Flame Test Wire

A small piece of inert metal wire is bent into a loop to support a small drop of solution. The small loop is dipped into a solution, and then the wire loop is placed into the outer flame. Flame test wires can easily become contaminated. Make sure your wire is clean before trying to observe new solutions. You can clean your wire by dipping it into HCl and then placing it in the flame. If it is clean, there will not be a large color change when the wire is placed in the flame.

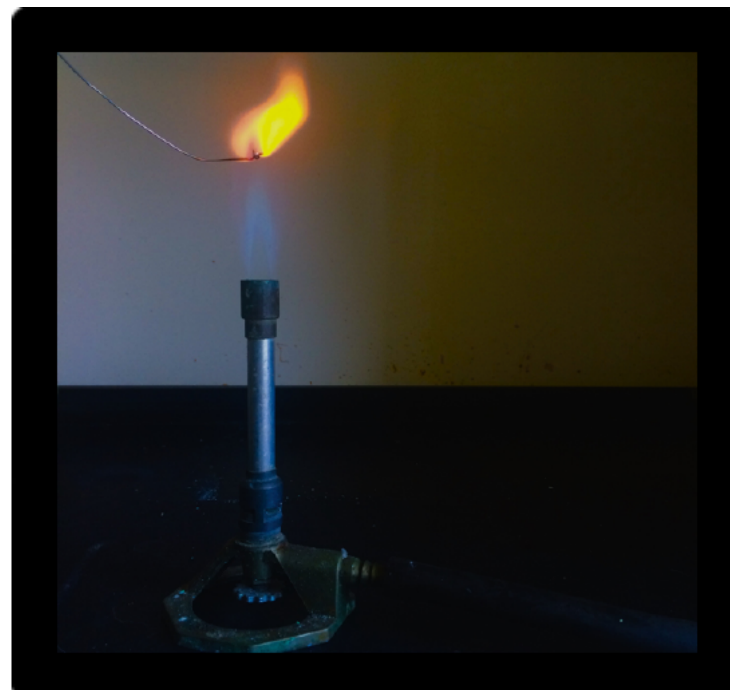

Figure 4.2: Flame Tests is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by LibreTexts.

Record your flame test observations for each of these solutions.

Potassium Chloride, Calcium Chloride, Strontium Chloride, Sodium Chloride, Copper Chloride, Lithium Chloride

Safety Considerations

Strong acids and bases are used in this laboratory along with toxic metals.Make sure to wash hands immediately if you come in contact with these materials.

Bunsen burners should be treated with care to avoid fires and burns.

Waste Disposal

All generated waste should be placed in a labeled waste container.

Pre-Lab Questions

1. Look up solubility rules. In the lab we will be using NaCl, $AgNO_3$, $BaCl_2$, and K_2CrO_4. Which of these do you expect to be soluble in water?

2. In question 1 we take about four different solutions. If we mix different combinations of these solutions together, which ones do you expect to react and not react based on solubility rules?

3. When copper is mixed with concentrated nitric acid copper ion is formed and NO_2 gas is also formed. Write the balanced Red-Ox reaction for this process, which is in acidic media.

4. What is the purpose of "wafting" as a technique in the laboratory?

5. If an acid is tested with red litmus paper, what will happen? If an acid is tested with blue litmus paper, what will happen?

Post-Lab Questions

1. Make sure all the balanced reactions that occurred during the lab are recorded in the laboratory notebook.

2. Write all the reactions as net-ionic equations and identify any spectator ions.

3. If someone gave you a liquid that they thought could be a solution of NaCl, HCl, or NaOH, how could you determine which one of the three is in the bottle. Include as many tests as possible to help confirm your results.

4. If someone gave you a liquid that they thought could be a solution of $NaNO_3$, NaCl, $Cu(NO_3)_2$, $CuCl_2$, or $Sr(NO_3)_2$, how could you determine which one of the four ions is in the solution? Include as many tests as possible to help confirm your results. Can you think of any other tests you could perform based on solubility rules?

What Is Contaminating the Water Supply? - Exploration Laboratory

Background

A local town is concerned that the water from their well has started to become contaminated. Preliminary testing has narrowed down the potential contaminant to one of the following salts:

- Calcium Chloride ($CaCl_2$)
- Potassium Chloride (KCl)
- Sodium Chloride (NaCl)
- Strontium Chloride ($SrCl_2$)
- Potassium Sulfate (K_2SO_4)
- Sodium Sulfate (Na_2SO_4)
- Sodium Acetate ($NaC_2H_3O_2$)
- Sodium Carbonate (Na_2CO_3)
- Potassium Carbonate (K_2CO_3)

Figure 5.1: "Urban Well Water" by FotoGrazio is licensed under CC BY-NC-ND 2.0.

Your job is to determine which contaminant is in the water supply, its concentration in the water, and how hazardous the contaminant is to the people consuming the water.

Experiment

You have learned the techniques you need to perform this determination during your earlier labs. Consult those procedures and your results to help in planning your experiments. Before you begin your experiments, you need to devise an experimental plan and procedure for what you will do.

This is a two-week lab. You will come up with the plan during the first laboratory session and start to carry out experiments. You will finish the experiments in the second week and write up your conclusions.

Remember that all conclusions need to be supported by evidence! For determining the identity of the salt make sure to rule out all other choices. For determining the concentration of the salt make sure to make at least three determinations so that you have a sense of the uncertainty in your measurement.

Available Materials

In addition to what you have in your lab drawer, you will have all the lab equipment from the first four labs available to you for this experiment. You do not need to use and should not use all the equipment in the lab.

Data Collection

When collecting data, keep everything well organized and labeled. Think about the experiments you will perform. What data will need to be recorded? Set up tables for yourself to help in organizing your data.

Safety Considerations

Talk through your experimental approach with your laboratory instructor before beginning to make sure you have appropriately accounted for safety. Check safety considerations from previous labs to aid in developing your approach to completing this lab safely.

Waste Disposal

Dispose of solid waste in the appropriately labeled solid waste container. Dispose of liquid waste in the appropriately labeled liquid waste container.

Pre-Lab Questions

1. How will you determine which salt is the contaminant? Which previous lab has the most relevant information for helping you?

2. How will you determine the concentration of the salt in the water? Which previous lab has the most relevant information for helping you prepare?

3. How will you determine the hazard level of the contaminant? Which previous lab has the most relevant information for helping you prepare?

4. Recap the safety consideration from each of the previous labs. Summarize the safety information from those labs here.

Post-Lab Questions

1. What is the contaminant in the water supply? Provide the evidence you have for your determination and the logic for making your determination and ruling out the other possibilities.

2. What is the concentration of the contaminant in the water supply? Report this value in two units (molarity and grams per liter).

3. What is the relative average deviation for the concentration?

4. How hazardous is the contamination? Cite outside resources you find. You should consider the identity and concentration of the contaminant when making this determination. Make sure to consider units when comparing your data with outside resources.

5. Based on your information, what is your recommendation for what the town should do now with the information you found. Make sure your recommendation is based on your evidence.

Titrations Technique Laboratory

Titration Background and Application

Quantitative analysis (determining the quantity/amount of a compound in a sample) is important in every science, engineering, and health field. If you are in the pharmaceutical industry, you need to determine how much of an active ingredient is in the drug you are providing. If you are in the food industry, the acid content in your preparations can be the difference between something being properly preserved or tasting horrible. In environmental chemistry, determining the concentrations of varies species that are dissolved in water is critical for understanding risks to wildlife and appropriately treating wastewater.

