat germ

A1. Heat water bath to 70°C.

A2. Weigh out 5.0 grams of wheat germ and mix with 20 mL DI water in a 50 mL conical tube. Allow to sit for 30 minutes and stir occasionally to lyse the cells and release the cellular contents.

A3. Centrifuge at 5000 x g for 5 minutes, making sure the centrifuge is properly balanced. You may need to place a counterweight directly opposite the sample tube in the centrifuge.

A4. Decant and save the supernatant by tilting the conical tube into a clean 50 mL conical tube and discard the pellet. Reserve 200 µL of the supernatant for downstream testing. Label as **Fraction 1**.

A5. Add 500 µL of 1.0 M $MgCl_2$ and stir to mix.

A6. Centrifuge 5000 x g for 5 minutes. Decant and save the supernatant then discard the pellet. Reserve 200 µL of supernatant then determine supernatant volume. Label as **Fraction 2**.

   a. Supernatant volume: _____ µL/mL

A7. Add 0.54 volume saturated $(NH_4)_2SO_4$ and slowly stir to mix, avoiding bubbles and foaming as this denatures the enzyme.

   a. Volume of saturated $(NH_4)_2SO_4$ to add: _____ µL/mL

   b. For example: if the supernatant volume in 6a is 5.0 mL, add 2.7 mL of saturated $(NH_4)_2SO_4$ (5.0 mL x 0.54 volume = 2.7 mL).

A8. Centrifuge 5000 x g for 5 minutes. Decant and save the supernatant then discard the pellet. Reserve 200 µL of supernatant then determine supernatant volume. Label as **Fraction 3**.

   a. Supernatant volume: _____ µL/mL

A9. Slowly add 0.51 volume saturated $(NH_4)_2SO_4$ and place the sample tube in a water bath until sample temperature reaches 60°C and hold for 2 minutes.

   a. Volume of saturated $(NH_4)_2SO_4$ to add: _____ µL/mL

A10. Immediately after 2 minutes, place the sample tube in an ice bath until sample temperature reaches 8°C.

A11. Centrifuge 5000 x g for 10 minutes. Discard the supernatant. The enzyme will be present in the insoluble pellet.

A12. Resuspend pellet in 5.0 mL DI water and mix until completely dissolved, then reserve 200 µL. Label as **Fraction 4**.

A13. If Part B is not performed on the same day, all fractions can be stored in a freezer.

A14. If using an acid phosphatase control, prepare a 1.0 mg/mL sample.

Part B: Protein concentration with BCA assay

B1. The microplate protocol can be performed per the Pierce BCA protein assay instructions.

B2. Prepare a BSA standard curve using the serial dilutions provided in Table 1. Use clean pipette tips between each dilution.

Standard #	Volume of Water (µL)	Volume and source of BSA (µL)	Final BSA concentration (mg/mL)
Reference	300	0	0
1	0	300 of stock	2.0
2	150	150 of stock	1.0
3	150	150 of Standard 2	0.5
4	150	150 of Standard 3	0.25
5	150	150 of Standard 4	0.125
6	150	150 of Standard 5	0.0625

Table 1. BSA standard curve 1:1 dilution preparation to provide a working range of 0.0625 mg/mL – 2 mg/mL.

B3. Prepare the BCA working reagent as follows where the control is included as an "unknown":

   a. (7 standards + 5 unknowns) x (2 replicates) x (200 µL) = 4.8 mL working reagent required

   b. Mix 50 parts BCA Reagent A with 1 part BCA Reagent B: 5.0 mL Reagent A with 100 µL Reagent B.

   c. Note: the working reagent will appear light green.

B4. Mix 25 µL of each standard and unknown with 200 µL working reagent. Incubate at 37°C for 30 minutes.

   a. Note: the solution will turn purple as the colorimetric reaction takes place.

B5. On the NanoDrop Ultra instrument, select the tab **Proteins**, open the **Protein BCA** application, and enter the following in the Setup:

   a. Curve type: Linear

   b. Replicates: 2

   c. Standard Amount: 6

   d. Enter the BSA concentrations listed in Table 1. The Reference is already included in the software. Press "Done" when finished.

   e. Clean the pedestals by wiping with a lint-free lab wipe. Make a Blank measurement with 2.0 µL of DI water.

   f. Follow the on-screen prompts for measuring the standards in 2.0 µL volumes, making sure to clean the pedestals with a lint-free lab wipe before each new measurement. Once the standards are complete, select **Run Samples** to measure the unknowns in 2.0 µL volumes.

   g. Record the reported concentrations for the unknowns in Data Table 1 along with the R^2 of the BSA standard curve.

Part C: Single-point phosphatase assay and absorbance measurements

C1. Label six 1.5 mL microcentrifuge tubes as **F1**, **F2**, **F3**, **F4**, **C**, and **B**, where F1-4 correspond to the four fractions from part A, C corresponds to the control (if using), and B corresponds to the blank.

C2. Add 125 µL of 0.09 M citrate buffer and 125 µL of PNPP to all tubes.

C3. Mix each tube and equilibrate to 37°C for 5 minutes in a water bath or heat block.

C4. Add 25 µL distilled water to tube **B** and place back in the 37°C bath or block.

C5. Add 25 µL of the acid phosphatase control to tube **C** (if using), immediately mix by inversion, place back in the 37°C bath or block, and start a timer for 5 minutes.

C6. Working quickly, add 25 µL of Fraction 1 to tube **F1** and continue with fractions 2-4, mixing by inversion and then placing the tubes in the 37°C bath or block.

C7. After exactly 5 minutes, add 1.0 mL 0.1 M NaOH to all tubes in the same order the enzyme solutions were added (e.g., B, C, F1, F2, F3, F4). Adding NaOH stops the enzymatic reaction from continuing.

C8. From the NanoDrop Ultra instrument home screen, select the tab **Custom** and select the application **UV-Vis**.

C9. Enter 750 nm for the **Baseline Correction**, enter 405 nm for the **Analytical Wavelength**, and add 405 nm as a **Monitored Wavelength**.

   a. The baseline correction anchors the spectrum to zero at 750 nm and is used to correct for a baseline offset due to the presence of light-scattering particles.

   b. The analytical wavelength represents the PNP peak at 405 nm and instructs the NanoDrop software to use the optimal pedestal pathlength depending on the absorbance intensity. Regardless of the pathlength used, all displayed absorbance measurements are normalized to a 1.0 cm pathlength for application to Beer's Law.

C10. Follow the on-screen prompts for measuring the blank (tube B) and the samples in 2.0 µL volumes on the microvolume pedestal, making sure to clean the pedestals with a lint-free lab wipe before each new measurement.

   a. *Note: the best practice after making a Blank measurement is to measure the blank solution as a sample. For the NanoDrop Ultra instruments, this measurement should be within ± 0.04 absorbance units at the analysis wavelength (405 nm). If the measurement is outside of this range, measure a new Blank.*

C11. Record the absorbance results in Data Table 2.

Part D: Calculate enzyme activity and specific activity

D1. Calculate the concentration of PNP in µmol/mL using Beer's Law, where pathlength (l) = 1.0 cm, extinction coefficient (ε) = 18,300 M^{-1} cm^{-1}, and use the reported absorbance in Data Table 2.

$$\text{PNP concentration}\left(\frac{\mu mol}{mL}\right) = \frac{A}{\varepsilon * l} * 1.0\varepsilon 3\left(\text{conversion from } \frac{mol}{L} \text{ to } \frac{\mu mol}{mL}\right)$$

   a. Record the concentration in Data Table 3.

D2. Calculate the enzyme activity in µmol/min using the total reaction time (5 minutes) and the total assay volume (1.275 mL).

$$\text{Enzyme Activity}\left(\frac{\mu mol}{min}\right) = \left(\frac{\text{PNP}\left(\frac{\mu mol}{mL}\right)}{\text{Reaction time (min)}}\right) * \left(\text{total assay volume (mL)}\right)$$

   a. Record the enzyme activity in Data Table 3.

D3. Calculate the total amount of enzyme in the reaction using the protein concentration from the BCA assay in Data Table 1 and the sample volume added to the kinetics reaction (25 µL or 0.025 mL).

$$\text{Total enzyme (mg)} = \left(\text{protein concentration}\left(\frac{mg}{mL}\right)\right) * \left(\text{sample volume (mL)}\right)$$

   a. Record the total enzyme amount in Data Table 3.

D4. Calculate the specific activity using the values calculated for enzyme activity and total enzyme.

$$\text{Specific activity}\left(\frac{\mu mol}{min \bullet mg}\right) = \frac{\left(\text{Enzyme activity}\left(\frac{\mu mol}{mL}\right)\right)}{\text{Total enzyme (mg)}}$$

   a. Record the specific activity in Data Table 3.

Lab Report

1. Hypothesize how the specific activity changes with each fraction number.

2. Record the R^2 of the BCA protein assay standard curve and the protein concentration in mg/mL reported by the NanoDrop Ultra spectrophotometer.

R^2 of the standard curve: _____

Fraction #	Control	1	2	3	4
Enzyme concentration (mg/mL)					

Data Table 1. BCA protein assay results.

3. Discuss the R^2 of the BSA standard curve. Is this value close to 1.0? If not, what are some steps to take to improve the R^2?

4. Record the PNP absorbance results reported by the NanoDrop Ultra spectrophotometer. The reported absorbances are normalized to a 1.0 cm pathlength.

Sample	Blank	Control	F1	F2	F3	F4
Absorbance at 405 nm						

Data Table 2. UV-Vis absorbance results.

5. What did you observe during the 5 minute kinetics experiment?

6. Why is it important to measure the blank solution as a sample with spectrophotometry?

7. Record the calculated PNP concentration, enzyme activity, total enzyme in the reaction, and specific activity.

Sample	Blank	Control	F1	F2	F3	F4
PNP Concentration (µmol/mL)						
Enzyme Activity (µmol/min)						
Total Enzyme (mg)						
Specific Activity (µmol/ min•mg)						

Data Table 3. PNP concentration, enzyme activity, and specific activity results.

8. Do your specific activity results agree with your experimental hypothesis from #1? Why or why not?

9. How does specific activity change in relation to the fraction number and the total enzyme amount? What does this tell you about enzyme purity?

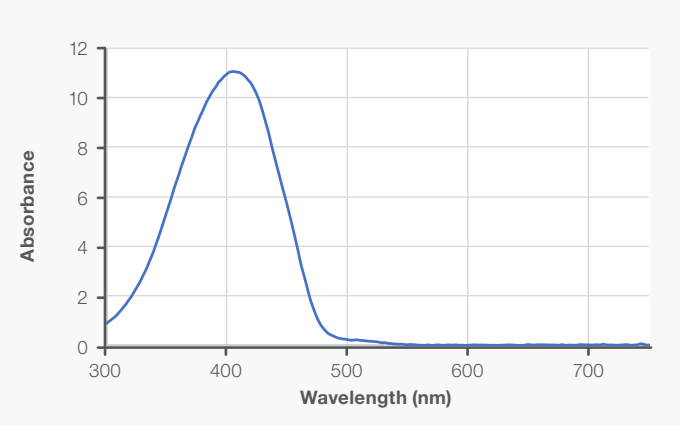
10. What do you predict would happen to specific activity if the reaction temperature was raised to 42°C?

11. Based on the specific activity of Fraction 4, does the enzyme need to be purified further? If so, what should be the next step in the purification procedure?

12. Is the enzyme present in Fraction 4 pure acid phosphatase? Why or why not?

Notes for Professors and Teaching Assistants

Expected PNP absorbance spectrum (405 nm peak):



References

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2. Robinson, P. K. (2015). Enzymes: Principles and biotechnological applications. *Essays in Biochemistry*, 59, 1–41. https://doi.org/10.1042/bse0590001
3. Moorman, V. R., & Brayton, A. M. (2021). Identification of individual components of a commercial wheat germ acid phosphatase preparation. *PLoS ONE*, 16(3), e0248717. https://doi.org/10.1371/journal.pone.0248717
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7. *Enzymatic Assay of Phosphatase, Acid* (EC 3.1.3.2). Document number SSPNPP02. (1995). Sigma-Aldrich.
8. Joyce, B. K., & Grisolia, S. (1960). Purification and properties of a nonspecific acid phosphatase from wheat germ. *The Journal of Biological Chemistry*, 235, 2278–2281

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Quantifying strawberry DNA with spectrophotometry

Introduction

Essential to all living organisms, DNA contains the genetic framework for cellular development and function. DNA extraction is an important laboratory method because genetic information is used in a variety of applications, from solving crimes to diagnosing genetic diseases. Extracted DNA can be used in gel electrophoresis, quantitative polymerase chain reaction (qPCR), sequencing, cloning, and more. To ensure these experiments function properly, the sample's DNA concentration must be known and there should be no contaminants.

Ultraviolet-visible (UV-Vis) spectrophotometry is a simple method to quantitate nucleic acid. A spectrophotometer measures the amount of light absorbed by a sample. The DNA bases (cytosine, guanine, adenine, and thymine) absorb light at a characteristic wavelength of 260 nm, which is in the ultraviolet range of the electromagnetic spectrum (Figure 1). To measure absorbance, a beam of light passes through the sample to a detector; the reported absorbance reading is directly proportional to sample concentration. Higher absorbance indicates that the light is being absorbed by more molecules in the sample.

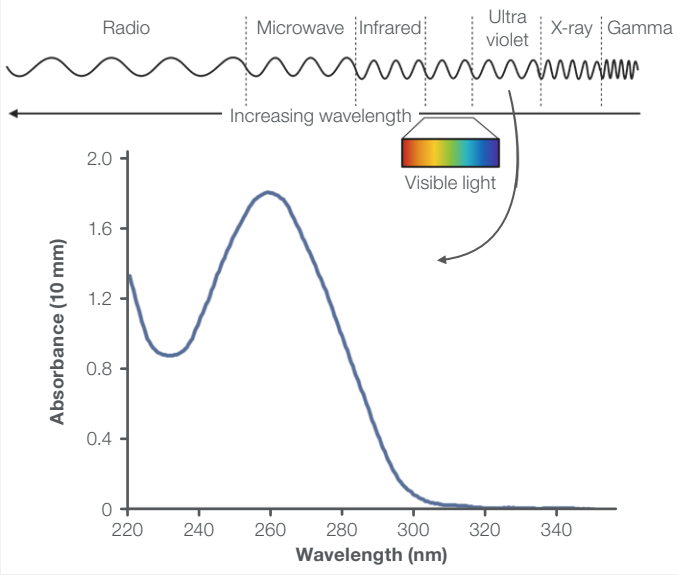


Figure 1. The electromagnetic spectrum and a DNA absorbance spectrum with the characteristic peak at 260 nm, in the ultraviolet range. Image created with BioRender.com.

Additionally, UV-Vis spectrophotometers provide information on DNA sample quality through purity ratios. The two most commonly reported ratios are A260/A280 and A260/A230. These ratios provide helpful quality metrics because the amino acids in proteins absorb light at 280 nm; leftover nucleic acid extraction material, such as phenols and salts, typically absorb light around 230 nm. For pure DNA, the expected A260/A280 purity ratio is ~1.8 while the expected A260/A230 purity ratio is 2.0–2.2. If protein contamination were present in a DNA sample, the A260/A280 purity ratio would be reduced due to the increased protein absorption at 280 nm. The purity ratios for extracted dsDNA should be close to expected, otherwise the sample would need additional purification before further experiments are performed.

Beer's Law

Beer's Law is used to relate absorbance and concentration using three variables in the following relationship:

$$A = \varepsilon bc \quad or \quad c = \frac{A}{\varepsilon b}$$

By rearranging Beer's Law, concentration (c) is determined by dividing the absorbance (A) by the pathlength (b) and the sample-specific extinction coefficient (ε). The extinction coefficient is a measure of how strongly a sample absorbs light; for dsDNA, the extinction coefficient is 0.020 $(ng/\mu L)^{-1}\ cm^{-1}$ at 260 nm. The pathlength is the distance the light travels through the sample. Traditional spectrophotometers typically use a 1 cm pathlength cuvette, but microvolume spectrophotometers use a range of pathlengths to accommodate higher sample concentrations. Note that one absorbance unit at 260 nm for dsDNA in a 1 cm cuvette is equal to a concentration of 50 ng/µL when applied to Beer's Law.

Experimental

The overall workflow for extracting strawberry DNA is outlined in Figure 2. In this experiment, the goal is to extract the DNA and then perform a quantity and quality assessment using a Thermo Scientific™ NanoDrop™ Lite Plus Microvolume UV Spectrophotometer.

A typical DNA extraction begins with a cell lysis step that breaks open the cell membrane, which is present in both plant and animal cells and consists of a phospholipid bilayer. Detergents, such as household dish soap, dissolve the lipid bilayer and break open the cell membrane, releasing the cellular contents, including the DNA. Since a cell also contains many proteins, DNA must be separated from the proteins and precipitated using salt and alcohol. The positively charged salt molecules stabilize the negatively charged DNA, making it insoluble in water. DNA is also insoluble in alcohol, so the addition of the salt and alcohol pulls DNA out of the solution, forming visible white clumps of DNA.

The strawberry genome is octoploid, which means there are eight copies of chromosomes;[1] the increased quantity of genetic material, compared to a diploid organism, makes strawberry DNA easier to visualize at the end of the extraction. The DNA can be spooled out using a rod or pelleted using a centrifuge, at which point it is finally resuspended in a buffer for future experiments.

Materials

- Dish soap
- Table salt
- Strawberries
- 15 mL conical tube or clear plastic cup (2x)
- 50 mL conical tube or clear measuring cup
- Eye dropper
- Cold isopropyl alcohol or ethanol (70% or higher – the higher the percentage, the more DNA will precipitate)
- Coffee filters
- Water
- Toothpick or glass rod
- Teaspoon
- Clear plastic bag
- Scissors or knife
- Lint-free lab wipes
- 1–10 µL pipettor and tips
- Optional: control dsDNA sample or gel electrophoresis DNA ladder

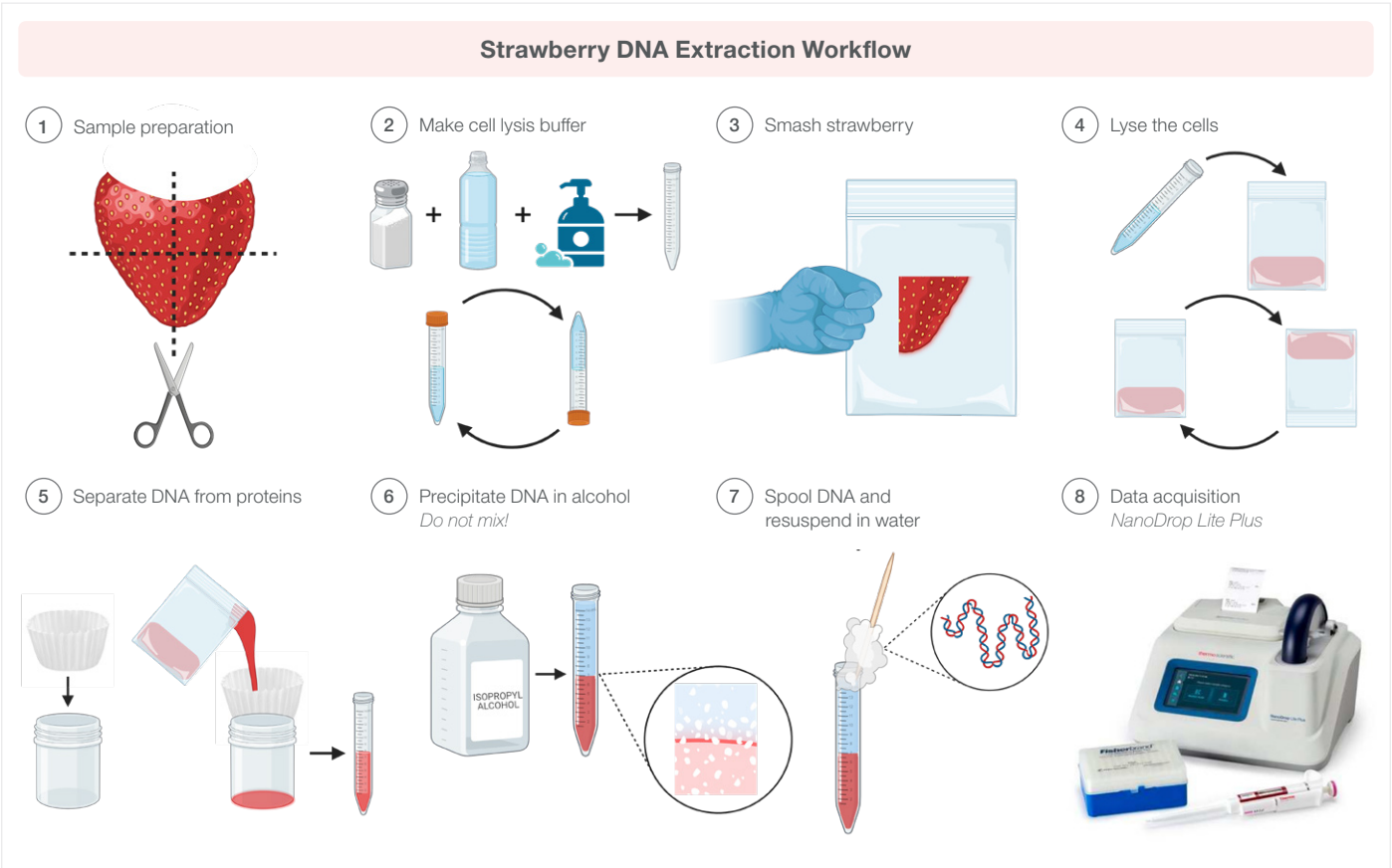


Figure 2. Strawberry DNA extraction workflow. Image created with BioRender.com.

Part 1. Prepare the reagents and samples

1. Cell lysis buffer
    a. In a 15 mL conical tube or clear plastic cup, mix 10 mL water with 1 teaspoon salt and dissolve by swirling.
    b. Add 10 drops of soap using an eye dropper and slowly mix to dissolve – avoid making bubbles.
2. Prepare strawberries
    a. Remove the green stem from one strawberry and cut into quarters.
    b. Add one quarter of a strawberry into a clear plastic bag. Note: using half of a strawberry will yield more DNA.
    c. Smash the strawberry until it is liquified.
3. Fill one 15 mL conical tube or clear plastic cup with isopropyl alcohol or ethanol and store on ice.

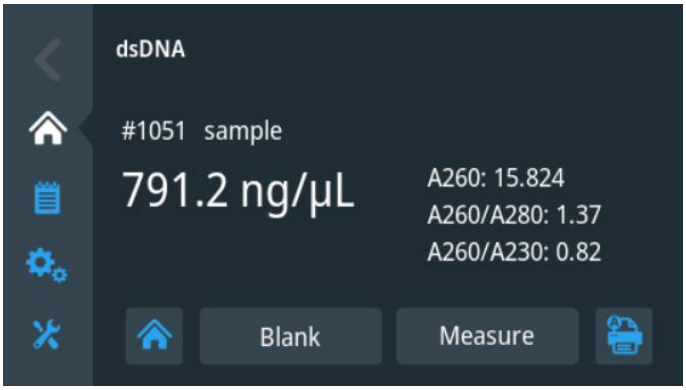
Part 2. DNA extraction

1. Cell lysis and DNA isolation
    a. Pour the cell lysis buffer into the bag containing the liquified strawberry.
    b. Mix by gently rotating the bag for 1-2 minutes – avoid making bubbles.
    c. Place a coffee filter on top of a clear plastic cup.
    d. Slowly pour the strawberry/buffer solution onto the filter and allow the solution to drip through the filter – this step may take several minutes to complete.
    e. Throw away the coffee filter and add the liquid to a 50 mL conical tube or clear measuring cup.
2. DNA precipitation
    a. Add an equal volume of isopropyl alcohol or ethanol to the strawberry solution from Step 1e by slowly pouring along the side of the tube or cup. Do not mix; allow the alcohol to layer on top of the strawberry solution.
    b. The DNA will start to rise into the top alcohol layer and form a cloudy, white precipitate.
    c. Use a toothpick or a glass rod to scoop and twirl the DNA out of solution and transfer to a clean tube or cup containing 1 mL of water.

Part 3. Making a measurement with the NanoDrop Lite Plus Spectrophotometer

1. Open the **Nucleic Acids** application on the NanoDrop Lite Plus Spectrophotometer and select **dsDNA**.
    a. Note: selecting a sample type applies the correct extinction coefficient to the Beer's Law equation for calculating concentration.

2. Baseline correction at 340 nm should be turned on.
3. Lift the pedestal arm, clean the top and bottom pedestal with a lint-free lab wipe, and pipette 2 µL of water onto the bottom pedestal surface. Lower the arm and select **Blank**.
4. Once the blank is complete, lift the pedestal arm and clean the top and bottom pedestals with a lint-free lab wipe.
5. Pipette 2 µL of the buffer solution (water) onto the bottom pedestal surface. Lower the arm and select **Measure**. If the A260 reading is outside of +/- 0.04 absorbance units, repeat step 3 to perform a new blank. Record the information in Data Table 1 in the lab report.
6. Lift the pedestal arm and clean the top and bottom pedestals with a lint-free lab wipe. Pipette 2 µL of the strawberry DNA onto the bottom pedestal surface. Lower the arm and select **Measure**. Clean the pedestals with a lint-free lab wipe. Record the information in Data Table 1 in the lab report.
7. Optional: If a control dsDNA sample or gel electrophoresis DNA ladder is available, pipette 2 µL onto the bottom pedestal surface, lower the arm and select **Measure**. Clean the pedestals with a lint-free lab wipe. Record the information in Data Table 1 in the Lab Report.



Disposal of chemicals: Check with the instructor before discarding any solutions. To dispose of solutions, pour down the sink while rinsing with water to dilute. Discard solids in the trash.

Reference
Yarnell, SH. Genetic and Cytological Studies on Fragaria. *Genetics* 16(5) p422–454 (1931). https://doi.org/10.1093/genetics/16.5.422

Lab report

Sample	Concentration (ng/µL)	A260	A260/A280	A260/A230
Water				
Strawberry DNA				
Control DNA (optional)				

Data Table 1.

Questions

1. What is the absorption wavelength for dsDNA?

   λ_{max} = _____ nm

2. What is the extinction coefficient for dsDNA? Include units.

   ε = _____

3. How does the NanoDrop Lite Plus spectrophotometer calculate sample concentration? Write the full equation.

4. What are the expected purity ratios for pure dsDNA?

   A260/A280 = _____

   A260/A230 = _____

5. Compare the purity ratios from the NanoDrop Lite Plus Spectrophotometer to the expected purity ratios for pure dsDNA. If the purity ratios deviate from expected, explain why.

6. Based on the data provided by the NanoDrop Lite Plus Spectrophotometer, would your dsDNA sample be optimal for sequencing? Why or why not?

7. Why is it important to check the data from the buffer solution after a blank?

8. Why was the addition of alcohol necessary in the dsDNA extraction?

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Chemistry

Food Dyes and Beer's Law

What makes your drink blue?

Introduction

The color of light

White light, as we see it, is a mixture of all the colors of the spectrum. We are used to seeing raindrops scatter white light into its colors to form a rainbow, or seeing "rainbows" of light on a wall from sunlight that has been scattered by cut glass or a prism. If you perceive an object as being colored, as opposed to white, it is because colors other than the one you see are being absorbed by the object.

For chemical solutions, we can use an instrument called a spectrophotometer to pass light through the solution and measure which wavelengths are absorbed. You can predict what wavelengths will be absorbed at a simple level by taking the visible spectrum and wrapping it into a circle to make a spectroscopist's color wheel. With this wheel, the color that you see is the opposite of the color that is absorbed. If you know what wavelengths of the visible spectrum correspond to which color, you can predict where in the spectrum a chemical will absorb even before doing the experiment.

The wavelength of light is measured in nanometers: 1 nm is 1 x 10^{-9} meters. The visible spectrum in Figure 2 shows which wavelengths correspond to which color of light.

UV-Visible spectrophotometers

Measuring how much of which wavelengths of light are absorbed by a substance, and getting useful information about that substance from the results, is the scientific discipline of spectroscopy. The visible spectrum is one is part of the electromagnetic spectrum that we can access with equipment found in a typical chemistry laboratory. The basic principles of spectrum analysis can also be applied to other instrumentation that examine the ultraviolet, infrared, and radio frequency regions.

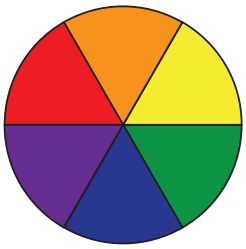


Figure 1. Color wheel.

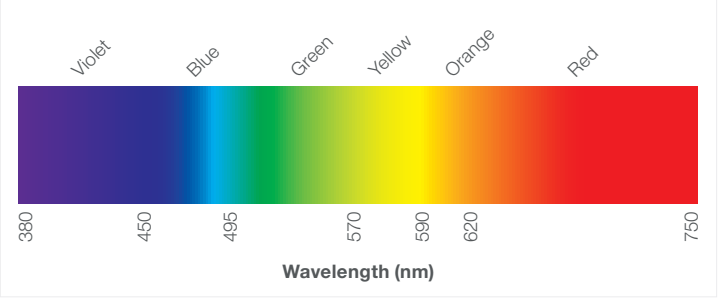


Figure 2. Visible spectrum.

In a visible spectrophotometer, we shine a beam of light into a solution containing the sample, and detect how much of it comes out of the other side of the solution. By comparing the amount of light transmitted by the pure solvent to the amount transmitted when the sample is dissolved in it, we can calculate a quantity called the **absorbance**. Absorbance is directly proportional to concentration, so if you know the proportionality constant, you can use it to calculate the concentration of a substance in solution. Being able to answer the "how much?" question means that a visible spectrophotometer is a tool for doing *quantitative* analysis.

Knowing exactly which wavelengths of light are absorbed by a substance also gives us information that can be used to tell one substance from another or to determine whether a sample is a pure substance or a mixture. Being able to answer the "what is it?" question means that a visible spectrophotometer is also a tool for doing *qualitative* analysis.

Absorbance and Beer's Law

When colored solutions are irradiated with white light, the solution selectively absorbs incident light of some wavelengths. The wavelength of light where the absorbance is highest is used as the analytical wavelength. Once the analytical wavelength for a particular solution is determined, we can learn more about the solution through the relationship between absorbance (**A**) and three variables:

$$A = \varepsilon bc$$

Beer's Law.

The three variables are concentration of the solution (**c**), the pathlength of the light through the solution (**b**), and the sensitivity of the absorbing species to the energy of the analytical wavelength. When the concentration is expressed in molarity and the path length is measured in centimeters, the sensitivity factor is known as the molar absorptivity (**ε**) of the particular absorbing species.

Visible spectrophotometers are capable of displaying data in either of two scales:

- Percent transmittance (**%T**), which is a linear scale
- Absorbance (**A**), which is a logarithmic scale

The linear %T scale can be converted to absorbance where T is the percent transmittance expressed as a decimal (e.g., 22% = 0.22):

$$A = -Log_{10}\ T$$

The most important lesson to take home from this logarithmic relationship is the realization that when the absorbance is 1.0, only 10% of the light beam's full intensity is reaching the detector and when the absorbance is 2.0, only 1% of the light beam is reaching the detector. The accuracy and sensitivity of low cost instruments starts to suffer at absorbance values higher than 1.5.