A widespread technique for quantitatively determining the amount of a compound in a sample is to use a titration. The basic ideas used in a titration are to identify the compound we are trying to quantify (we call this compound the analyte) and then slowly add a species that reacts with the analyte (we call this species the titrant). When we are adding the titrant, it is critical to keep track of how much we have added. We keep adding the titrant until all the analyte has reacted with it, and then we make sure to stop adding titrant. We call the point when we have added the exact amount of titrant to react with the analyte the equivalence point of the titration.

Now we know exactly how much titrant has been added. By knowing the amount of titrant we have added, we also know the amount of analyte in the sample by using stoichiometry.

The moles of the titrant added are stoichiometrically equivalent to the moles of the analyte.

If they have a 1:1 ratio in the balance equation, then the moles of titrant are equal to moles of analyte. If they have a different ratio in the balanced equation, then we just account for that different ratio by using the coefficients in the balanced equation. Either way at the end point we have added the perfect amount of titrant so that both the titrant and analyte completely react with neither of them remaining.

Titrations allow us to determine the number of moles of a compound in a sample. Here is the key equation to understanding a titration where the coefficients refer to the balanced chemical equation of the titrant reacting with the analyte:

$$\text{moles of analyte} = \frac{\text{coefficient of analyte}}{\text{coefficient of titrant}} \times (\text{moles of titrant})$$

Buret Introduction

For a titration to be quantitative, we need to determine how many moles of titrant we have added. A buret is a commonly used piece of scientific equipment to quantify the volume of liquid that has been delivered. We put a solution into the buret that has a known molarity. We then determine the number of moles by

$$\text{moles of titrant} = (\text{molarity of titrant}) \times (\text{volume of titrant (L)})$$

The buret has three main parts: 1. The tip of the buret is where the liquid leaves the end of the buret, 2. The stopcock is the valve near the tip of the buret that starts and stops the flow of liquid from the buret, and 3. The neck of the buret has volume markings that allow us to determine how much liquid has left the buret.

Figure 6.1: 50 mL buret.

When you are reading a buret, it is critical to make sure that you take the time to look closely at the markings and understand the spacing. In the picture in Figure 6.2 each milliliter is marked (19, 20, 21, and 22). In addition, there are nine lines in-between each milliliter value, so each of these lines represents one-tenth of a milliliter. When reading the buret in Figure 6.2, I see that the bottom of the meniscus is in between the 1st and 2nd line after the 20 mL mark. Notice that the bottom of the meniscus is likely to not fall exactly on a marking which allows us to interpolate the value to get the correct value along with an estimate of the uncertainty.

My thought process for reading the buret is first to note that the value is somewhere between 20 and 21 mL; then I go a step further and see it is between 20.1 and 20.2 mL, finally I determine the recorded value by estimating the last digit. It looks as if it is closer to 20.2 mL than 20.1 mL so I

would read it as 20.18 mL. Someone else might have a slightly different estimation of the last digit maybe they think it is 20.16 mL or 20.19 mL. This slight disagreement is fine and gives an approximation of the uncertainty of the measurement. I would record this value with four significant digits since my last recorded digit should be the one that has uncertainty.

Buret readings should be recorded to 2 decimal places.

We determine the volume of liquid delivered by reading the volume at the beginning of the titration and then reading the volume again at the end of the titration. The volume delivered is then the difference between those two readings:

Figure 6.2: "Buret" by photobunny is licensed under CC BY-NC-ND 2.0.

$$\text{volume of titrant} = (\text{final volume}) - (\text{initial volume})$$

Buret Preparation

We prep the buret by first rinsing with water multiple times and then rinsing multiple times with the titrant solution we are using. To rinse the buret, we use about 15 mL of liquid for each rinsing. Put the liquid into the buret with the stopcock closed (perpendicular to the tip). Then slowly tilt the buret so that the liquid touches all of the interior surface and finally allow the liquid to run out the tip of the buret.

Once the buret has been rinsed with water and the titrant solution, then the buret can be filled with the titrant solution. Make sure the liquid level is within the volume markings and that the tip of the buret is filled with liquid and does not contain an air bubble.

Analyte Preparation

In a titration, the chemical we are trying to quantify (analyte) is either a solid or in a solution. For a titration to work appropriately, we need the analyte to be dissolved in a solution. If you have a solid analyte the first step is to dissolve it in a solvent (very often water).

Since we are trying to be quantitative in this technique, it is also critical to know the amount of analyte we are using during the titration. If the analyte is a solid, then the easiest approach is to measure the mass of solid using a balance. If the analyte is a solution, then the easiest approach is to use a volumetric pipet to know the volume of analyte that will be titrated.

One other factor to keep in mind when we are preparing an analyte is that we want to ensure that we have an appropriate amount of analyte for the titrant solution being used. In general, we want the titrations to require somewhere between 10 and 50 mL of solution delivered from the buret. This is because most burets can at most deliver a maximum of 50 mL before being refilled. We also want the titration to take at least 10 mL so that we get smaller uncertainties and more significant figures for the measurement.

Indicator

For the titration to work, we need some way of determining when we have added exactly the right amount of titrant to completely react with the analyte. Indicators are the common approach to determine when we have reached the equivalence point. For the titrations we are performing in this laboratory (acid as the analyte and base as the titrant), we will use an acid-base indicator that is one color at low pH values and a different color at high pH values. Phenolphthalein is a commonly used indicator because it is colorless when the pH is below 8.3 and is pink when the pH is above 8.3.

As we add a base to solution the pH will increase. In a titration where the analyte is an acid the pH will remain low until all of the acid has reacted and then it will rise rapidly. The goal of the indicator is to show us exactly when all the acid has reacted by changing color. We want to control our delivery of the titrant, so the indicator in the solution goes from colorless to pink when just one drop or less of

titrant is added. Making sure that we are only adding one drop or less when the solution changes color ensures that we are doing our best at finding the equivalence point.

Common Errors To Avoid

Here are some common mistakes that you want to avoid when performing a titration.

1. Make sure the tip of your buret is fully filled with solution before starting the titration. It is common for air bubbles to collect in the tip of the buret when filling. If you don't remove the air bubbles before beginning the titration, then at some point the air bubble will run out of your buret and instead of delivering 0.5 mL of solution, you will have delivered 0.5 mL of air.

2. Remember the indicator. The titration only works if you can tell when the equivalence point has been reached.

3. Make sure to appropriately read the volume. Take the time to interpret the markings on the buret and read the volume directly. Near the top of the buret is marked 0 mL and near the bottom of the buret is marked 50 mL. Record the current location of the bottom of the meniscus when reading.

4. Avoid parallax errors when reading the buret. Parallax errors are avoided by ensuring that your eye and the meniscus are at the same height when reading the buret. This likely means you need to move either the location of your eye or the location of the buret to appropriately take a reading. You can take the buret out of the clamp to appropriately read the volume.

5. Go slow at the end of the titration. Remember we want the solution to change colors when we have only added a drop or less.

Performing the Titration

Here are the general steps for performing a titration:

1. Clean the buret and fill with the titrant solution.
2. Add a known amount of analyte to an Erlenmeyer flask. The total volume should usually be between about 25 and 50 mL in the Erlenmeyer before beginning the titration. You can always add more water to achieve the appropriate volume because adding water does not change the number of moles of analyte.
3. Add 2 or 3 drops of indicator to the analyte flask.
4. Record the initial volume on the buret to 2 decimal places.
5. Use the buret to deliver titrant solution to the analyte flask while swirling to mix.
6. Find the end point by having one single drop or less cause a color change.
7. Record the final volume on the buret to 2 decimal places.
8. Properly dispose of waste solution in Erlenmeyer flask.

Techniques for Adding One Drop or Less

Here are a few different techniques that can be used to add one drop or less of titrant to the Erlenmeyer flask.

1. Slowly open the stopcock and monitor for a single drop to fall out of the buret tip and then close the stopcock. This allows you to add exactly one drop.
2. Slowly open the stopcock and monitor for a single drop to emerge from the tip. Close the stopcock before the drop falls and then rinse the partial drop into your flask using a water squirt bottle. This allows you to deliver a volume that is smaller than a drop and should only be used when you are very close to the end point of the titration.
3. Quickly turn the stopcock half of a turn in one quick motion. Start with the stopcock closed and then rapidly turn the stopcock 180 degrees so that it is closed again.

Safety Considerations

Acids and bases can be harmful to both your skin and eyes.

Wear eye protection and wash hands regularly to avoid having harmful materials come into contact with your eyes.

If you spill acids or bases on your skin, immediately wash thoroughly with soap and water.

Disposal of Waste

All waste and excess reagents should be disposed of in the labeled waste container in the room.

Laboratory Activities

This week's lab is designed to help you learn, demonstrate, and improve on your titration techniques.

Part One

Show proper cleaning and filling of buret with water and read the buret appropriately: Each person should demonstrate how to do this to their laboratory partner. Your laboratory partner needs to sign-off in your notebook that it is done properly. When checking your lab partner look for the following items:

- **Is about 15 mL of liquid used to clean?**
- **Are inside walls coated with liquid and run out of tip?**
- **Is rinsing repeated at least twice?**
- **Is buret filled with water and meniscus is inside of volume markings?**
- **Is the tip of buret filled with water and free of air bubbles?**
- **Is the volume of buret recorded appropriately?**
- **After both lab partners have cleaned the buret with water, rinse and fill your buret with NaOH for the remaining parts of the experiment.**

Part Two

Determine the concentration of an HCl solution via a titration with 0.100 M NaOH. Here HCl is the analyte and NaOH is the titrant. Each laboratory partner should successfully complete one titration of the HCl solution following the general steps on the previous page. Use a 25.00 mL volumetric pipet to add the analyte to the Erlenmeyer flask. Check that your laboratory partner does the following when completing their titration:

- **Is analyte added to flask with volumetric pipet?**
- **Is indicator added to the flask?**
- **Is the initial volume of buret recorded to 2 decimal places in notebook?**
- **Does the solution turn color with the addition of one drop or less of titrant solution?**
- **Is the final volume of buret recorded to 2 decimal places?**
- **Is waste solution appropriately disposed of?**

Sample Data Table for Part Two

- **Volume of HCl Added to Flask (mL):**
- **Molarity of NaOH (M):**
- **Initial Volume Reading of Buret (mL):**
- **Final Volume Reading of Buret (mL):**
- **Volume Added (mL):**
- **Moles of NaOH Added (moles):**
- **Moles of HCl in Flask (moles):**
- **Molarity of HCl (M):**

Part Three

Determine the concentration of an H_2SO_4 solution via titration with 0.100 M NaOH. Here H_2SO_4 is the analyte and NaOH is the titrant. Each laboratory partner should successfully complete one titration of the H_2SO_4 solution following the general steps on the previous page. Use a 10.00 mL volumetric pipet to add the analyte to the Erlenmeyer flask. Add additional water to the flask to bring the total volume to somewhere between 25 and 50 mL. Check that your laboratory partner does the following when completing their titration:

- **Is analyte added to flask with volumetric pipet?**
- **Is the indicator added to the flask?**
- **Is the initial volume of buret recorded to 2 decimal places?**
- **Does the solution turn color with the addition of one drop or less of titrant solution?**
- **Is final volume of buret recorded to 2 decimal places?**
- **Is waste solution appropriately disposed of?**

Sample Data Table for Part Three

- **Volume of H_2SO_4 Added to Flask (mL):**
- **Molarity of NaOH (M):**
- **Initial Volume Reading of Buret (mL):**
- **Final Volume Reading of Buret (mL):**
- **Volume Added (mL):**
- **Moles of NaOH Added (moles):**
- **Moles of H_2SO_4 in Flask (moles):**
- **Molarity of H_2SO_4 (M):**

Calculations

1. **Determine volume of titrant added.** This is determined by subtracting the final volume of a titration from the initial volume of the titration.

2. **Determine moles of titrant added.** This is determined by multiplying the molarity of the titrant by the volume in liters of the titrant found in the previous calculation.

3. **Determine the moles of analyte in the Erlenmeyer flask.** This is determined by using the moles of titrant added in the previous calculation and multiplying it by the appropriate stoichiometric ratio using the balanced equation. You need to write the balanced equations to appropriately answer this question. The products of the reaction in Part 2 are H_2O and NaCl. The products of the reaction in Part 3 are H_2O and Na_2SO_4.

4. **Determine the molarity of the analyte that was titrated.** This is determined by taking the moles of analyte in the previous calculation and dividing it by the volume in liters of analyte pipetted into the Erlenmeyer flask.

Pre-Lab Questions

1. How do you avoid parallax errors when reading scientific glassware?

2. What is the role of an indicator in a titration?

3. How many decimal places should be included in your reading of the buret?

4. What are the balanced chemical equations for HCl reacting with NaOH and H_2SO_4 reacting with NaOH?

5. In the part 3 titration if the initial volume reading is 1.25 mL and the final volume reading is 23.81 mL, what is the concentration of H_2SO_4 in the solution? Remember that the molarity of NaOH is 0.100 M and that you need to make sure to consult the balanced equation.

Post-Lab Questions

1. What were the concentrations of HCl and H_2SO_4 in the two solutions?

2. We used a 25.00 mL volumetric pipet for the HCl and a 10.00 mL volumetric pipet for the H_2SO_4. Why do you think we used different volumes for the two titrations when the molarities of the two acids were similar?

3. If you were going too quickly as the equivalence point was reached and accidentally added more than a drop to cause the color to change, how would that error impact your calculated molarity of the acid (too high, too low, no change)? Explain the logic for your answer.

4. If you did not rinse your buret with NaOH before filling it, how would that impact the actual molarity of the NaOH in the buret?

5. What part of performing the titration did you find most difficult? We will be performing titrations again the next two weeks. What tips/tricks do you want to make sure to remember or get additional help with for the next lab?

References

John C. Goeltz and Lia A. Cuevas. "Guided Inquiry Activity for Teaching Titration Through Total Titratable Acidity in a General Chemistry Laboratory Course." *Journal of Chemical Education*, 2021, *98* (3), 882-887. DOI: 10.1021/acs.jchemed.0c01198.

What Is the Acidity of Vinegar? - Exploration Laboratory

Background

We are tasked with determining two aspects of vinegar. The first is determining how the acetic acid (CH_3COOH) concentrations compare between different brands of vinegar. In other words, can I swap one brand of white-distilled vinegar for a different brand of white-distilled vinegar and expect to see the same results? The second question is whether the labels are correct on bottles of vinegar. In other words, are the manufacturers truthful with their labeling?

Vinegars are labeled with the percent of their acidity. Acetic acid is the only acid in white-distilled vinegars. Percent acidity is a weight-to-weight percent, meaning that if a vinegar is labeled as 5% acidity, then it has 5 grams of acetic acid for every 100 grams of solution.

Figure 7.1: "Vinegar" by HomeSpot HQ is licensed under CC BY 2.0.

Experiment

Acetic acid reacts with sodium hydroxide to make water and sodium acetate. It is an ideal candidate for using the titration techniques we practiced last week.

This is a two-week lab where you are tasked with answering the two questions above. In the first week of the laboratory, you will plan your experiments and start to carry them out. In the second week of the laboratory, you will finish carrying out data collection and analyzing the data to answer the two questions posed above.

Available Materials

0.100 M NaOH; burets; 100 mL volumetric flasks; 5, 10, and 25 mL volumetric pipets; phenolphthalein; three different vinegars.

Data Collection

When collecting data, keep everything well organized and labeled. Think about the experiments you will be performing. What data will need to be recorded? Set up tables for yourself to keep the data organized.