Transmittance (or %T) itself is determined by the instrument by dividing the detector signal when measuring the sample (**I**) by the signal recorded for a "blank" solution ($\mathbf{I_0}$).

$$T = \frac{I}{I_0}$$

Transmittance.

When we work with cuvettes or test tubes where the path through the liquid is exactly 1 cm, the value of "b" in the equation for Beer's Law is simply 1, so it effectively drops out of the equation and simplifies it to A = εc. This means that:

- If you were to measure the absorbance of several solutions of known concentration, and plot the absorbance on the y-axis and concentration on the x-axis, the slope would be the molar absorptivity (ε) of the sample in solution.
- If you know the molar absorptivity, you can calculate the concentration (c) of a solution with ease by simply dividing the absorbance by ε (c = A/ε).

Purpose

In this experiment, you will make different kinds of measurement on various food dyes:

1. A scan of the visible spectrum recorded using a Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer* will show you which wavelengths are absorbed by each sample. You will identify a peak or peaks in the scan and record the wavelength of each peak. Officially, the wavelength at the top of the peak is called the "wavelength of maximum absorbance", which is abbreviated to λ_{max} (spoken as "lambda max").
2. A single point measurement recorded at λmax will be used to calculate the concentration of red, yellow, green, and blue food dyes in a solution. You will be able to determine which chemical dye was used in the solution samples and whether the dye is a single chemical food dye or a mixture of dyes.
3. Given a stock solution of known concentration, you will make a Beer's Law plot by diluting the solution. You will then take a sports drink or soft drink and determine the molar concentration of the Blue No. 1 dye found in it. From this calculation and the molar mass of your dye, you will determine the mass of Blue No. 1 dye found in 591 mL of the solution – equivalent to a 20 fluid ounce bottle.

Experimental

Procedure

Making a measurement with the Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer*

1. Turn on the instrument and allow it to complete its startup sequence. Let the instrument warm up and stabilize for at least 30 minutes. Set up the experiment you want to perform in the spectrophotometer software. Obtain a square plastic cuvette or glass test tube to use in your experiments. If using a test tube cuvette, use a pen to place a mark near the top if the cuvette is not already marked with a white line. The mark allows you to ensure consistent placement into the instrument.
2. Add liquid to the cuvette until there is ~3 cm of liquid in the bottom (4 cm for test tubes). If plastic transfer pipettes are available, use one. The exact liquid level in the cuvette is not critical for good measurements as long as it is above 3 cm. Do not waste solution or risk spills by over-filling the cuvette.
3. Place the cuvette in the sample stage of the SPECTRONIC 200 Visible Spectrophotometer. If using a plastic cuvette, the clear sides should be on the right and left. If using a test tube cuvette, place it so that the mark faces to the right.
4. After the warm-up period, follow steps 2 and 3 using water or the appropriate "blank" solvent. Zero the instrument by pressing the autozero button.
5. For each subsequent measurement, empty and rinse your cuvette, shaking out as much of the rinse solvent as possible. When preparing samples, never return excess solution to the stock bottle. Pour all waste or excess into the appropriate waste receptacle. Follow steps 2 and 3 using your sample.

SPECTRONIC 200 Visible Spectrophotometer

*SPECTRONIC 200 Spectrophotometers are available on loan from Thermo Fisher Scientific™ at no cost. We will ship it to you, and you ship it back after one week. If you are interested in this program, please visit: thermofisher.com/spec200freetrial

Part 1. Scan the dyes

Prior to the lab, your instructor should have prepared dye solutions using the four packs of liquid food dyes from McCormick® Food Coloring containing red, yellow, blue and green dyes [1]. The actual concentration of the dye solutions is arbitrary, but they should be chosen to ensure the largest peak in each solution lies within the absorbance range of the spectrophotometer.

1. Run a scan of each dye solution from 400 nm to 700 nm.
2. Record the wavelength (λ_{max}) and absorbance at each peak in the spectrum. If the color is due to a mixture of dyes, two λ_{max} peaks will be present.
3. Enter this information in Data Table 1 in the Lab Report.

Data analysis: Determination of the dyes used in McCormick food coloring

Use the reference spectra in the Appendix to determine which chemical dye(s) are used to make each of the four colors from McCormick. Some of the colors are pure substances and some are mixtures of dyes. Enter your answers in Data Table 2 in the Lab Report.

Calculations: Molar concentration of dyes present in each solution

Use the Beer-Lambert Law equation (A = εbc), your measured absorbance values, and the molar absorptivity values in Table 1 below to calculate the molar concentration of each dye present in the four solutions tested. Write your answers in Data Table 2.

You will need to know the pathlength (b). If you have a standard square plastic cuvette the pathlength is 1 cm. If you are measuring in test-tube cuvettes or ordinary test tubes (without a pre-printed white line to help you to align them consistently) the pathlength will not be 1 cm. If this is the case, use a metric ruler to measure the pathlength of your cuvette and record it on the Lab Report.

FD&C Dye	Molar Mass ($g \cdot mol^{-1}$)	ε ($L \cdot cm^{-1} \cdot mol^{-1}$)
Red 3 or Erythrosine (cherry red)	898	31,000
Red 40 or Allura Red AC (orange-red)	496	25,900
Yellow 5 or Tartrazine (lemon-yellow)	534	27,300
Yellow 6 Sunset Yellow (orange)	452	25,900
Green 3 Fast Green FCF (sea green)	809	43,000
Blue 1 Brilliant Blue FCF (bright blue)	793	130,000
Blue 2 Indigotine (royal blue; Indigo Carmine)	466	111,000

Table 1.

Part 2. Create a Beer's Law plot for Blue No. 1 dye

What is the relationship between the absorbance of a colored solution and its molar concentration? You will prepare a series of solutions of known concentration, measure their absorbance at λmax, and plot the data.

Record the concentration of the stock solution: _____
(This will be given by the instructor.)

Dilutions: Take approximately 40 mL of the Blue No. 1 dye stock solution to your bench and prepare dilute solutions from it according to Table 2. These solutions will be your known concentrations of the dye. Calculate the molar concentrations of your solutions and enter them in Data Table 3 in the Lab Report. Report the concentrations as μM. Find the absorbance of your five solutions using the spectrophotometer and record in Data Table 3.

Solution	Dilution Ratio (mL stock/mL water)
1 (stock solution)	10 mL/0 mL
2	8 mL/ 2 mL
3	6 mL/ 4 mL
4	4 mL/ 6 mL
5	2 mL/8 mL

Table 2.

Using your absorbance readings and the molar concentrations, construct a Beer's Law plot (plot the molar concentrations of your known solutions on the x-axis and the absorbance data on the y-axis). Use a spreadsheet program or a graphing calculator to plot your data and determine a best-fit line (trend line) to calculate the slope of your line. Record the slope of the line in the Lab Report.

Part 3. What's in that drink?

1. Obtain about 5 mL of the blue colored drink.
2. Measure the absorbance of the drink at λ_{max} for Blue Dye No. 1 and record it on the Lab Report.
3. Calculate the concentration of Blue No. 1 dye in the drink using the Beer's Law plot from Part 2.
4. Calculate the mass of dye present in a 20 oz (591 mL) bottle of the drink.
5. Record your calculations and answers in the Lab Report.

Disposal of chemicals:

All of the food dyes can be flushed down the sink with plenty of water.

Further reading/reference material

1. Sigman SB, Wheeler DE (2004) The quantitative determination of food dyes in powdered drink mixes. A high school or general science experiment. J Chem Educ 81: 1475–1478.

Lab Report

Food Dyes and Beer's Law

Name: _____

Date: _____

Section No. or Lab Period: _____

Part 1. Scan the dyes

Color of Solution	λ_{max} (nm)	Absorbance
Red		
Yellow		
Green		
Blue		

Data Table 1.

Record the pathlength of your cuvette: _____ cm

Color of Solution	Dye(s) contained in solution	Pure substance or mixture?	Conc. (mol/L)
Red			
Yellow			
Green			
Blue			

Data Table 2.

Questions

1. What was the wavelength of light absorbed by the blue colored solution at its λ_{max}?

2. Using the information in the introduction, determine the color of light this corresponds to in the visible light spectrum.

3. How is the color of light absorbed by the colored solution related to its perceived color? Is there a connection between these two?

4. Show your concentration calculations for any two of the dyes listed in the table. Label the calculation with the name of the dye, box your answer, and write neatly!

Part 2. Create a Beer's Law plot for Blue No. 1 dye

Solution	Dilution Ratio (mL stock/mL water)	Molar Conc. (μM)	Measured Absorbance
1 (stock solution)	10 mL/0 mL		
2	8 mL/2 mL		
3	6 mL/4 mL		
4	4 mL/6 mL		
5	2 mL/8 mL		

Data Table 3.

Plot of Absorbance vs. Concentration for Blue No. 1 dye (Beer's Law plot)

Staple your printed graphs to this report sheet and record the required data and answers in the spaces below:

1. Record the slope of the best-fit line: _____

2. Write the full equation (y = mx + b format) for the best fit line on the graph you just created using the slope.

3. The slope of the line is derived from the molar absorptivity (ε) of the dye and the path length (b) of the sample in the spectrophotometer. What is the path length of your cuvette?

   Record the pathlength of your cuvette: _____ cm

4. Use the slope of the line to determine the molar absorptivity (ε) of Blue No. 1 dye. Use the equation for Beer's Law to derive and include the units. Note that absorbance has no units. Show your calculation here:

Name: _____

Part 3. What's in that drink?

Absorbance of the blue drink: _____ at _____nm

1. Determine the molar concentration of the Blue Dye in the drink. Show all work.

2. Determine the mass of Blue #1 Dye found in 591 mL of the drink. Show all work.

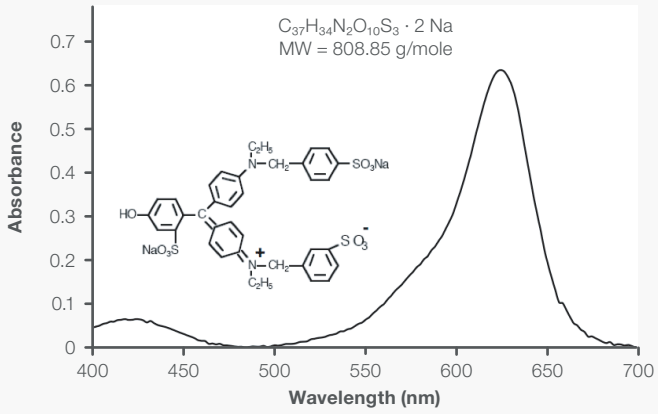
Remember:

- Staple hand-drawn or printed graphs to your lab report
- Staple the two sheets of the lab report together before you hand them in

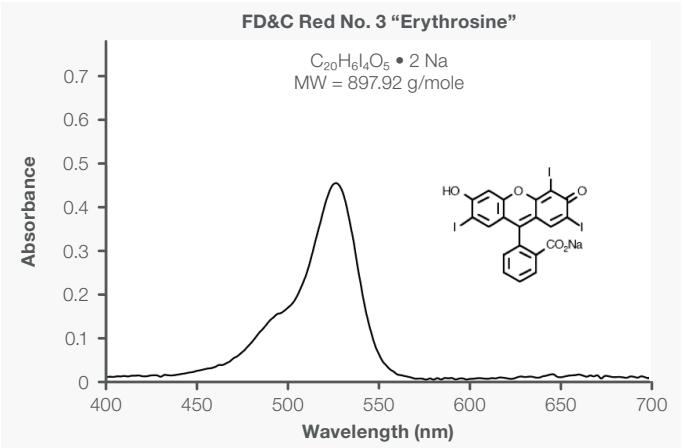
Appendix

Reference spectra for FDA food dyes

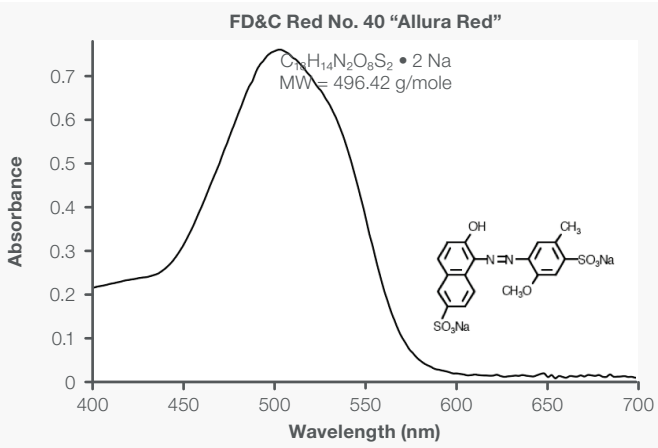
FD&C Green No. 3 “Fast Green”



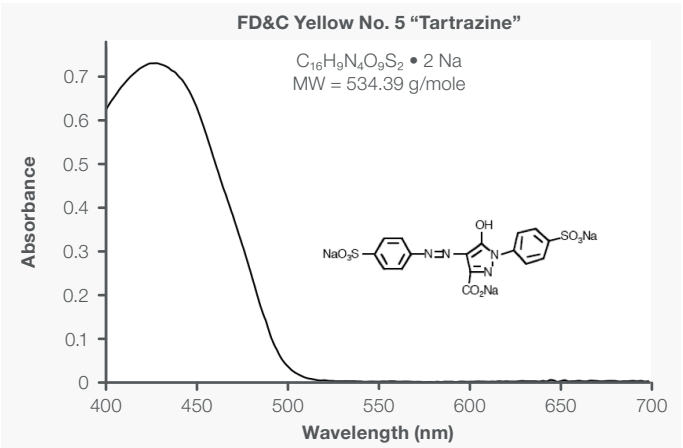
FD&C Red No. 3 “Erythrosine”



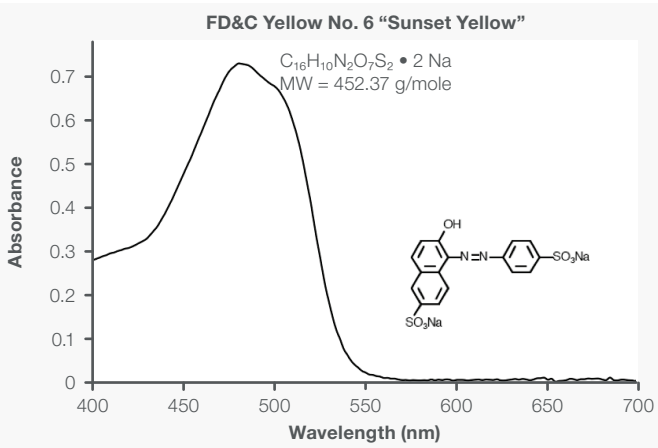
FD&C Red No. 40 “Allura Red”



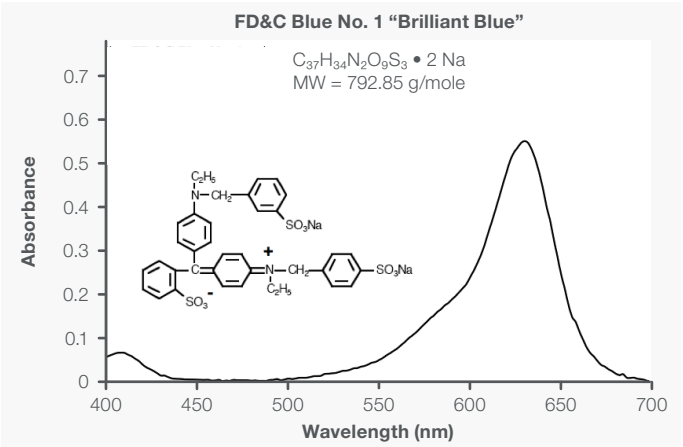
FD&C Yellow No. 5 “Tartrazine”



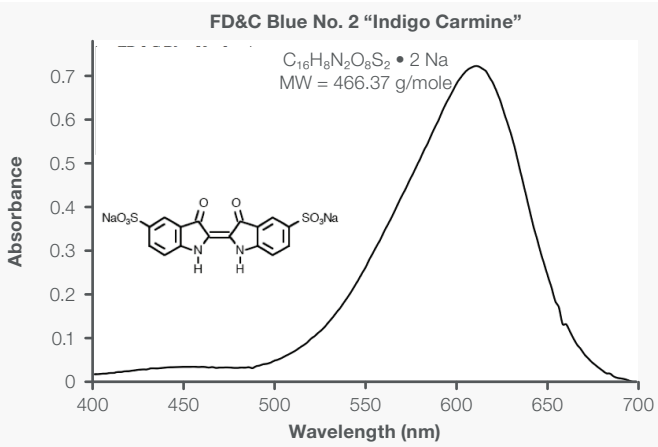
FD&C Yellow No. 6 “Sunset Yellow”



FD&C Blue No. 1 “Brilliant Blue”



FD&C Blue No. 2 “Indigo Carmine”



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Chemical Analysis of Brass

Alloys and their composition

Introduction

Alloys

An alloy is a substance made by mixing two or more metallic elements into a single crystalline structure. Alloys are often advantageous to produce because they retain properties similar to those they exhibit in their pure state while also producing additional desirable properties due to the controlled nature of the mixed elements. Since alloys are mixtures, their composition can vary widely. One example of an alloy is brass, which is composed primarily of copper (Cu) and zinc (Zn). Some brasses also have small amounts of other types of metals, such as tin (Sn). Brass with a high copper content is often used to produce musical instruments due to its malleability, workability, and resistance to corrosion. Naval brass is a particular mixture of copper, zinc, and tin and is commonly used for various hardware components. The added tin imparts an extra degree of corrosion resistance. Other common applications of brass include plumbing fittings, electrical applications, and architectural or design use where a golden finish is desired. In each case, the composition of the brass is tailored to give the best physical properties for the application.