We want to be able to quantify both the value and the repeatability of your measurements (relative average deviation). You should have at least three trials for each of the three vinegar samples.

Additional Considerations

Think back to last week's titrations. We said that titrations should take between 10 and 50 mL of titrant. That means if we are using 0.100 M NaOH, that we can deliver between 0.00100 moles and 0.00500 moles of NaOH to create a quality titration. Therefore, the moles of acid in the analyte in the Erlenmeyer flask must be completely reacted with somewhere between 0.00100 and 0.00500 moles of NaOH.

Commercial vinegar samples contain roughly 5% by weight of acetic acid. If we assume the solution has a density of 1.0 g/mL, then the approximate molarity of acetic acid in vinegar is

$$\frac{5\ g\ CH_3COOH}{100\ g\ Solution} \times \frac{1.0\ g}{1\ \text{mL}} \times \frac{1000\ \text{mL}}{1\ L} \times \frac{1\ \text{mol}\ CH_3COOH}{60.05\ g\ CH_3COOH} = 0.83\ M\ CH_3COOH$$

Think about this when determining how much acetic acid solution to use. If you have too many moles of acid, you can either use a smaller volume of the acid or dilute the acid in a volumetric flask and then titrate the diluted acid.

Safety Considerations

Acids and bases can be harmful to both your skin and eyes.

Wear eye protection and wash hands regularly to avoid having harmful materials come into contact with your eyes.

If you spill acids or bases on your skin, immediately wash thoroughly with soap and water.

Disposal of Waste

All waste and excess reagents should be disposed of in the labeled waste container in the room.

Pre-Lab Questions

1. What is the balanced chemical equation for the reaction of acetic acid with sodium hydroxide?
2. If you perform a titration that uses 25.00 mL of 5% acetic acid and 0.100 M NaOH, how many mL of NaOH will be required to reach the equivalence point.
3. In the example calculation on the previous page we assumed that the density is 1.0 g/mL. You determined the density of a solution in a previous lab. What was the procedure you used to make that determination?
4. Look back at previous labs. What are the safety considerations you should use for this lab? Write out an appropriate safety precautions section based on your past experience.

Post-Lab Questions

1. What are the average molarities of acetic acid in each of the vinegars?
2. What are the percent acidities of acetic acid in each of the vinegars?
3. What are the relative average deviations of the percent acidities for each of the vinegars?
4. How do the vinegars compare with regards to the acid content? Answer this question using your data and explaining your conclusions.
5. Are the labels on the vinegars truthful? Answer this question using your data and explaining your conclusions.
6. Is there a relationship between the cost of a vinegar and its acid content?
7. In recipes is it appropriate to substitute one vinegar for a different vinegar? Answer this question using your data and explaining your conclusions.

References

John C. Goeltz and Lia A. Cuevas. “Guided Inquiry Activity for Teaching Titration Through Total Titratable Acidity in a General Chemistry Laboratory Course.” *Journal of Chemical Education*, 2021, *98* (3), 882-887. DOI: 10.1021/acs.jchemed.0c01198.

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Absorption Spectroscopy Technique Laboratory

Absorbance Spectroscopy Background

We saw previously how to use a titration to quantitatively determine the amount of a compound of interest in a sample. Here we will learn about absorbance spectroscopy, which is another widely used technique for quantitative analysis. One example of an application where absorbance spectroscopy can be used is in determining the nitrate concentration in water. Large amounts of nitrate can enter waterways via run-off from fertilizer application in farming. High concentrations of nitrate in drinking water can lead to deleterious health impacts. Excessive nitrate in water has also contributed to harmful algae blooms in lakes. The western basin of Lake Erie is one example where algae blooms have contributed to drinking water problems.

Figure 8.1: "Harmful algae bloom. Kelley's Island, Ohio. Lake Erie" by NOAA Great Lakes Environmental Research Laboratory is marked with Public Domain Mark 1.0.

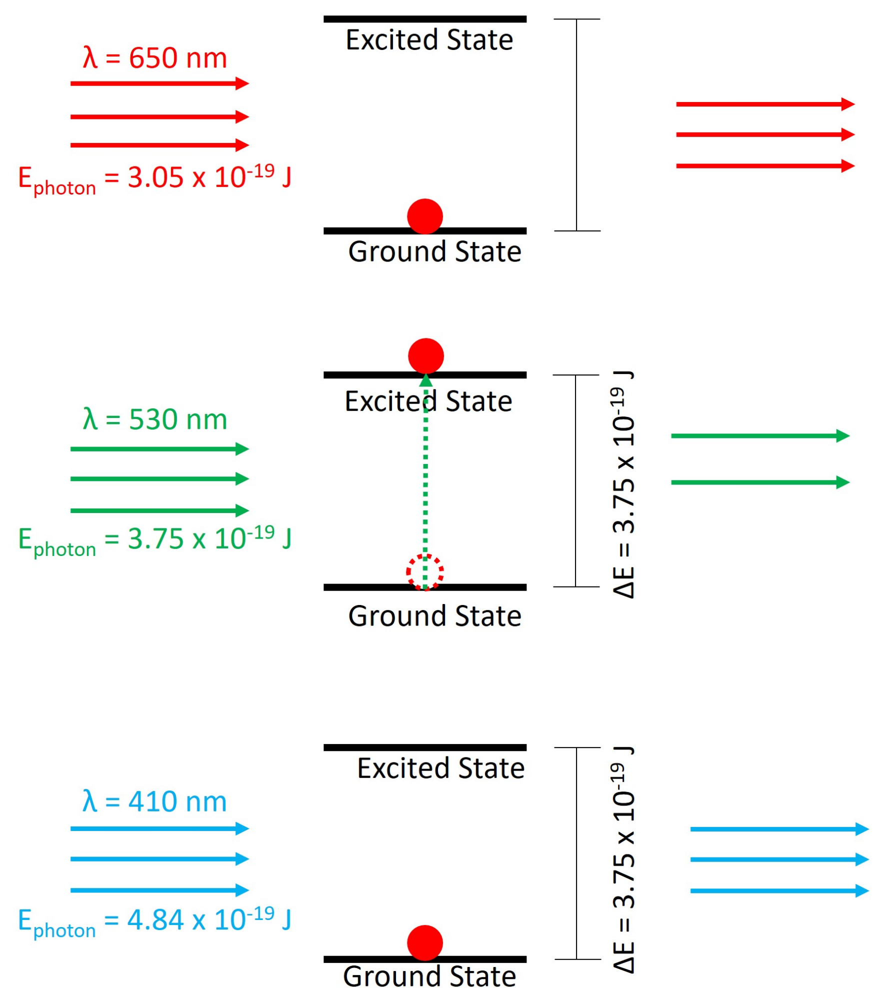

Figure 8.2: Energy level diagram that shows how different wavelengths of light interact with a molecule.

Absorbance spectroscopy uses the quantity of light that passes through a sample to determine how much of a particular substance is in a mixture. The use of light to quantify the amount of chemical is beneficial because it allows for remote sensing where we can determine quantities of different species even if they are in other solar systems. The basic idea is that atoms and molecules can absorb light when the energy of the photon of light is

equal to some energy level difference in the atom or molecule. In figure 8.2 the red light does not have enough energy to excite the electron to the higher energy level. The blue light has too much energy and therefore does not excite the electron to the higher energy level. But the green light has exactly the right amount of energy to excite the electron to the higher energy level. This molecule will absorb green light but will not absorb red or blue light.

Ultraviolet-Visible Absorbance Spectroscopy

Many different wavelengths (IR, microwave, x-ray, etc.) of light can be used in absorbance spectroscopy. One of the most widely used wavelength ranges for absorbance spectroscopy is light in the ultraviolet and visible (UV-Vis) regions of the electromagnetic spectrum. The UV-Vis region has energies of photons that are often similar to energies required to promote an electron from one energy level to another energy level. In UV-Vis absorption spectroscopy we measure how much light passes through a sample at all of the different wavelengths.

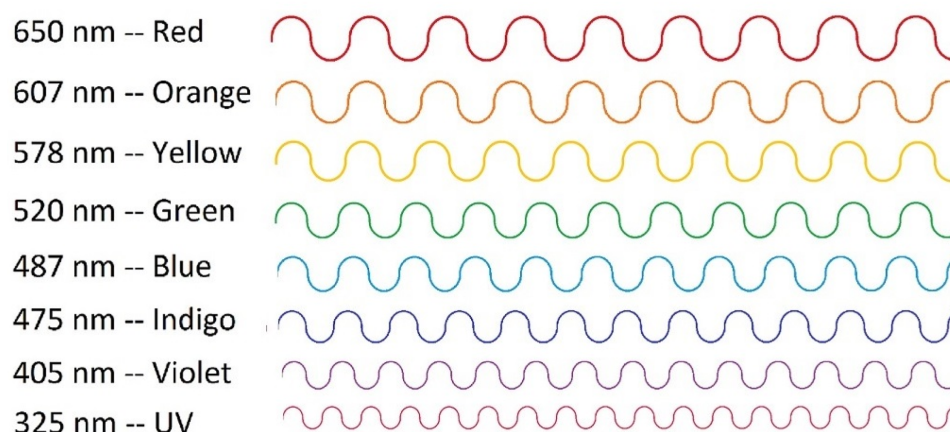

Figure 8.3: Derived from "File:Light wave harmonic diagram.svg" by Rubber Duck (☮ • ✍) is licensed under CC BY-SA 3.0.

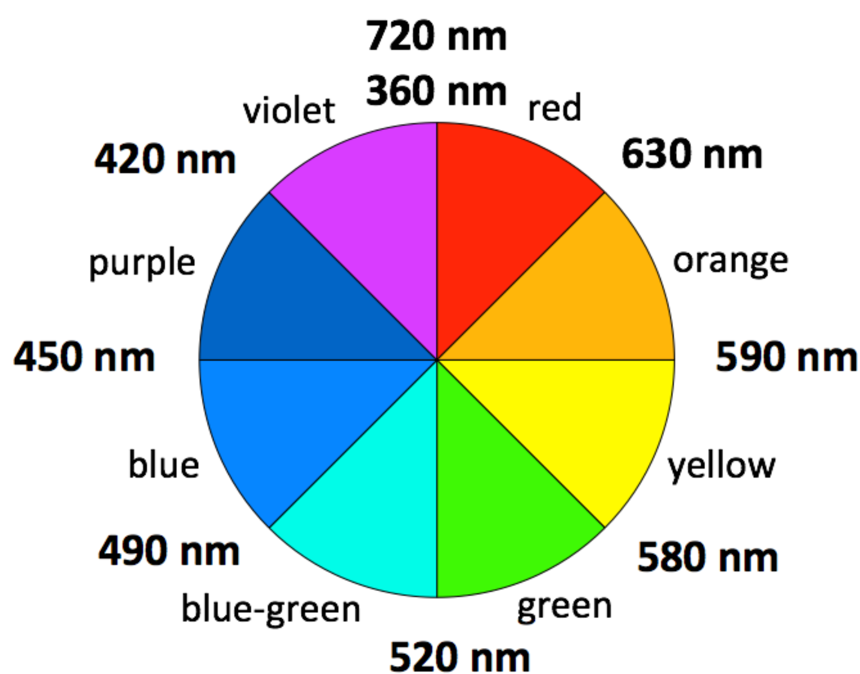

Figure 8.4: "File:Color wheel wavelengths.png" by Tem5psu is licensed under CC BY-SA 4.0.

If we look at a liquid sample and observe it to have a specific color with our eye, the light that is being absorbed by the sample is the complement of that color on a color wheel. Using figure 8.4 as a guide, we can demonstrate how this works. A solution that appears yellow to our eye, for example, is absorbing the complementary color purple. We would expect a yellow solution to show strong absorbance for photons near 435 nanometers.

UV-Vis Spectrophotometer

A UV-Vis spectrophotometer is a widely used instrument to take absorbance measurements. There are many commercial manufacturers of these instruments, but they all have the same basic working components: a light source, a way to select the wavelength of light, a sample holder, and a way to detect the amount of light.

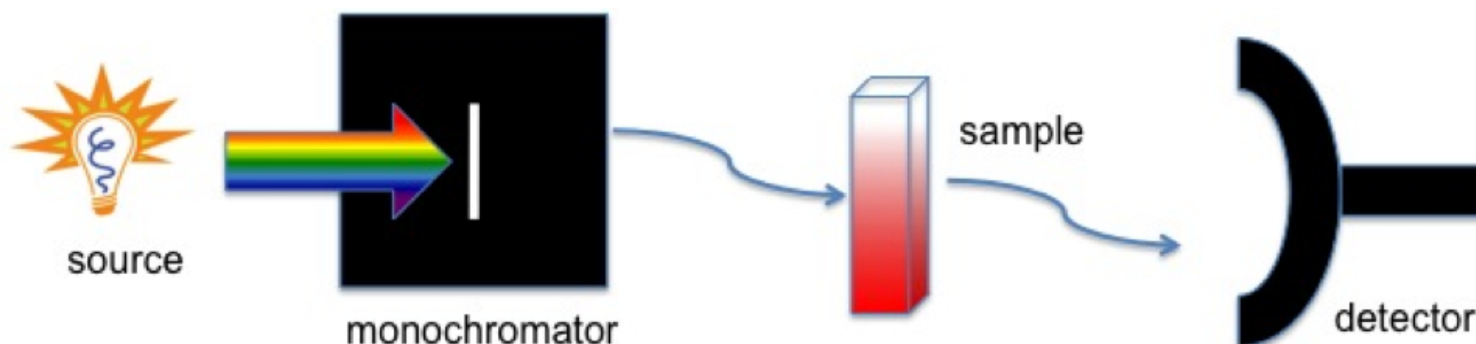

Figure 8.5: 4.4: UV-Visible Spectroscopy is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by Pavan M. V. Raja & Andrew R. Barron (OpenStax CNX).

The light source, wavelength selector, and light detector are all typically controlled by the instrument's computer interface, while the specifics will depend on the instrument being used. The typical sample holder for UV-Vis absorption spectroscopy experiments is a cuvette. A cuvette is small container - typically made of quartz, glass, or plastic - with a known width (commonly 1 cm). If measurements are being done in the visible region, then glass or plastic will work well, but quartz must be used if measurements are performed in the ultraviolet region of the spectrum. Proper care and use of a cuvette are two of the most important steps for achieving accurate and reliable results in UV-Vis spectroscopy.

Use of a Cuvette

The entire technique relies upon how much light makes it through the sample. The light passes through the walls of the cuvette and the sample before being detected by the instrument. This makes the walls of the cuvette a critical part of the measurement. Think about trying to look through glasses that have a fingerprint on them. The amount of light that reaches your eye changes because of the presence of the fingerprint! When working with the cuvette our goal is to minimize the impact of the cuvette walls on the measurement and to make the impact from the walls consistent for each measurement. To accomplish this, use the following guidelines:

1. Choose a cuvette that is free from scratches.
2. Always use the same cuvette for all your measurements.
3. When handling the cuvette, avoid touching the surfaces the light will pass through. Many cuvettes will have frosted sides and clear sides. Handle the cuvette on the frosted sides and make sure the light goes through the clear sides.
4. When cleaning, rinse the cuvette a few times with the solution you are about to fill it with. This minimizes the impact of residual solution left in the cuvette.
5. Only use non-abrasive materials when cleaning the cuvette (tissues and other items that don't scratch are the best to use for cleaning).
6. Make sure to fill the cuvette so that the light is going through the solution. Typically filling to three-quarters full is sufficient.
7. Wipe any drips or liquid from the outside of the cuvette using a soft tissue.
8. Always put the cuvette into the instrument with the same orientation. Typically, there is a mark on the cuvette, and you can always ensure that the mark is facing the same direction.