Oxidation of metals

Since the copper and zinc in brass exist in their elemental forms, they need to be oxidized to their ionic forms to get them into solution. For active metals including zinc, this can be done by adding an acid (H^+) where the following reaction takes place:

$$Zn_{(s)} + 2H^+_{(aq)} \rightarrow Zn^{2+}_{(aq)} + H_{2\,(g)}$$

Less active metals like copper require a stronger oxidizing agent. Concentrated nitric acid (HNO_3; ~15 M) at room temperature or hot 7.0 M nitric acid are each strong enough to oxidize copper by the following reaction:

$$Cu_{(s)} + 4HNO_{3\,(aq)} \rightarrow Cu(NO_3)_{2\,(aq)} + 2NO_{2\,(g)} + 2H_2O_{(l)}$$

Not all metal cations are soluble in acidic aqueous solution. One example would be tin which is a common component of brass. Tin(II) ions (Sn^{2+}) are formed by the oxidation of metallic tin in nitric acid and are soluble in aqueous solution. However, they slowly undergo additional oxidation to form tin(IV) oxide, which is insoluble in water. In this experiment, you can assume that your brass samples are composed of only copper and zinc.

Metal coordination chemistry

Since metal ions carry a positive charge, negatively charged species in a solution, like anions or negatively charged atoms of complex ions, will be attracted to them.

If the metal is a *d*-block element (transition metal) this means that the electron clouds of the ligands overlap with the d-orbitals in the metal to different extents. The d-orbitals split, with the d_{xy}, d_{xz} and d_{yz} forming one group and the $d_{x^2-y^2}$ and d_{z^2} forming a second group at a different energy level. The energy gap between these energy levels is very often in the range of a photon of visible light. If a photon with the right energy strikes an electron in the lower energy orbital, that photon can be absorbed and the electron is promoted to the higher orbital. This absorbance gives the metal ion solution its color and allows us to measure the concentration by colorimetry. By changing the metal or the ligand, we change the energy gap which affects both the color/wavelength of light absorbed and how strongly it is absorbed.

Experimental

Purpose

In this experiment, you will perform an analysis of three brass samples to determine the copper content of each by dissolving brass in a solution and measuring the absorbance at a particular wavelength after workup.

Reagents

- Brass Samples
- 7.0 M Nitric acid (HNO_3)
- 0.1 M Copper(II) sulfate ($CuSO_4$)
- 2.0 M Ammonia (NH_3)

Part 1. Getting brass samples into solution

1. You will start with one of the samples derived from three different brass sources. Label a 100 mL beaker with the identity of the brass sample you will analyze in it. Weigh ~0.5 g of the brass directly into the labeled beaker.
   Record the identity and exact mass of the sample in Data Table 1 of your Lab Report.
2. Carefully pipette 10.0 mL of 7.0 M HNO_3 into the beaker.
3. Perform this step in a fume hood or in a well ventilated area. Cover the beaker with a watch glass and set it on a hot-plate set to low. Observe and record what happens.

   Warning: *Do not inhale the fumes that rise from the beakers: they are corrosive.*

4. While the brass is reacting, proceed to Part 2.

Figure 1. Scan settings.

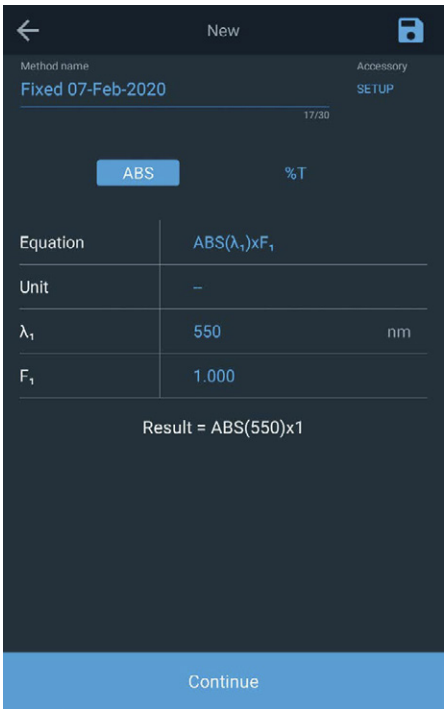

Figure 2. Fixed settings.

Part 2. Preparation of standard $[Cu(NH_3)_4]^{2+}$ solutions

1. Pipette 3 mL of a 0.1 M $CuSO_4$ solution into a clean cuvette. Blank the instrument with water and then record an absorbance spectrum of the $CuSO_4$ solution by selecting the Scan method in the GENESYS software and using the settings from Figure 1. Use the cursor to determine the wavelength of maximum absorbance, λ_{max} and record the data in the Lab Report.
2. For each solution, use a pipette to add the amount of 0.1 M $CuSO_4$ indicated in the table below to a 25 mL volumetric flask.

Standard solution	Volume of 0.1 M $CuSO_4$
1	1.0 mL
2	2.0 mL
3	4.0 mL
4	5.0 mL

3. Using 2.0 M NH_3, dilute the solutions to the mark on the volumetric flask. The final deep blue color indicates formation of the $[Cu(NH_3)_4]^{2+}$ complex. Place the stopper in the flask and mix the solutions well by inverting and swirling.
4. Transfer the prepared solutions to clean, dry, labeled 50 mL beakers and save for analysis.
5. Measure your standards using the GENESYS UV-Vis Spectrophotometer.
    a. Using Standard Solution 4, perform a scan using the same settings from Figure 1.
    b. Record the wavelength of maximum absorbance (λ_{max}) for $[Cu(NH_3)_4]^{2+}$.
    c. Record the measured absorbance at this wavelength for your solution in Data Table 2.
    d. Either in Fixed mode (Figure 2), or in Live Display mode, measure the absorbance of the other three standard solutions at λ_{max} and record the data in Data Table 2.
6. Using your concentration and absorbance readings, construct a Beer's Law plot. Use a spreadsheet program or a graphing calculator to plot your data and determine a best-fit line to calculate the slope of your line. Record the slope of the line in the Lab Report.

Part 3. Analysis of the brass samples

1. After all the brass has reacted away, pipette ~3 mL of the solution into a clean, dry cuvette and record its absorbance spectrum using the settings from Part 2. Use the cursor to determine the wavelength of maximum absorbance, λ_{max} and record the result in Data Table 3 of the Lab Report. Discard the sample.
2. With the remaining solution:
    a. Pipette 2.0 mL of the solution into a 100 mL volumetric flask. Be as careful as you can to deliver exactly 2.0 mL.
    b. Add 20 mL of 2.0 M NH_3 solution to the volumetric flask and swirl the contents. Record your observations in detail.
    c. Add additional 2.0 M NH_3 solution to make the volume up to the mark. Stopper the flask and invert it several times to mix the contents thoroughly.
    d. Pour the contents of the flask into a labeled beaker.
    e. Pour or pipette ~3 mL of this solution into a clean, dry cuvette. Record the absorbance of the solution using the Fixed or Live Display method at the same wavelength that you used for the $[Cu(NH_3)_4]^{2+}$ standards in Data Table 4 of the Lab Report.
    f. Complete Part 3 for all three brass samples.
3. Report the values in your Lab Report:
    a. Identity of the brass sample
    b. Mass of brass used in your experiment
    c. Absorbance of your solution
    d. λ_{max} used to make the measurement

Disposal of chemicals:

Check with your instructor before discarding any solutions. All solutions can be poured down the sink and rinsed with lots of water to dilute. Discard solids in the trash.

Lab Report
Chemical Analysis of Brass

Name: _____

Date: _____

Section No. or Lab Period: _____

Part 1. Getting brass samples into solution

Questions

1. Record the identity of the brass and the exact mass of the brass you weighed out in your beaker:

Sample number	Identity of brass	Mass of brass (g)
1		
2		
3		

Data Table 1.

2. What happens when the brass reacts with the nitric acid solution?

Part 2. Preparation of standard $[Cu(NH_3)_4]^{2+}$ solutions

1. Record the wavelength of maximum absorbance (λ_{max}) for the 0.1 M $CuSO_4$ solution:

   λ_{max} ($CuSO_4$ solution): _____ nm

2. Is color of the $[Cu(NH_3)_4]^{2+}$ solution the same as the color of the 0.1 M $CuSO_4$ solution?

   ☐ Yes ☐ No

3. Record the wavelength of maximum absorbance (λ_{max}) for $[Cu(NH_3)_4]^{2+}$.

   λ_{max} ($[Cu(NH_3)_4]^{2+}$ solution): _____ nm

4. Calculate the concentration of Cu in mol/L of each of the standard solutions and record the results:

Standard nolution	Measured absorbance	Concentration of Cu solution (mol/L)
1		
2		
3		
4		

Data Table 2.

5. Record the slope of the best-fit line:

   _____

6. Write the full equation ($y = mx + b$ format) for the best fit line on the graph you just created using the slope.

7. Use the slope of the line to determine the molar absorptivity (ε) of the $[Cu(NH_3)_4]^{2+}$ complex. Use the equation for Beer's Law ($A = \varepsilon bc$) to derive and include the units. Note that absorbance has no units. Show your calculation here:

8. Is the peak in the spectrum of the 0.1 M $CuSO_4$ solution the same peak that you saw in the $Cu(NH_3)_4]^{2+}$ standards?

   ☐ Yes ☐ No

Remember:

- Staple hand-drawn or printed graphs to your lab report
- Staple the two sheets of the lab report together before you hand them in

Name: _____

Part 3. Analysis of the brass samples

1. Record the wavelength of maximum absorbance (λ_{max}) for the dissolved brass samples before adding ammonia.

Sample number	λ_{max} before ammonia (nm)
1	
2	
3	

Data Table 3.

1. Were these wavelengths the same as the wavelength of maximum absorbance (λ_{max}) for $[Cu(NH_3)_4]^{2+}$?

   ☐ Yes ☐ No

2. Record the absorbance for the dissolved brass samples after adding ammonia using the same wavelength that you used for the $[Cu(NH_3)_4]^{2+}$ standards.

Sample number	Measured absorbance
1	
2	
3	

Data Table 4.

3. Using this absorbance values and the previously prepared calibration curve, determine the concentration of copper in mol/L in the brass solutions. Show your calculations here:

4. Using this concentration, determine the number of moles of copper in your 100 mL unknown brass solutions. Show your calculations here:

5. Using the moles of copper in the 100 mL unknown brass solutions, determine the mass of copper found in the original solid brass samples. (Hint: you used 1/5 of the 10 mL dissolved brass solution to prepare the 100 mL unknown brass solution.) Show your calculations here:

6. Calculate the mass percentages of copper and of zinc in your original brass samples using the mass of each original brass sample and the calculated mass of copper in each brass sample. Show your calculations here:

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Spectrophotometric Determination of Trace Iron in Solution

Measure the concentration of iron using a calibration curve

Introduction

Background

The ability to measure the concentration of iron in aqueous solutions in a quick and efficient way is important to many industries. Manufacturing industries where metal parts need to be cleaned may need to determine the level of iron in their waste streams for environmental compliance. Governments at all levels have an interest in testing wastewater, natural waters, and drinking waters to determine iron content to ensure compliance with the law and to ensure the safety of the water supply for wildlife and the human population.

Well-equipped, modern laboratories may perform iron content analysis by atomic emission spectroscopy in an inductively coupled plasma (ICP) spectrometer. Flame atomic absorption spectrometry also can be used, but iron solutions are notorious for clogging the burner with iron oxide when the concentration exceeds a certain level. Both of these techniques require a significant investment in instrumentation and a sustained laboratory infrastructure involving compressed gases and control of exhaust vapors. Fortunately, the solution chemistry underlying a colorimetric determination of iron content is simple enough to be reduced to kit form and performed in the field with hand-held equipment or in the lab with a low-cost visible spectrophotometer and simple glassware.

Preparing the colored iron complex

The colorimetric determination of iron content involves the measurement of the ferrous ion (Fe^{2+}) when it forms a complex with three molecules of 1,10-phenanthroline, also called *ortho*-phenanthroline or abbreviated as phen.

The chemical structure and numbering scheme for 1,10-phenanthroline is shown in Figure 1.

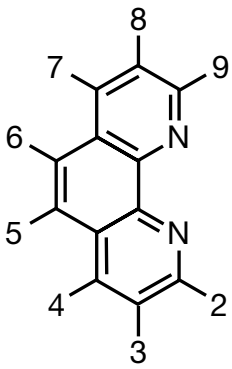

Figure 1. Numbering scheme for positions in 1,10-phenanthroline.

The complex formed by 1,10-phenanthroline and Fe^{2+}, ferrous tris(1,10-phenanthroline)iron(II) or $[Fe(phen)_3]^{2+}$, is a bright orange color. A 3D model of the structure of the complex is shown in Figure 2.

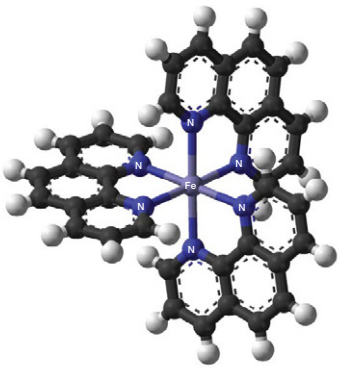

Figure 2. 3D model of the $[Fe(phen)_3]^{2+}$ complex.

In addition to its Fe^{2+} (ferrous ion) form, iron also can exist in a Fe^{3+} (ferric ion) form. To perform a *total iron* measurement, it is essential to reduce any Fe^{3+} in solution to Fe^{2+} before adding the phenanthroline to form the complex. The chosen reducing agent in this experimental protocol is hydroxylamine hydrochloride, which reacts with Fe^{3+} by *Reaction 1:*

$$2Fe^{3+}_{(aq)} + 2NH_3OH^{+}_{(aq)} \rightarrow 2Fe^{2+}_{(aq)} + N_{2\,(g)} + 2H_2O_{(l)} + 4H^{+}_{(aq)}$$

Reaction 1.

Upon adding the phenanthroline, *Reaction 2* occurs:

$$Fe^{2+} + 3\ phen \rightarrow [Fe(phen)_3]^{2+}$$

Reaction 2.

Using Beer's Law to determine the concentration of an unknown

To determine the concentration of iron in an unknown solution, we must first *calibrate* the method with the spectrophotometer using Beer's Law:

$$A = \varepsilon bc$$

Beer's Law.

where

A = the absorbance reported by the spectrophotometer

ε = the extinction coefficient, a value that describes how strongly the particular compound absorbs photons at the particular wavelength, typically with units of ($L{\cdot}cm^{-1}{\cdot}mol^{-1}$)

b = the pathlength of the cuvette in cm, where typically a 1 cm pathlength cuvette is used

c = the concentration of the solution in mol/L ($mol{\cdot}L^{-1}$)

A Beer's Law plot can be constructed by preparing a series of solutions of known concentration and graphing the absorbance of each solution on the y-axis versus concentration on the x-axis:

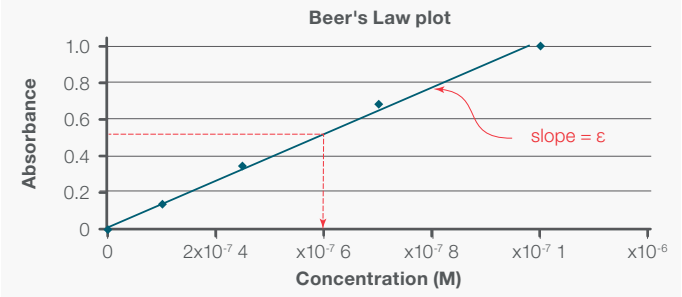

Figure 3. Example of a generic Beer's Law Plot.

Comparing the equation for Beer's Law to the plot, we see that the slope of the line is equal to ε. We can use the plot to calculate the concentration of an unknown solution in one of two ways:

1. Use the plot itself to select the point on the y-axis representing the measured absorbance. Trace a horizontal line from that point to the plot line, then draw a vertical line straight down to the concentration on the x-axis. The point where this intersects the x-axis represents the concentration of the unknown solution.
2. Use the equation of the line. If A = εbc and b=1, then c = A/ε. Use a spreadsheet program or a graphing calculator to plot your data and determine a best-fit line (trend line) to calculate the slope of your line. This slope equals ε. Divide the measured A value for the unknown by ε to calculate the concentration of the unknown solution.

Experimental

Purpose

In this experiment, you will perform an analysis of an iron-containing solution with an unknown concentration by reducing all the iron in solution to its ferrous form, determining λ_{max}, and creating a standard curve of absorbance versus concentration to calculate the concentration of the unknown solution.

Reagents

- Ammonium iron(II) sulfate, hexahydrate
- Hydroxylamine hydrochloride
- 1,10-phenanthroline
- Sodium acetate

Part 1. Prepare the reagents and standards

1. Prepare solutions prior to beginning this experiment, using deionized water for all dilutions.

- **100 mg/L iron solution:** Dissolve 0.7022 g of ammonium iron(II) sulfate, hexahydrate in water in a 1 L volumetric flask.
- **10 mg/L iron working solution:** Pipet 5 mL of the 100 mg/L iron solution into a 50 mL volumetric flask and fill to the mark with water.
- **0.3 M hydroxylamine hydrochloride solution**
- **0.25 % 1,10-phenanthroline solution:** Stir the solution to ensure that all solids have dissolved, using heat if necessary.
- **1.0 M sodium acetate solution**

2. Set up six 50 mL volumetric flasks and pipette reagents into them as follows:

Flask no.	10 mg/L iron solution (mL)	0.3 M hydroxylamine hydrochloride solution (mL)
1	0.0	1.0
2	2.0	1.0
3	5.0	1.0
4	8.0	1.0
5	14.0	1.0
6	20.0	1.0

Stopper each flask, then invert repeatedly for 2 minutes to allow the reaction to complete.

3. To each flask, add:

- 5.0 mL of 1.0 M sodium acetate solution. Stopper and invert the flask to mix.
- 5.0 mL of 0.25% 1,10-phenanthroline solution. Stopper and invert the flask to mix.
- Add deionized water to the mark. Stopper and invert several times to mix.

Part 2. Determine the proper analytical wavelength

1. Pipet 3 mL of the solution from Flask 1 into a cuvette. This solution will be your blank.
2. Wipe the outside faces of the cuvette with a laboratory tissue and place the cuvette into the square cuvette stage of the GENESYS UV-Vis Spectrophotomer sample compartment.
3. Close the lid of the GENESYS UV-Vis Spectrophotometer. Select the Scan method and use the settings shown in Figure 1. Select Continue and then select Blank to record a baseline.
4. Once the blank measurement has completed, open the lid, remove the cuvette with the blank solution and set it aside.
5. Pipet 3 mL of the solution from Flask 6 into a cuvette. Wipe the outside faces of the cuvette with a laboratory tissue and place the cuvette into the square cuvette stage of the GENESYS UV-Vis Spectrophotometer sample compartment. Close the lid and press Measure to record the scan.
6. Once the scan is completed, use one of the cursor lines to select the wavelength corresponding to the largest absorbance value. Record the wavelength of maximum absorption, known as λ_{max} in the Lab Report (Figure 2).
7. Print the spectrum if your GENESYS UV-Vis Spectrophotometer is equipped with a printer (Figure 3).

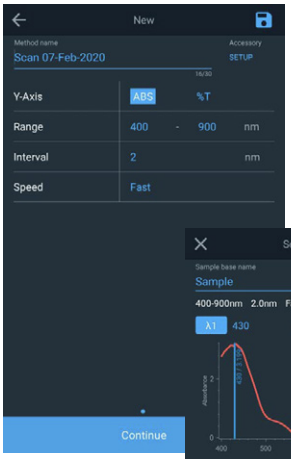

Figure 1.
Scan method settings

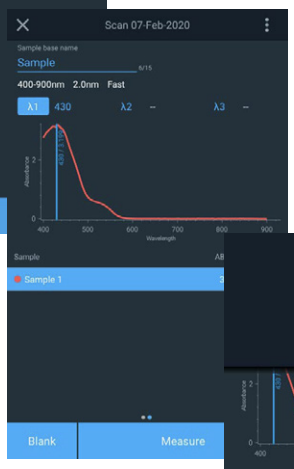

Figure 2.
Scan results

Figure 3.
Export or print

Part 3. Prepare the Beer's Law plot

1. Calculate the concentration of iron in mM in each of Flasks 1 through 6 and enter the values in the Lab Report.
2. Select the Quant method in the GENESYS Software using similar settings to what is shown in Figure 4. Set the analysis wavelength at the λ_{max} as determined in Part 2. Enter the concentration values for the iron standard solutions you calculated in the previous step.
3. Use a cuvette with blank solution from Flask 1 to record a blank value.
4. Prepare and measure the absorbance of the cuvettes containing the solutions from Flasks 2 through 6. Record the values in Data Table 1 in your Lab Report.
5. The software will automatically generate a Beer's Law plot from the entered concentration data and measured absorbance values. Record the slope of the line in your lab report as ε (Figure 5). Use this plot to complete Part 4.

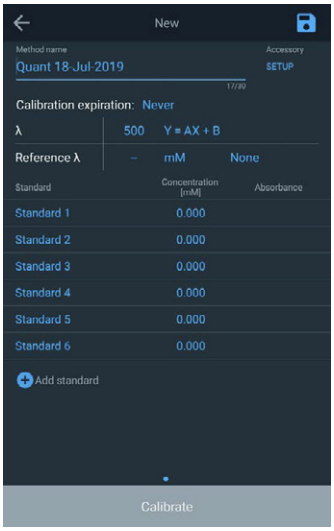

Figure 4.
Quant method settings

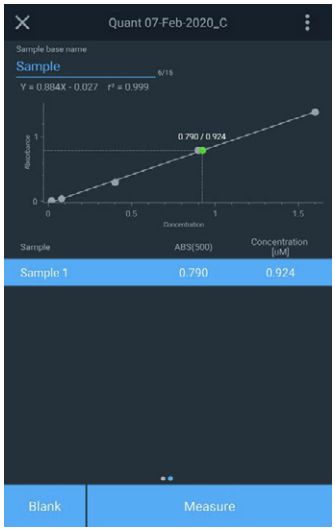

Figure 5.
Beer's Law plot.

Part 4. Determine the iron concentration in an unknown sample

1. Clean out one of the volumetric flasks.
2. Pipet 5.0 mL of an unknown iron solution into the flask. Follow the procedure used in Part 1, steps 2 and 3, to prepare the solution for measurement.
3. Measure your sample using the calibration curve obtained in Part 3.
4. Record the absorbance of the unknown iron sample.
5. Use the iron concentration reported by the software to calculate the actual concentration of your unknown iron sample. Remember to include the effect of diluting 5 mL of the unknown to 50 mL before you made your measurement when you calculate the concentration of the unknown.

Disposal of chemicals:

Check with your instructor before discarding any solutions. All solutions can be poured down the sink and rinsed with lots of water to dilute. Discard solids in the trash.

Lab Report

Spectrophotometric Determination of Trace Iron in Solution

Name: _____

Date: _____

Section No. or Lab Period: _____

Questions

1. What is the wavelength of maximum absorption for your iron standard solutions?

   λ_{max} = _____ nm

Flask no.	Fe^{2+} concentration	Measured absorbance
1		
2		
3		
4		
5		
6		

Data Table 1.

2. What is the extinction coefficient for your iron standard solutions?

   ε = _____

   (don't forget to include the units)

3. What is the measured absorbance of the unknown [Fe2+]solution?

   A = _____

4. What is the concentration of the unknown [Fe^{2+}] solution?

   c = _____

   (don't forget to include the units)

5. What is the absolute error between your value for the unknown [Fe^{2+}] solution and the true value obtained by your instructor?

6. Calculate the percent error from question 4.

7. Why was it necessary to add hydroxylamine and sodium acetate to the solution used to record the blank?

8. Many concentrations in this experiment were given in units of mg/L. Express 10 mg/L iron using units of mol/L.

Remember:

- Staple hand-drawn or printed graphs to your lab report

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Kinetics

Application note

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.

$$mA \rightarrow xB + yD$$

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 (eqn 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).

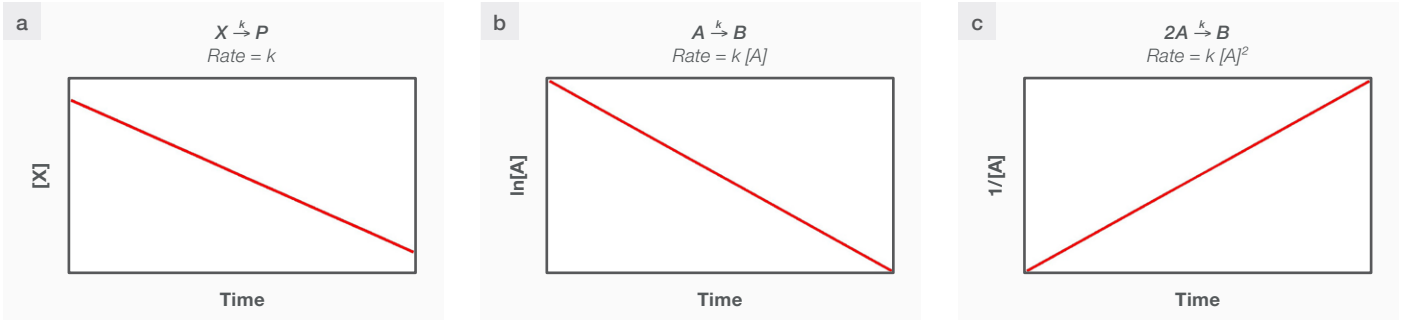

Figure 1. Concentration dependence as a function of time for (a) zeroth order (b) first order and (c) second order reactions.

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),

$$A = lc\varepsilon$$

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.

$$A + B \xrightarrow{k} D$$

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.

$$NaOH + C_{25}N_3H_{30}Cl \rightarrow NaCl + C_{25}N_3H_{30}OH$$

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 [NaOH] >> [$C_{25}N_3H_{30}Cl$]? 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™ 40 or 50 UV-Visible 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 ($C_{25}N_3H_{30}Cl$)
- 0.15 M Sodium Hydroxide (NaOH)
- DI or Nanopure water

Safety

Eye protection and gloves should be worn at all times when handling 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 in the 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 so 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. Setup the "Fixed" application on the UV-Visible instrument with the following parameters (See Figure 2):
    - Equation: $ABS(\lambda_1)xF_1$
    - 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 1: 0.06 M NaOH
    - NaOH stock solution 1: 0.03 M NaOH
9. Setup 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.

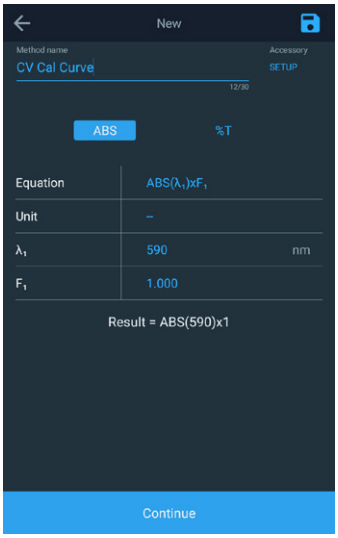

Figure 2. Fixed application parameters for the Genesys instruments.

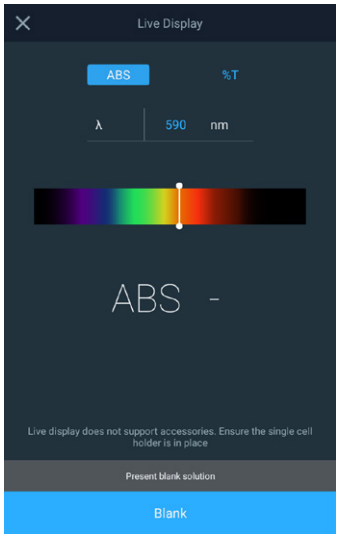

Figure 3. Live display application for the Genesys instruments.

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.

$[C_{25}N_3H_{30}Cl]$ (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 (˚C)
1						
2						
3						
4						

Table R2. Reaction Sample Information.

Write down your observations from the experiment below:

Time (s)	$A_{590\ nm}$	$[C_{25}N_3H_{30}Cl]$ (μM)	$\ln([C_{25}N_3H_{30}Cl])$	$1/[C_{25}N_3H_{30}Cl]$ (μM^{-1})

Table R3. Sample 1 Reaction Data: Room Temperature, 40 mM NaOH.

Time (s)	$A_{590\ nm}$	$[C_{25}N_3H_{30}Cl]$ (μM)	$\ln([C_{25}N_3H_{30}Cl])$	$1/[C_{25}N_3H_{30}Cl]$ (μM^{-1})

Table R4. Sample 2 Reaction Data, Room Temperature, 20 mM NaOH.

Time (s)	$A_{590\ nm}$	$[C_{25}N_3H_{30}Cl]$ (μM)	$\ln([C_{25}N_3H_{30}Cl])$	$1/[C_{25}N_3H_{30}Cl]$ (μM^{-1})

Table R5. Sample 3 Reaction Data: Room Temperature, 10 mM NaOH.

Time (s)	$A_{590\ nm}$	$[C_{25}N_3H_{30}Cl]$ (μM)	$\ln([C_{25}N_3H_{30}Cl])$	$1/[C_{25}N_3H_{30}Cl]$ (μM^{-1})

Table R6. Sample 4 Reaction Data: Ice Bath, 40 mM NaOH.