Blanking the Instrument

The entire technique is built on measuring how much light makes it through the sample. This requires us to know how much light would make it to the detector if our sample were not present. To accomplish this, we set a reference point that we will call the "blank," which is how much light reaches the detector when a cuvette contains everything except the compound of interest. Often pure water is sufficient to be used as a blank for simple measurements.

When blanking the instrument, we measure how much light reaches the detector and then set that amount of light to 100% transmission. That means if the detector measures that specific amount of light, then we can assume all of the light made it through at that particular wavelength. If the sample absorbs some of the light at that wavelength, then we should measure a smaller amount of light reaching the detector.

It is important to note that a separate blank measurement is required for each wavelength because the light source gives off different amounts of light at each wavelength.

Beer's Law

The key equation for making UV-Vis absorption experiments quantitative is Beer's Law:

$$A = \epsilon \cdot l \cdot c$$

where *A* is the absorption of the sample, *ε* is the molar absorptivity, *l* is the path length of the sample, and *c* is the concentration of the absorbing molecule in the sample.

The molar absorptivity, *ε*, is a measure of how well a particular molecule absorbs a particular wavelength of light. The wavelengths where a molecule absorbs large amounts of light have high molar absorptivity, and the wavelengths where the molecule does not absorb light have zero molar absorptivity. The molar absorptivity is a property of a molecule and changes as the wavelength changes. Typical units of molar absorptivity are $\frac{1}{cm \cdot M}$.

The path length, *l,* is determined by the sample holder and is usually consistent from one measurement to the next. In our experiments the path length is determined by what cuvette is used. This will typically be 1 cm for most UV-Vis spectrophotometers.

The concentration, *c,* is typically expressed as a molarity. It is the concentration of the molecule that is absorbing the light.

The absorbance, *A*, is a measure of how much light made it through the sample. It is calculated by taking the negative log base 10 of the fraction of light that made it through the sample at a certain wavelength.

We can consider a few scenarios to understand how absorbance is calculated. If 50% of the light makes it through the sample - meaning that when we measure the sample half as much light comes through as compared with the measurement of the blank - then the absorbance would be equal to -log(0.50)=0.30. If 100% of the light makes it through the sample, then the absorbance would be equal to -log(1)=0. Absorbance is a logarithmic scale, which means that when the absorbance doubles, the amount of light making it through the sample is 10 times smaller! An absorbance of 1 means that 10% of the light made it through the sample. An absorbance of 2 means that 1% of the light made it through the sample.

Because of measurement limitations, it is usually best to keep absorbances between 0 and 1 to avoid errors.

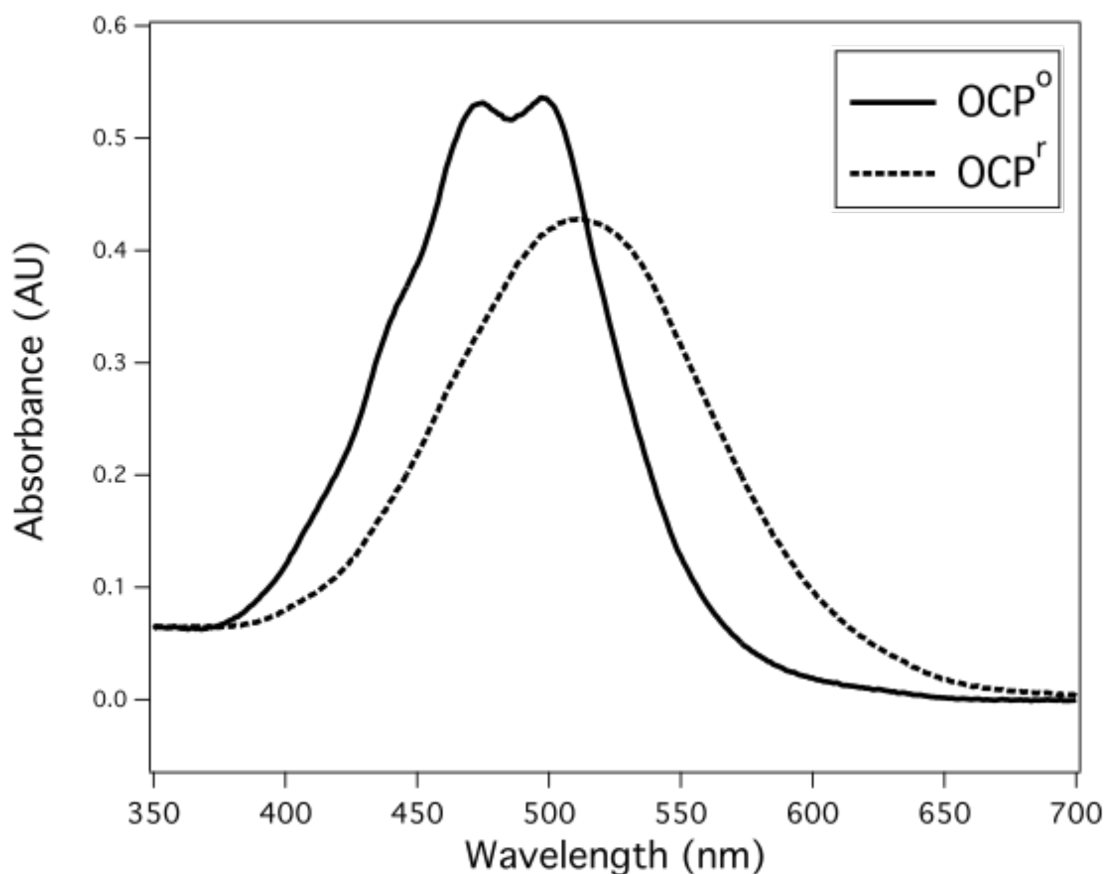

Figure 8.6: "File:Orange Carotenoid Protein spectra of orange vs red form.svg" by Ryan Leverenz and Cheryl Kerfeld is licensed under CC BY-SA 4.0.

Absorbance Spectrum

The absorbance spectrum of a solution is a measure of how much light that solution absorbs at each wavelength. Figure 8.6 shows absorbance spectra for an orange and red forms of orange carotenoid protein.

This absorbance spectrum is taken by measuring the absorbance of the solution at different wavelengths while keeping the path length and concentration constant. If we look at the dotted line (red form) in the figure, we see that the solution has zero absorbance between about 680 and 700 nm, which means that all of the light at those wavelengths pass through the sample.

We will typically be interested in measuring the wavelength of maximum absorbance, which is the wavelength where the absorbance has its largest value. This is the location where molar absorptivity, ε, of the compound is the largest. The wavelength of maximum absorbance for the dotted line in Figure 8.6 is about 525 nm.

If we change the concentration of our sample, the shape of the curve does not change because the shape of the curve is determined by the identity of the compound through its molar absorptivity, ε. When we change the concentration all the points on the absorbance spectrum curve move up or down by the same factor. If the solution was diluted so the concentration is half of the original, then all the absorbance values would just be half as big. Using Figure 8.6 as an example, if the concentration was half of the original, the wavelength of maximum absorbance would still be 525 nm, but the absorbance would now be about 0.2.

Calibration Curve

In most cases we are trying to measure the absorbance of an unknown solution to determine the concentration of a molecule in the solution. The best way to accomplish this is to measure the absorbance spectra of solutions where we know the concentrations. These measurements then allow us to construct a graph for how concentration and absorbance are related.

When we create this graph, it is important to use the wavelength of maximum absorbance because that will give us the biggest change in signal as the concentration changes, and it is also insensitive to small wavelength changes.

In constructing a calibration curve for the red form of orange carotenoid protein, we would keep the measuring wavelength the same (525 nm) and measure how the absorbance changes at this wavelength as we change the concentration. This would lead to a graph like Figure 8.7

Here the data points represent the absorbances at each concentration, and the dotted line represents a line of best fit with the equation of the line displayed on the graph.

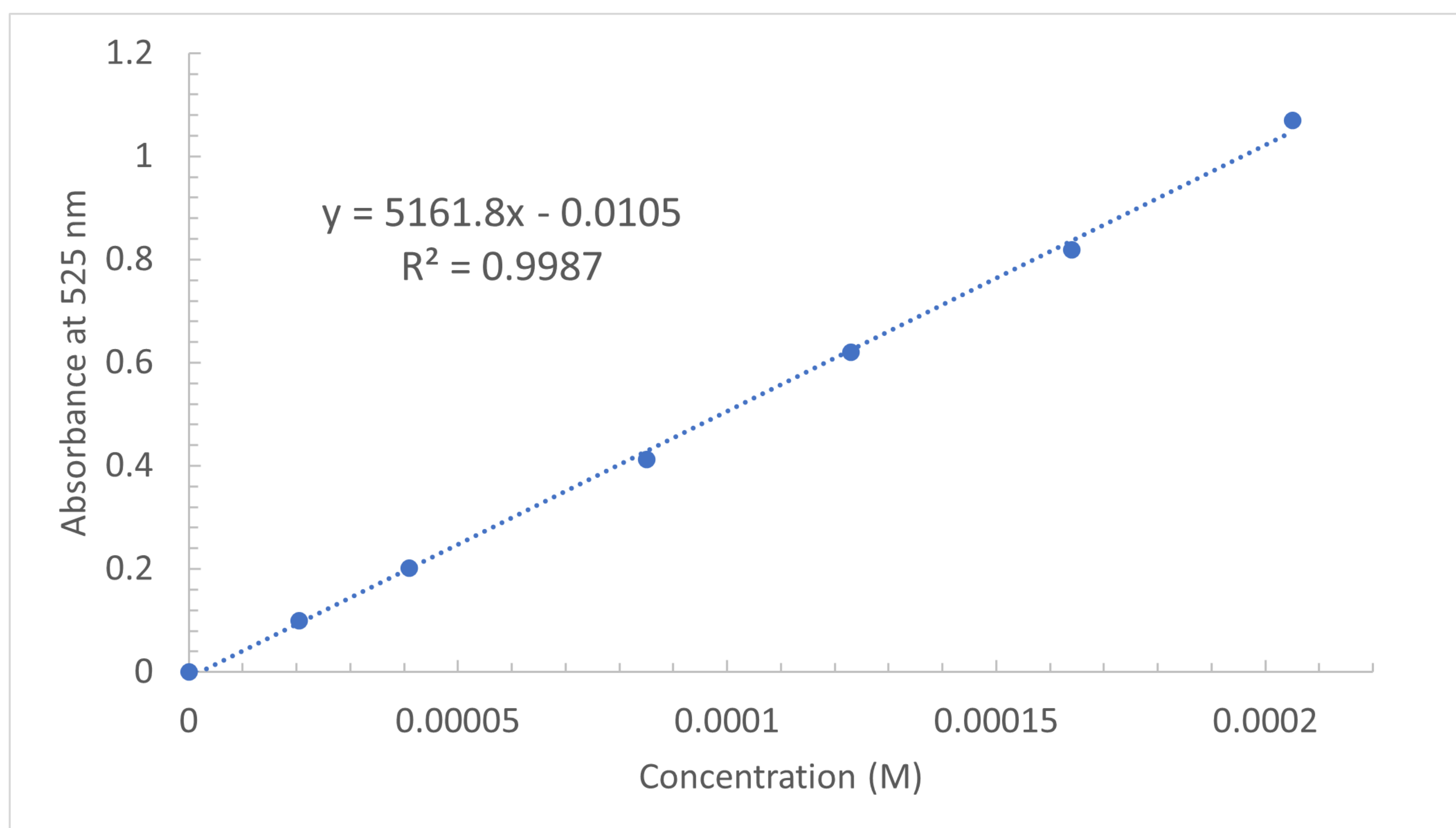

Figure 8.7: Example calibration curve for the red form of orange carotenoid protein. The line of best fit for the data is $y = 5161.8x - 0.0105$ with $R^2 = 0.9987$. The raw data is available as a supplementary resource.

This calibration curve graph and the equation for the line now allow us to determine the concentration of an unknown sample by measuring just its

absorbance at 525 nm. In the equation for the line, the y-variable represents the absorbance, and the x-variable represents the concentration.

Measuring a Solution of Unknown Concentration

If we take and measure an unknown solution and find that it has an absorbance of 1.5 at a wavelength of 525 nm, then we can use that information to determine the concentration. One important point we should always keep in mind is that we want the measurement of the unknown to fall in between our lowest and highest measurements. In this example, an absorbance of 1.5 is higher than my highest point on the calibration curve. Since this is the case, I would dilute the unknown by taking say 10 mL of the unknown and diluting it to a new volume of 25 mL.

Now when I measured the absorbance of the diluted unknown, I get an absorbance of 0.587. This value can be used with my calibration curve because it falls within the measurements I have already taken. Now I can use the equation for the line to calculate the concentration of the diluted unknown.

$$0.587 = 5161.8x - 0.0105$$

Rearranging to solve for “x” which represents the concentration of the diluted unknown gives:

$$x = \frac{0.587 + 0.0105}{5161.8}$$

This gives that the concentration of the unknown sample is 0.000116 M. This number makes sense since I could approximate it by finding the point on the dotted line that has an absorbance of 0.587 and reading off the concentration.

In order to know the concentration of the original unknown, we must take into account the dilution using

$$M_1V_1 = M_2V_2$$

This example gives

$$M_1 = \frac{0.000116\ M \times 25.00\ \text{mL}}{10.00\ \text{mL}} = 0.000289\ M$$

General Procedure and Tips for UV-Vis Absorption Measurements

1. Turn on the instrument and provide ample time for the lamp to warm up and become stable (usually 10 minutes).
2. Find one cuvette to use for all measurements. Note the orientation, so you can consistently place it in the sample holder.
3. Rinse the cuvette multiple times with water and then fill it with the blank solution. We will use distilled water as the blank for this experiment.
4. Make sure the cuvette walls are free of interfering species (water drops, fingerprints, scratches, etc.)
5. Make sure the solution is clear. Remember: In chemistry a clear solution means one that is homogenous and free from solids or gas bubbles. The presence of solids or gas bubbles will scatter light and interfere with measurements. Cloudy is the opposite of clear. If your solution appears cloudy, you must make it clear before taking the measurement.
6. Place the cuvette into the instrument and blank the instrument. Make sure the orientation is correct.
7. Rinse the cuvette multiple times with the new solution that will be measured and then fill it with that solution.
8. Make sure the cuvette walls are free of interfering species (water drops, fingerprints, scratches, etc.).
9. Make sure the solution is clear. A green solution can be clear because clear does NOT mean colorless. See the note above in point 5.
10. Place the cuvette into the instrument and measure the absorbance. Make sure the orientation is correct.

Safety Considerations

Wear eye protection and wash hands regularly to avoid having harmful material come into contact with your eyes.