Post-Lab - Data Analysis and Questions

1. 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.

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**).

3. 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.

4. 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: _____

5. Using the data from samples 1, 2 and 3, determine the reaction order for OH^- (Include all calculations). Round to the nearest whole number.

6. Were the reactions' orders determined in questions 3 and 4 whole numbers? What sources of error could have led to a fractional number?

7. 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: _____

8. Compare the calculated rate constant for Samples 1 and 4. What affect did changing the temperature of the reaction have? Why?

9. What would you expect to happen if the concentration of NaOH was decreased to 10 µM?

Complex Reaction Monitoring with UV-Visible Absorption Spectroscopy: $KMnO_4$ + Sugar

Introduction

Kinetic Analysis

The study of chemical kinetics involves the monitoring of reactant and/or product concentrations over time in order to better understand how quickly or slowly a reaction proceeds. By learning how quickly a product is formed, information about the optimal reaction conditions (*e.g.*, temperature, starting material concentrations, etc.) can be better understood. Furthermore, kinetic analyses can aid in understanding the overall mechanism of the reaction, as well as alternative reaction pathways.

One of the simplest examples is a first order reaction, as shown in Eq. 1, where one species (A) reacts to form a product (B).

$$A \xrightarrow{k_1} B$$

Equation 1.

k_1 refers to the rate constant for the reaction; it is a value which correlates the rate of a reaction to the concentration of the starting materials. The rate constant is the inverse of the reaction time constant (τ) and is used to describe how fast or slow a reaction proceeds. This value is also useful when comparing rates for reactions performed under varying conditions.

Using the chemical equation, a differential rate law can be written which describes the change in a species concentration as a function of time (Eq. 2),

$$\frac{d[A(t)]}{dt} = -k_1[A(t)]$$

Equation 2.

where *[A(t)]* is the concentration of the species of interest at a given reaction time; in this case (*A*) is the reactant from Eq. 1. When reactions are monitored experimentally, the integrated form of the rate law is needed. By integrating the differential rate law, a function can be generated relating the measured concentration of the species of interest as a function of time. This function will describe how the species is lost or produced overtime. For the generic first order reaction described in Eq. 1, *[A(t)]* will be lost according to an exponential function shown in the integrated rate law (Eq. 3).

$$A(t) = A_0 e^{-kt}$$

Equation 3.

As described previously, the rate constant (*k*) is often used as a descriptor for the speed of the reaction. Experimentally, by fitting the species concentration measured as a function of time, the integrated rate law is fit to the data and the rate constant can be determined from the fitting parameters. It is important to note that the reaction order (*i.e.*, first, second, etc.) is not determined by the chemical equation, but instead is determined experimentally. The chemical equation can aid in developing a model that can be checked by analyzing the measured data.

While a first order reaction is fairly simple, many reaction mechanisms can often be more complicated and involve multiple steps before the overall reaction is complete. For example, a starting material may initially react to form an intermediate species which can then react further to form a final product as shown in first order consecutive reaction outlined in Eq. 4.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} D$$

Equation 4.

This consecutive reaction involves a more complicated analysis than a first order reaction as the intermediate (B) is involved in both steps of the reaction. Consequently, the rate constants k_1 and k_2 will both contribute to the rate law. The magnitude of these rate constants will have direct implications not only for the speed of the reactions, but for the maximum intermediate concentration possible.[1] This value is highly dependent on the rate of the first and second reaction steps. As shown in Figure 1, changing the rate constants alters the reaction profile drastically, including changing the maximum intermediate concentration produced over the course of the reaction.

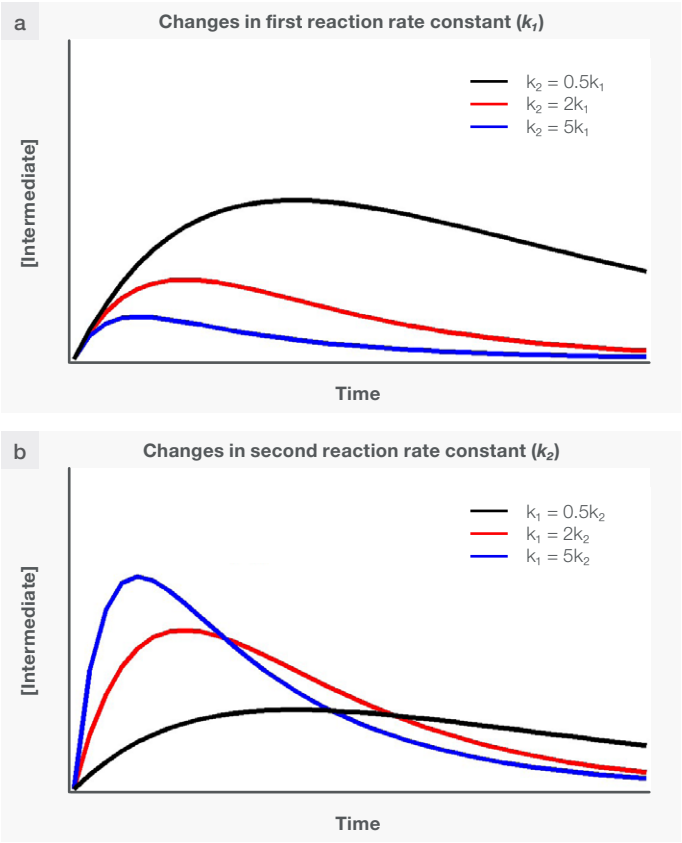

Figure 1. (a) Changes in the intermediate concentration over time for reactions with different k_1 and the same k_2. (b) Changes in the intermediate concentration over time for reactions with different k_2 and the same k_1.

In some circumstances, the intermediate species is the compound that needs to be isolated. Under these circumstances the reaction should be quenched before significant product is formed. Ideally, the reaction would be quenched when the maximum intermediate concentration is generated, requiring knowledge of when in the reaction process this occurs. As shown in Figure 1, the reaction profile is highly dependent on the rates of both first and second reaction, including the time at which the maximum intermediate concentration is formed. This value can be calculated if the rate constants are known for both steps of the reaction.

This concentration maximum correlates to the point at which the rate of change in the intermediate species is zero. Along with knowledge of the differential rate law for the intermediate species, this fact can be used to generate an equation (Eq. 5) relating the rate constants of the first and second reactions (k_1 and k_2, respectively) to the time at which the intermediate concentration reaches a maximum (t_{max}).[1]

$$t_{max} = \frac{1}{(k_1 - k_2)} \ln\left(\frac{k_1}{k_2}\right)$$

Equation 5.

UV-Visible Spectroscopy

As kinetic analysis requires the ability to monitor the concentration of compounds while a reaction progresses, one of the most common methods to monitor these reactions is UV-Visible absorption spectroscopy. In this technique, light spanning the UV-Visible range of the electromagnetic spectrum will interact with the sample. If the energy of incident photons are sufficient, electrons from the ground state can be promoted to the excited state; a process referred to as absorption (Figure 2).

As the energy levels for a small molecule are discrete, only photons with the appropriate energy can be absorbed by the molecule. The energy of a photon (E) is related to the wavelength of light (λ) though Eq. 6,

$$E = \frac{hc}{\lambda}$$

Equation 6.

where c is the speed of light and h is Plank's constant. Therefore, only specific regions of the electromagnetic spectrum can be absorbed by a molecule, leading to a unique absorption spectrum for the species studied. The energy spacing can be larger or smaller depending on the molecule studied, and will therefore result in different characteristic absorption spectra between different molecules. This can be helpful when measuring samples containing multiple species in solution, including reaction mixtures.

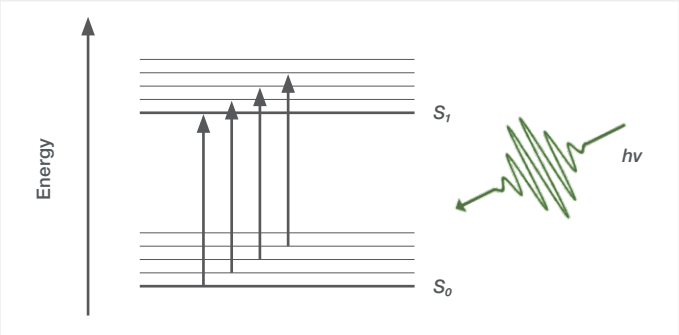

Figure 2. Generic energy level diagram depicting absorption of light for a small molecule.

Because of the absorption of photons by the sample, the intensity of the light observed exiting the sample (I) will be smaller than the initial intensity of the light before interacting with the sample (I_o) as shown in Figure 2. The loss of light due to absorption can be described through Beer's law (Eq. 7),

$$A = log\left(\frac{I_0}{I}\right)$$

Equation 7.

where the log of the ratio between the light intensity before and after the sample, I_0 and I, respectively, is equivalent to the absorbance of the sample (A). This is generally how a spectrophotometer calculates the absorbances of a measured sample. In these measurements, I_0 is typically established by measuring the intensity of a "blank" or a cuvette containing the solvent and all components other than the analyte of interest.

Beer's law can also be represented as Eq. 8,

$$A = cl\varepsilon$$

Equation 8.

where c is the concentration of the analyte, l is the path length the light travels through and ε is the extinction coefficient, a unique parameter for the substance studied. Through this form of Beer's law, absorbance is shown to be directly proportional to the concentration of the absorptive substance. By measuring the absorbance over time, the concentration of the species participating in the reaction can be directly monitored. However, it is important to know that not all molecules absorb in the UV-Visible range. Consequently, this analysis method is only pertinent when either one or all species participating in the reaction can absorb in the UV-Visible region.

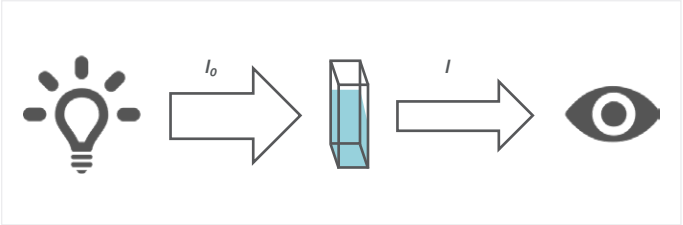

Figure 3. Generic depiction of light absorption within a liquid sample.

Reaction Scheme

Herein, the reduction of manganese-containing species will be studied using UV-Visible absorption spectroscopy in order to determine the rate constants for the reaction in the presence of different reducing sugars. While the mechanism is complex and dependent on a variety of parameters,[2,3] Eq.'s 9-11 describe one assumed reaction scheme.[4]

$$Mn(VII)\ (aq) + e^- \xrightarrow{k_1} Mn(VI)\ (aq)$$

Equation 9.

$$Mn(VI) + 2e^- \xrightarrow{k_2} Mn(IV)$$

Equation 10.

$$Mn(IV) \xrightarrow{k_3} MnO_2\ (s)$$

Equation 11.

In this scheme, the Mn^{7+} species (MnO_4^-, permanganate) is reduced to a Mn^{6+} species before the final reduction to a Mn^{4+} species by a reducing agent under alkaline conditions. Eq. 11 outlines the formation of insoluble MnO_2 from the soluble Mn^{4+} species.[4] Each manganese-containing compound absorbs strongly in the UV-Visible spectral region, allowing for all pertinent species to be monitored through this technique. Through the course of this experiment, this complex reaction will be monitored and the rate constants, k_1 and k_2, will be analyzed.

References

1. Engel, T.; Reid, P., *Physical Chemistry*; Pearson, 2014.
2. Simándi, L.I.; Jáky, M.; Savage, C.R.; Schelly, Z.A., Kinetics and Mechanism of the Permanganate Ion Oxidation of Sulfite in Alkaline Solutions. The Nature of Short-Lived Intermediates, *JACS*, **1985**, 107, 4220 – 4224.
3. Dash, S.; Patel, S.; Mishra, B.K., Oxidation by Permanganate: Synthetic and Mechanistic Aspects, *Tetrahedron*, **2009**, 65, 707 – 739.
4. Fernández-Terán, R.J.; Sucre-Rosales, E.; Echevarria, L.; Hernández, F.E., A Sweet Introduction to the Mathematical Analysis of Time-Resolved Spectra and Complex Kinetic Mechanisms: The Chameleon Reaction Revisited, *J. Chem. Ed.*, **2022**, 99, 2327 – 2337.

Experimental

Materials

- Thermo Scientific™ Evolution™ One Spectrophotometer
- Cuvette (1.0 cm, quartz. Need 3.0 mL volume)
- Pipetter and appropriate pipette tips
- Lint-free lab wipes
- Appropriate Glassware for preparing stock and sample solutions
- DI or Nanopure water
- Potassium permanganate
- Sodium Hydroxide
- D(+)-glucose
- D(+)-sucrose

Note: This analysis requires the ability for a student to fit the data with complex equations. Ensure students have access to data analysis software which allows for fitting to multiple exponential functions.

Safety

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

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

Instructions

Part A: Starting Material UV-Visible Spectra:

A1. Turn on the Evolution One spectrophotometer.

A2. Make the following stock solutions:

   a. 0.75 mM $KMnO_4$
   b. 400 M NaOH
   c. 700 mM D(+)-glucose
   d. 700 mM D(+)-sucrose

A3. Select the “Scan” application in the instrument software and set up the instrument using the following parameters in the settings window:

   a. “Measurement” tab:
      i. 100%T Baseline
   b. “Instrument” tab:
      i. Data Mode: Absorbance
      ii. Start Wavelength: 700 nm
      iii. End Wavelength: 300 nm
      iv. Data Interval: 1.0 nm
      v. Bandwidth: 1.0 nm
      vi. Integration Time: 0.1 s
      vii. Derivative: None
      viii. Smooth: None

A4. Prepare the following samples in DI water:

   a. 2.5 mM D(+)-glucose
   b. 2.5 mM D(+)-glucose
   c. 70 mM NaOH
   d. 0.25 mM $KMnO_4$

A5. Fill a clean 1.0 cm quartz cuvette with DI water to collect the blank measurement.

A6. Measure the blank solution, then empty and dry the cuvette.

   Note: Use gloves when handling the cuvette, and clean off the surface using a lint free lab tissue. Fingerprints and dust/lint can alter the blank and sample measurements. A light stream of air or N_2 can help dry the cuvette quickly.

A7. Measure each sample solution described in step A-4. Be sure to measure DI water as well.

A8. End the experiment and export the data as a .csv file (select “Spectrum, Comma Separated Values” for the raw data). This data will be used for your report.

Part B: Permanganate Reaction Kinetics:

B1. Prepare 3.0 mL of the following blank samples:

a. 70 mM NaOH, 2.5 mM D(+)-glucose

b. 70 mM NaOH, 2.5 mM D(+)-sucrose

B2. Select the "Kinetics" application in the instrument software and set up the instrument using the following parameters in the settings window:

a. "Type" tab:

i. Multiple Wavelengths

ii. Time Mode

b. "Measurement" Tab:

i. Time Units: Seconds

ii. Integration Time: 0.25 s

iii. Dwell Time: 0.75 s

iv. Stages: See Table 1

Stages	Start Time (s)	End Time (s)	Interval (s)
1	0	120	1.0 s
2	120	300	5.0 s

Table 1. Stages for $KMnO_4$ reaction in the presence of D(+)-glucose.

c. "Instrument" Tab:

i. Data Mode: Absorbance

ii. Bandwidth: 1.0 nm

iii. Wavelengths: 600 nm, 520 nm, 375 nm

B3. Fill a clean 1.0 cm quartz cuvette with the blank solution containing D(+)-glucose from step B-1a.

B4. Measure the blank solution, then empty and dry the cuvette.

B5. Measure a sample solution containing the following components:

a. Sample 1: 0.25 mM $KMnO_4$, 2.5 mM D(+)-glucose, 70 mM NaOH

Note: It will be easier to add the D(+)-glucose, NaOH and DI water to a cuvette and then place the cuvette in the instrument ***before*** *pipetting the $KMnO_4$ stock solution. As the reaction is rapid, the measurement should be started as soon as $KMnO_4$ is added to the solution.*

B6. End the experiment and export the data as a .csv file (select "Spectrum, Comma Separated Values" for the raw data). This data will be used for your report.

B7. Reserve the reaction product for analysis later.

B8. Rinse the cuvette three times with DI water. Make sure the cuvette is fully dried after the last rinse.

B9. Fill the cleaned 1.0 cm quartz cuvette with the blank solution containing D(+)-sucrose from step B-1b.

B10. Select the "Kinetics" application and set up the instrument using the following parameters in the settings window:

a. "Type" tab:

i. Multiple Wavelengths

ii. Time Mode

b. "Measurement" Tab:

i. Time Units: Seconds

ii. Integration Time: 0.25 s

iii. Dwell Time: 0.75 s

iv. Stages: See Table 2

Stages	Start Time (s)	End Time (s)	Interval (s)
1	0	120	1.0 s
2	120	600	5.0 s

Table 2. Stages for KMnO4 reaction in the presence of D(+)-sucrose.

c. "Instrument" Tab:

i. Data Mode: Absorbance

ii. Bandwidth: 1.0 nm

iii. Wavelengths: 600 nm, 520 nm, 375 nm

B11. Measure the blank solution, then empty and dry the cuvette.

B12. Measure a sample solution containing the following components:

a. Sample 1: 0.25 mM $KMnO_4$, 2.5 mM D(+)-sucrose, 70 mM NaOH

B13. End the experiment and export the data as a .csv file (select "Spectrum, Comma Separated Values" for the raw data). This data will be used for your report.

B14. Reserve the reaction product for analysis later.

B15. Rinse the cuvette three times with DI water. Make sure the cuvette is dried well after the last rinse.

Part C: Reaction Product UV-Visible Spectra:

C1. Select the "Scan" application in the instrument software and set up the instrument using the following parameters in the settings window:

a. "Measurement" tab:

i. 100%T Baseline

b. "Instrument" tab:

i. Data Mode: Absorbance

ii. Start Wavelength: 700 nm

iii. End Wavelength: 300 nm

iv. Data Interval: 1.0 nm

v. Bandwidth: 1.0 nm

vi. Integration Time: 0.1 s

vii. Derivative: None

viii. Smooth: None

C2. Fill a clean 1.0 cm quartz cuvette with DI water to collect the blank measurement.

C3. Measure the blank solution, then empty and dry the cuvette.

C4. Measure the two previously reserved reaction products from Part B (steps B7 and B14). Be sure to measure DI water as well.

C5. End the experiment and export the data as a .csv file (select "Spectrum, Comma Separated Values" for the raw data). This data will be used for your report.

**This procedure is based on the procedure outlined by Fernández-Terán et al.[4]*

Lab Report

Ensure that you have saved your experimental data and results where you can access them. Your results should be included in the final laboratory report, as well as the following:

1. Include plots of the absorption spectra of all starting materials, including DI water.
2. Include a plot of the absorption spectra of the final product for both reactions (D(+)-glucose and D(+)-sucrose).
3. Do the spectra of the final reaction products for both reactions match one another? Include additional observations for the absorption spectra included.
4. Using equations 9 – 11 from the introduction, solve and include the integrated rate laws for $Mn(VII)_{(aq)}$, $Mn(VI)_{(aq)}$ and $Mn(IV)_{(aq)}$. For the purposes of this experiment an integrated rate law for formation of $MnO_{2(s)}$ will not be required.

   Note: Electrons will not need to be included in the integrated rate laws.

5. Using the integrated rate laws and your knowledge of kinetics, fit the absorbance data as a function of time. Report k_1 and k_2 based on the fits for each data set. Do these results fit well to the data set? If not, why?

   Note: The data will need to be fit in a software program which allows fitting with single or multi-exponential functions. If the fit function does not appear to fit well, a constant offset may need to be added into the fit function to produce the appropriate fit (see Eq. 12 for an example).

$$[A] = A_0 e^{-k_1 t} + C$$

Equation 12.

   Hint: For some of the fitting functions, the fits may not need to span the full data set. Choose the appropriate region in which to fit the data.

6. Which reactant, D(+)-glucose or D(+)-sucrose, led to a faster overall reaction? Why?
7. Calculate and include t_{max} for each sample. Is there a trend in the t_{max}?
8. Based on the fit results, does the proposed mechanism in equations 9-11 make sense? Why/why not?
9. If the reaction needed to be slowed down, how could this be achieved?

Note: All axes for the reported graphs should be properly labeled, include appropriate units and significant figures.

Notes for Professors and Teaching Assistants:

Due to the complex nature of the permanganate reaction, as well as the overlap between the respective spectra of each manganese species analyzed in this experiment, the data does not perfectly fit to a standard two-step consecutive reaction if the entire data set is used. To avoid potential confusion, it is advised the data sets are fit using the time ranges outlined in Table 3. Fits can be performed in most commercially available data analysis software with curve fitting capabilities. Figure 4 depicts an example data set for the permanganate reaction performed in the presence of D(+)-glucose (Figure 4a) and D(+)-sucrose (Figure 4b).

Analysis Wavelength (nm)	Time Range (s)	
	Reaction with D(+)-glucose	Reaction with D(+)-sucrose
375	0 – 250	0 – 100
**520	0 – 20	0 – 250
600	0 - 300	0 – 600

Table 3. Recommended time ranges for curve fitting.

***Note, the loss and formation of the reactant and intermediate, respectively, are rapid for the permanganate reaction involving D(+)-glucose. Care should be taken to ensure the data fits only reflect the early dynamics, specifically for the reactant where late dynamics involve the growth of an additional species.*

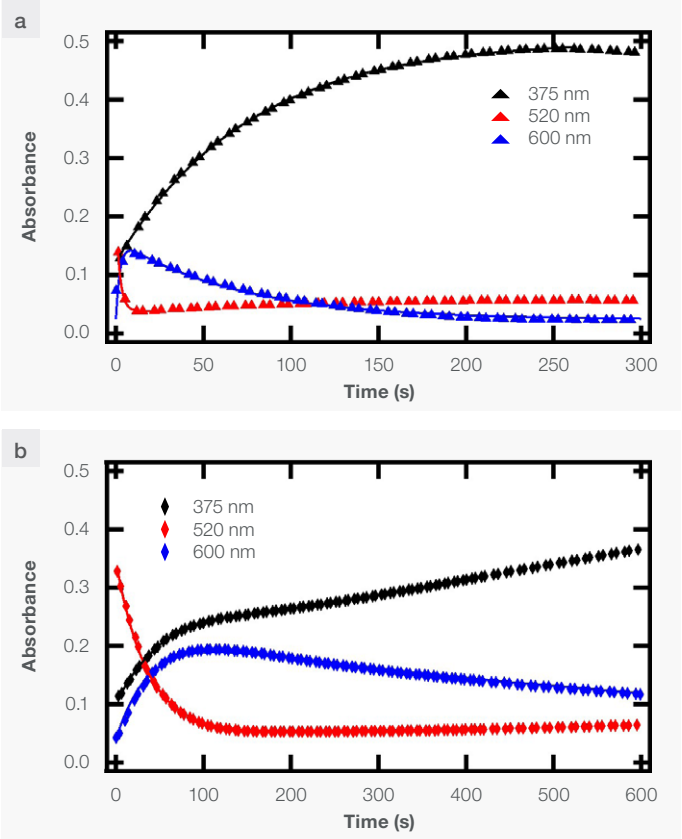

Figure 4 - Absorbance as a function of time for the permanganate reaction samples in the presence of (a) D(+)-glucose and (b) D(+)-sucrose. For a more clear visualization of the overlapping fit, some data points are excluded from these figures.

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Kinetics of Blue Dye with Hypochlorite Bleach

How does bleach keep your whites white?

Introduction

Blue No. 1 dye consists of a single molecule with a strong absorbance band whose wavelength of maximum absorbance (λ_{max}) is at 632 nm. The dye molecule is colored because it has an electronic structure called a chromophore that corresponds to an electron occupying a filled orbital that is separated from an empty orbital by an energy gap. If a photon of the correct energy hits the molecule, the photon is absorbed and that excited electron jumps up into the empty orbital.

If the electronic or physical structure of the dye molecule is altered, it can affect the size of the energy gap between the filled and empty orbitals. While small changes in the energy gap might make the molecule appear to be a different color, larger changes, such as oxidizing a portion of the molecule, can inactivate the chromophore completely and cause the color to go away.

A common product that is used to oxidize molecules is liquid bleach. Bleach is a dilute solution of sodium hypochlorite (NaOCl) that reacts with colored compounds to turn them into colorless ones. An example of this would be how bleach is utilized to remove stains in clothing.

In this experiment, you will study the reaction between blue food dye and hypochlorite bleach:

$$dye + bleach \rightarrow colorless\ products$$

Equation 1.

With this reaction we can write the rate law as:

$$Rate = k[dye]^m\ [bleach]^n$$

Equation 2.

Beer's Law tells us that concentration is proportional to absorbance, so the concentration of the dye ([dye])can be monitored during the reaction by monitoring the absorbance at 632 nm.

In **Part A**, you will determine the order of reaction with respect to [dye]. The experiment is set up so that the concentration of bleach ([bleach]) is much larger than [dye] that it effectively remains constant throughout the reaction. This has the effect of simplifying *Equation 2* to:

$$Rate = k'[dye]^m$$

Equation 3.

where $k' = k\ [bleach]^n$

k' is called a *pseudo rate constant*

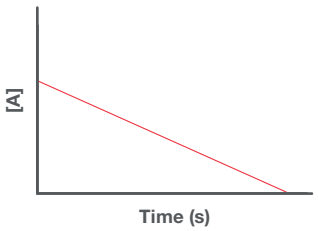
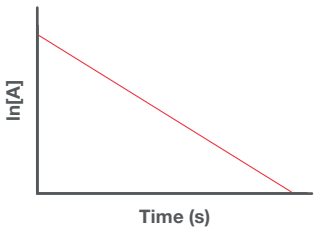
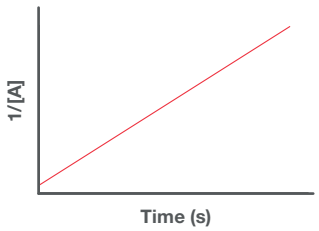
Recording the absorbance as a function of time after mixing the reactants allows the creation of integrated rate plots using the reactant concentration versus time data. The complexity of kinetics that define the order of reaction can be assigned using the integrated rate laws for zero, first, and second order reactions based on the best linear fit according to Table 1 below.

The order of [dye] in the reaction can be determined by which of the following graphs gives the best straight line:

- For zero order: Plot absorbance (Abs) or concentration of dye ([dye]) vs. time; the reaction is zero order in [dye] (***m*** in *Equation 3* = 0)
- For first order: Plot the natural logarithm of absorbance ((ln(Abs)) or concentration of dye (ln[dye]) vs. time; the reaction is first order in [dye] (***m*** = 1)
- For second order: Plot the inverse of absorbance (1/Abs) or concentration of dye (1/[dye]) vs. time; the reaction is second order in [dye] (***m*** = 2)

In **Part B**, you will monitor a second reaction where the [bleach] is doubled (while keeping the [dye] the same as in Part A) and the rate of reaction is measured again by computing an *instantaneous initial rate* close to the start of the reaction. This rate can be calculated by dividing the change in concentration over a short period of time by the number of seconds. Comparing the instantaneous initial rates at the same point in time to those in Part A will enable the observation of what effect doubling [bleach] has on the rate of reaction. If ***n***, the order with respect to [bleach], is zero, *Equation 2* tells indicates that doubling the [bleach] will have no effect on the measured rate, because any number to power zero equals 1. Similarly, if ***n***=1, the rate should double when we double [bleach] ($2^1 = 2$) and if ***n***=2 the rate should quadruple ($2^2 = 4$).

You will have experimental data for the measured instantaneous initial rate for both [dye] and [bleach] at the time you measured the rate. Since you will now know ***m*** and ***n*** in *Equation 2*, you can plug in all the known quantities and solve for the rate constant *k*.

Reaction Order	Zero	First	Second
Description	The rate is independent of the reactant concentrations.	Rate is proportional to the concentration of one reactant.	Rate is proportional to the square of the concentration of a single reactant.
Rate Law	Rate = k	Rate = k [A]	Rate = k $[A]^2$
Integrated Rate Law*	$[A] = -kt + [A]_0$	$\ln[A] = -kt + \ln[A]_0$	$\frac{1}{[A]} = kt + \frac{1}{[A]_0}$
Half-Life	$t_{1/2} = \frac{[A]_0}{2k}$	$t_{1/2} = \frac{\ln 2}{k}$	$t_{1/2} = \frac{1}{k[A]_0}$
Linear Plot*	[A] vs t	ln[A] vs t	$\frac{1}{[A]}$ vs t
Y intercept*	$[A]_0$	ln $[A]_0$	$\frac{1}{[A]_0}$
Slope*	$-k$	$-k$	k
Example plot*			

* The integrated rate laws for zero, first, and second reaction orders can be fit to the equation for a straight line, y = mx + b, where m is the slope and b is the Y intercept. $[A_0]$ is the initial concentration and [A] is the concentration at another time point.

Table 1.

In **Part C**, you will repeat the experiment from Part A at two slightly different temperatures. The rate law does not change as you change temperature, but the rate constant *k* does. You will calculate the rate constants at these two additional temperatures, then make a plot of ln(*k*) (y-axis) versus 1/T (x-axis, temperature in Kelvin).

This plot comes from the Arrhenius Equation:

$$ln(k) = -\frac{E_a}{R}\left(\frac{1}{T}\right) + ln[A]$$

Equation 4.

The slope of the line is the negative of E_a (the activation energy for the reaction) divided by R (the gas constant = 8.314 J·mol^{-1}·K^{-1}).

Kinetics is a complicated topic, however by understanding it and designing experiments carefully, we can determine all the rate parameters for a reaction is a straightforward way.

Purpose

This experiment consists of three different parts which can be completed in order or in separate sessions:

1. Part A: Determination of the order of reaction with respect to the concentration of [dye]
2. Part B: Determination of the order of reaction with respect to the concentration of [bleach]
3. Part C: Determination of the activation energy for the reaction E_a

SPECTRONIC 200 Visible Spectrophotometer

*SPECTRONIC 200 Spectrophotometers are available on loan from Thermo Fisher Scientific™ at no cost. We will ship it to you, and you ship it back after one week. If you are interested in this program, please visit:

thermofisher.com/spec200freetrial

Experimental

Procedure

Making a measurement with the Thermo Scientific™ SPECTRONIC™ 200 Visible (Vis) Spectrophotometer*

1. Turn on the instrument and allow it to complete its startup sequence. Let the instrument warm up and stabilize for at least 30 minutes. Set up the experiment you want to perform in the spectrophotometer software. Obtain a square plastic cuvette or glass test tube to use in your experiments. If using a test tube cuvette, use a pen to place a mark near the top if the cuvette is not already marked with a white line. The mark allows you to ensure consistent placement into the instrument.
2. Add liquid to the cuvette until there is ~3 cm of liquid in the bottom (4 cm for test tubes). If plastic transfer pipettes are available, use one. The exact liquid level in the cuvette is not critical for good measurements as long as it is above 3 cm. Do not waste solution or risk spills by over-filling the cuvette.
3. Place the cuvette in the sample stage of the SPECTRONIC 200 Visible Spectrophotometer. If using a plastic cuvette, the clear sides should be on the right and left. If using a test tube cuvette, place it so that the mark faces to the right.
4. After the warm-up period, follow steps 2 and 3 using water or the appropriate “blank” solvent. Zero the instrument by pressing the autozero button.
5. For each subsequent measurement, empty and rinse your cuvette, shaking out as much of the rinse solvent as possible. When preparing samples, never return excess solution to the stock bottle. Pour all waste or excess into the appropriate waste receptacle. Follow steps 2 and 3 using your sample.

Reagents

- McCormick® Blue No. 1 dye solution
  - Approximately a 10.0 x 10^{-6} M stock solution can be prepared by diluting 18 drops of dye to 1 L of water.
  - The actual concentration of the dye solution can be calculated using the molar absorptivity of the dye where ε = 130,000 L·cm^{-1}·mol^{-1}.
- Clorox® brand sodium hypochlorite bleach
  - The concentration of the bleach solution can be estimated as 1.00 M.
  - The exact concentration of bleach in the bottle can be calculated using the concentration of sodium hypochlorite by weight and the density.

Part A. Determine the order of reaction in [dye]

1. Obtain four well-matched cuvettes for measuring in the spectrophotometer and a cuvette rack for cuvette storage. Ensure that the optical faces are free of scratches and that the cuvettes are clean and not stained from use in previous experiments.
    a. Fill one cuvette with distilled water to serve as your blank. Store it in position 1 of your cuvette rack.
2. Place 25 mL of the stock blue dye solution in a beaker, add **2 mL** of water, and mix. Fill the second cuvette with this solution and set it aside. This will serve as your "Standard of comparison". Place it in position 2 of your cuvette rack.
3. Place 25 mL of the stock solution and **1 mL** of water in a second beaker. Measure and record the temperature of this solution. Obtain **1 mL** of bleach and prepare to perform the following steps quickly.
    a. Set the spectrophotometer to read in Live Display mode at 632 nm.
    b. Wipe the optical faces of the blank cuvette from Step 1 with a lint-free laboratory tissue and place it in the sample stage. Ensure that the optical faces are in the beam. Close the lid and zero the spectrophotometer (see specific instructions for using the spectrophotometer on the previous page). Once the blank measurement is complete, remove the blank cuvette and return it to position 1 of your cuvette rack.
    c. Add 1 mL of bleach to the solution in the beaker from Step 3. Mix thoroughly with a stirring rod and start the stopwatch.
    d. Fill cuvettes 3 and 4 with the solution from step 3c.
    e. Place cuvette 3 in position 3 of your cuvette rack for you to observe the reaction progress.
    f. Wipe the optical faces of the cuvette 4 with a lint-free laboratory tissue, place it in the sample stage in the spectrophotometer and close the lid.
    g. Record the absorbance every 5 seconds for the first 60 seconds, then every 20 seconds until cuvette 3, which you've observed throughout the reaction, appears colorless. Remember that cuvette 4 in the spectrophotometer contains a duplicate of the reaction and looks exactly like the contents of cuvette 3. Record your observations of what happens to the intensity of the color in cuvette 3.
4. Retain cuvettes 1 and 2 and their contents in the cuvette rack, discard the solutions in cuvettes 3 and 4, and then rinse the cuvettes 3 and 4 with distilled water.
5. Transfer your data to a spreadsheet program and use it to generate the plots and complete the calculations necessary to answer the questions on the Lab Report.

Part B. Determine the order of reaction in [bleach]

1. Place 25 mL of the dye stock solution in a third beaker. Obtain 2 mL of bleach and prepare to perform the following steps quickly.
    a. Set up your spectrophotometer and measure the blank cuvette as outlined in Part A (steps 3a and 3b) if you have not already done so.
    b. Add 2 mL of bleach to the solution, mix, and start the stopwatch.
    c. Fill cuvettes 3 and 4 with this solution.
    d. Place cuvette 3 in position 3 of your cuvette rack for you to observe.
    e. Insert cuvette 4 into the spectrophotometer.
    f. Record the absorbance every 5 seconds for the first 60 seconds, then every 10 seconds until cuvette 3 appears colorless. Observe cuvette 3 as the reaction progresses.
2. Retain cuvettes 1 and 2 and their contents in the cuvette rack, discard the solutions in cuvettes 3 and 4, and then rinse cuvettes 3 and 4 with distilled water.
3. Transfer your data to a spreadsheet program and use it to generate the plots and complete the calculations necessary to answer the questions on the Lab Report.

Part C. Determine the activation energy of the reaction

1. Place 25 mL of the stock solution and **1 mL** of water in a fourth beaker. Obtain **1 mL** of bleach and prepare to perform the following steps quickly.
    a. Set up your spectrophotometer and measure the blank cuvette as outlined in Part A (steps 3a and 3b) if you have not already done so.
    b. Prepare a hot water bath by heating approx. 150 mL of water in a 250 mL beaker on top of a hotplate.
    c. Warm the beaker containing the 25 mL of dye solution in a warm water bath prepared
    d. Warm the solution to 5°C above (± 1°C) room temperature. Record the room temperature and the temperature of the heated solution.
    e. Add 1 mL of bleach to the solution, mix, and start the stopwatch.
    f. Fill cuvettes 3 and 4 with this solution.
    g. Place cuvette 3 in position 3 of your cuvette rack for you to observe.
    h. Insert cuvette 4 into the spectrophotometer.
    i. Record the absorbance every 2 seconds for the first 60 seconds, then every 10 seconds until cuvette 3 appears colorless. Observe cuvette 3 as the reaction progresses.
2. Repeat the same procedure from Part C step 1 (a–i) with a solution that was warmed to 10°C above (± 1°C) room temperature. Make sure to record the exact temperature.
3. Transfer your data to a spreadsheet program and use it to generate the plots and complete the calculations necessary to answer the questions on the Lab Report.
4. Discard all solutions then clean and return cuvettes and all laboratory glassware.

Lab Report

Kinetics of Blue Dye with Hypochlorite Bleach

Name: _____

Date: _____

Section No. or Lab Period: _____

Part A. Determine the order of reaction in [dye]

Questions

1. Enter your time and absorbance data for the entire reaction into a spreadsheet program to compute columns C, D, and E. Use the value of the molar absorptivity ε = 130,000 $L \cdot cm^{-1} \cdot mol^{-1}$ to compute the concentration of column C.

	A	B	C	D	E
1	**Time (s)**	**Absorbance**	**[dye] (M)**	**ln[dye]**	**1/[dye] (M-1)**
2	0				
3	5				
4	10				

Spreadsheet example.

2. Use the spreadsheet program to make zero, first, and second order rate plots. Put a line of best fit, with the corresponding rate equation and R^2 values, on the graph with the best straight line of the three.

3. Staple printouts of your integrated rate law graphs to this page when you submit this report.

4. Report the temperature of your dye solution at the start of the experiment: _____

5. How long did the reaction take to reach completion (no more color visible in cuvette 3)?

6. Based on your integrated rate plots, what is the order of reaction with respect to [dye]?

   ☐ Zero ☐ First ☐ Second

7. What is the value of the rate constant *k´* from the integrated rate plot?

8. Look at either your absorbance (Abs) or [dye] vs. time data. Determine the half-life of the reaction across three different absorbance intervals by the time it takes for a chosen absorbance or concentration value to decrease by half.

From (Abs) or [dye]	To (Abs) or [dye]	Half-Life (seconds)

Data Table 1.

   a. Are the values approximately the same, or radically different?

   b. If the half-lives are similar, calculate the mean of the three values.

9. Calculate the value of the rate constant *k´* from the half-life that you measured in question 8 on the previous page. Use the appropriate equation based upon the order of reaction that you determined in question 6 on the previous page.

10. Calculate the percent error between the value of *k´* obtained from the plot (question 7 on the previous page) and the half-life equation (question 9 above).

Remember:

- Staple hand-drawn or printed graphs to your lab report
- Staple the two sheets of the lab report together before you hand them in

Name: _____

Part B. Determine the order of reaction in [bleach]

1. Use a spreadsheet program to make:
   a. A plot of [dye] vs. time
   b. The appropriate integrated rate plot for the order of reaction that you determined in Part A. Put a line of best fit, with the corresponding rate equation and R^2 values, on the second plot.
2. Staple printouts of your plots to this page when you submit this report.
3. For the reactions in both Part A and Part B, calculate the *instantaneous initial rate* at the same time point close to the start of both runs that corresponds to the change in [dye] over a period of time. For example, if you started recording data when the stopwatch was at 20 seconds, you might choose the time interval between 30 and 35 seconds.
   a. To calculate this use the [dye] vs. time data in your spreadsheet for the reactions with 1 mL (Part A) or 2 mL of bleach (Part B):

$$\textit{Instantaneous initial rate} = \frac{\Delta[dye]}{\Delta time}$$

4. Report the instantaneous rates that you calculated. Include the correct units.

Part	Instantaneous initial rate	Units
A		
B		

Data Table 2.

5. What happened to the instantaneous initial rate of the reaction when you doubled the concentration of bleach?
6. Use this data to calculate the order of reaction with respect to [bleach]. Write the full rate law in the form of *Equation 2* with the values of *m* and *n* filled in.
7. Into your full rate law from question 6 on the previous page, solve for the value of the rate constant *k*. Remember to include the correct units for the rate constant. Use the following values to insert into the equation:
   a. The instantaneous initial rate from the data in Part A or Part B that you used in question 3 on the previous page.
   b. The [dye] (from column C in the spreadsheet) at the point where you calculated the instantaneous rate.
   c. The [bleach] (calculated from the concentration of the stock solution and the dilution that occurred when you added it the dye solution in Part B (step 1b) of the Experimental procedure).
8. In Part B we added twice the amount of bleach to the reaction compared to Part A to achieve a concentration of bleach that was double what we used in Part A. With that difference in mind, explain the purpose of the 1 mL of water that was added in Part A, but not in Part B?

Name: _____

Part C. Determine the activation energy for the reaction

1. For each data set, use a spreadsheet program to make the appropriate integrated rate plot for the order of reaction that you determined in Part A. Put a line of best fit, with the corresponding rate equation and R^2 values, on each plot.
2. Staple printouts of your plots to this lab report when you turn it in.
3. Use the slope of the best fit line to calculate *k´* and divide this value by [bleach] to get the value of *k*. Do this for the data obtained in Part A and in Part C. You will have three values of *k*.

Plot	Slope	*k´*	[bleach]	*k*
1				
2				
3				

Data Table 3.

4. Make an Arrhenius plot (see Equation 4) with ln(*k*) on the y-axis and 1/T (in Kelvin) on the x-axis.

Plot	Temperature		1/T	*k*	ln(*k*)
	(°C)	(K)			
1					
2					
3					

Data Table 4.

5. The slope of this plot is equal to

$$-\frac{E_a}{R}$$

Record the slope of the plot: _____

6. Calculate the value of E_a, which is the activation energy of the reaction.

7. The Arrhenius Equation can be rewritten for data at two temperatures as:

$$ln\left(\frac{k_2}{k_1}\right)=\frac{E_a}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)$$

   a. Use your data at room temperature as T_1 and at room temperature +10°C as T_2 to determine E_a using this equation.

8. Calculate the percent error between the values of E_a determined in question 6 and Question 7a.

   a. Which of the two values would you have more confidence in? Explain why.

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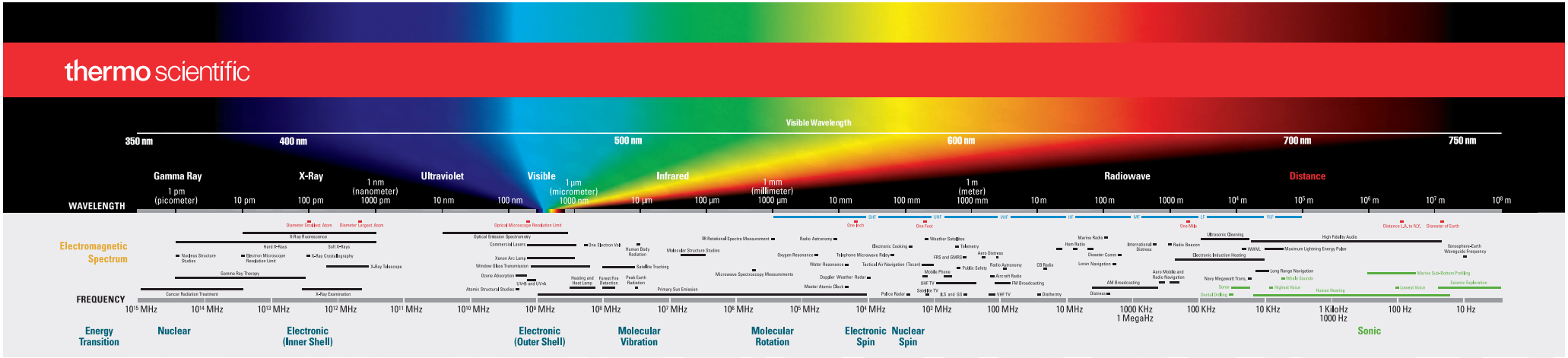
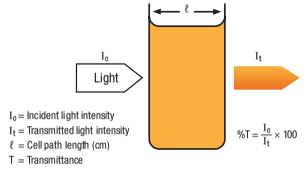
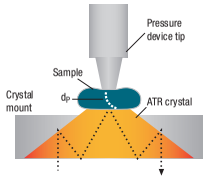


Spectroscopy techniques

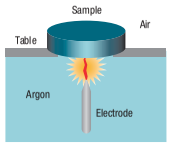
Transmittance



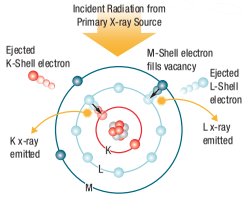
ATR



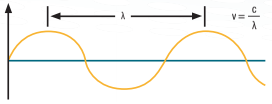
OES excitation stand



X-ray fluorescence process

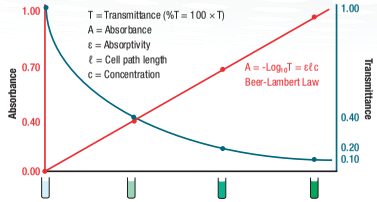


Wave properties

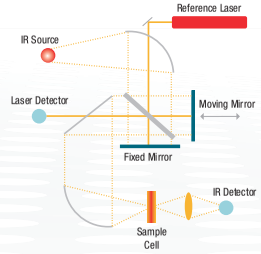


λ (lambda) = Wavelength *(distance between two peaks)*
ν (nu) = Frequency *(number of peaks passing a point in unit time)*
c = Speed of light in a vacuum

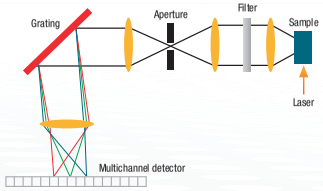
Transmittance and absorbance depend on concentration



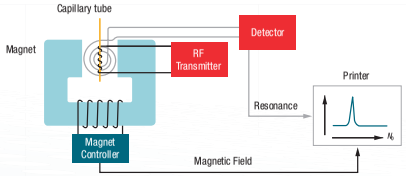
Optical diagram of conventional FTIR spectrometer



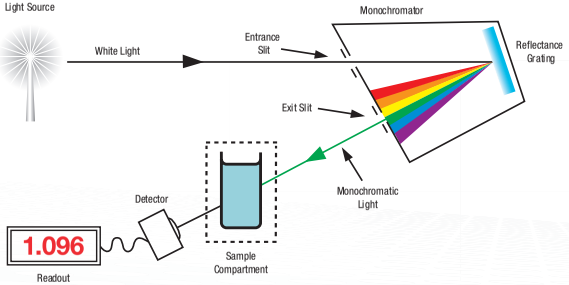
Optical diagram of conventional Raman spectrometer



Optical diagram of a typical NMR spectrometer



Optical diagram of conventional spectrophotometer



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