Disposal of Waste

All waste and excess reagents should be disposed of in the labeled waste container in the room.

Laboratory Activities

This week's lab is designed to help you learn, demonstrate, and improve on your UV-Vis absorption spectroscopy techniques.

Part One

Measure the absorbance spectrum of the standard solution of Red Dye Number 40 for wavelengths from 400 to 700 nm. Use the provided solution of known concentration of Red Dye Number 40 to measure the absorbance spectrum.

Sample Data Table for Absorbance Spectra of Dye Solution

Wavelength (nm)	Absorbance
400	
425	
450	
475	
500	
525	
550	
575	
600	
625	
650	
675	
700	

The data table can be downloaded from Google Drive: Sample Data Tables.

Record the wavelength of maximum absorbance. This wavelength is probably not one of the wavelengths listed in the table above.

Wavelength of Maximum Absorbance (nm):

Part Two

Make a dilution of the dye solution of known concentration, so that the concentration of the dye is half of the original concentration. Perform this dilution, using volumetric pipets and volumetric flasks.

- **Volume of Original Dye Solution Used (mL):**
- **Molarity of Original Dye Solution (M):**
- **Volume of Volumetric Flask for Diluted Dye Solution (mL):**
- **Molarity of Diluted Dye Solution (M):**

Measure the absorbance spectrum of the diluted dye solution.

Sample Data Table for Absorbance Spectra of Diluted Dye Solution

Wavelength (nm)	Absorbance
400	
425	
450	
475	
500	
525	
550	
575	
600	
625	
650	
675	
700	

The data table can be downloaded from Google Drive: Sample Data Tables.

Record the wavelength of maximum absorbance. This wavelength is probably not one of the wavelengths listed in the table above.

Wavelength of Maximum Absorbance (nm):

Part Three

Prepare four additional diluted solutions. The most dilute solution you prepare should be 1/10th of the concentration of the original solution. Measure the absorbance values of all these solutions at the wavelength of maximum absorbance.

Sample Data Table for Measuring Absorbance Values for Dilutions

Solution Number	Volume of Original Dye Solution Used (mL)	Volume of Diluted Dye Solution (mL)	Molarity of Diluted Dye Solution (M)	Absorbance at wavelength of maximum absorbance
1				
2				
3				
4				

The data table can be downloaded from Google Drive: Sample Data Tables.

Part Four

Measure the absorbance spectrum for the solution of unknown dye concentration.

Absorbance Value at λ_{max} for Unknown Dye Solution:

This absorbance is likely higher than any of the solutions you have previously measured. To determine the concentration of the unknown solution accurately, we will need to dilute it and then measure the absorbance value of the diluted solution. Pick the volumes for dilution based on what you found above.

- **Volume of Unknown Dye Solution Used (mL):**
- **Volume of Volumetric Flask for Diluted Unknown Dye Solution (mL):**
- **Absorbance Value at λ_{max} for Diluted Unknown Dye Solution:**

Calculations

1. Determine the molarities of all the solutions in parts one, two, and three. Do this by using $M_1V_1 = M_2V_2$

2. **Make a calibration curve with a line of best fit.** Make a scatter plot with the absorbance values at λ_{max} on the y-axis and the concentrations on the x-axis, using the data from parts 1 – 3. Use a spreadsheet program to draw a line of best fit and display the equation for the line and the R^2 value on the graph.

3. **Determine the concentration of dye in the diluted unknown solution.** Use the absorbance value for the diluted unknown dye solution and the line of best fit to determine this value.

4. **Determine the concentration of dye in the original unknown solution.** Use the concentration of dye in the diluted unknown solution and the volumes used in part 4 along with the dilution equation to make this determination.

Pre-Lab Questions

1. How are absorbance and the transmission of light through the sample related? As the % transmission decreases, what happens to the absorbance?

2. What is the purpose of the blank in a UV-Vis absorption experiment?

3. Copper solutions have a blue appearance. At what wavelengths of light do you expect a copper solution to show maximum absorbance?

4. A solution originally has a concentration of 1.3×10^{-4} M. That solution is diluted by taking 2.0 mL of it and diluting to a volume of 10.00 mL with a volumetric flask. What is the concentration of the diluted solution?

5. Using the graph in figure 7, if a solution of the red form of orange carotenoid protein measured an absorbance of 0.313 at 525 nm, what is the concentration of the protein in the solution?

Post-Lab Questions

1. What color of light shows the maximum absorbance by the dye? What color of light shows the smallest absorbance by the dye?

2. Compare the absorbance spectra data in parts 1 and 2. Does the data demonstrate Beer's Law? Explain why or why not?

3. If you accidentally left a fingerprint on the cuvette when measuring the absorbance of the unknown diluted solution, how would that impact your determination of the concentration of the unknown? Explain your answer.

4. Why do we measure at the wavelength of maximum absorbance when preparing the calibration curve and not the wavelength of minimum absorbance?

5. What parts of UV-Vis absorption spectroscopy did you find most difficult? We will be using this technique again the next two weeks. What tips/tricks do you want to make sure to remember or get additional help with for the next lab?

References

Sigman SB and Wheeler DE. "The Quantitative Determination of Food Dyes in Powdered Drink Mixes. A High School or General Science Experiment." *Journal of Chemical Education*, 2004, 81: 1475–1478. DOI: 10.1021/ed081p1475.

Thermo Scientific "Food Dyes and Beer's Law" Lesson Plan, 2019, FL53099, https://assets.thermofisher.com/TFS-Assets/MSD/Scientific-Resources/FL53099-food-dyes-beers-law-qc-lesson-plan.pdf Accessed December 13, 2022.

What Is the Dye Composition of a Drink?- Exploration Laboratory

Background

There are many naturally occurring and artificial dyes approved for human consumption by the United States Food and Drug Administration. In this lab you will determine how many milligrams of certain dyes are present in a powdered drink mix. We will focus our attention on three dyes that are commonly used: Yellow Dye 5, Red Dye 40, and Blue Dye 1. Ultimately you will determine how much dye you are ingesting if you drink one serving of the drink. A rival company wants to know what dyes (Yellow 5, Red 40, or Blue 1) are in the powdered drink mix and how much of each dye is in the powdered drink mix.

Figure 9.1: "Kool Aid ready" by Andrea Black (Lacuna) is licensed under CC BY-SA 2.0.

Experiment

Because we are trying to quantify molecules that have specific colors, this aim makes using UV-Vis spectrophotometry an ideal tool.

This is a two-week lab where you are tasked with answering the questions above. In the first week of the laboratory, you will plan your experiments and start to carryout experiments. In the second week of the laboratory, you will finish carrying out data collection and analyzing the data to answer the questions.

Available Materials

Stock solutions of known concentrations of Yellow Dye 5, Red Dye 40, and Blue Dye 1, Mohr pipets, 10 mL and 100 mL volumetric flasks, powdered drink mixes, and spectrophotometers.

Data Collection

When collecting data, keep everything well organized and labeled. Think about the experiments you will be performing. What data will need to be recorded? Set up tables for yourself to keep the data organized.

Additional Considerations

Think back to last week's measurements. We measured the absorbance spectrum and created a calibration curve for a single dye.

One complication when two dyes are present is that the absorbances from the dyes can overlap. Thus, we need to correct for the overlap since the absorbance at a wavelength of maximum absorbance can come from two sources (each dye). Each dye will have its own wavelength of maximum absorbance, but then it may also have some absorbance at the maximum of the other dye and interfere.

The easiest way to correct for this is to look at the absorbance spectra of the pure dyes by themselves to find the relationships between the two wavelengths. For example, Yellow Dye 5 has a maximum absorbance near 425 nm and Red Dye 40 has a maximum absorbance near 505 nm. Unfortunately, Yellow Dye 5 also has a small absorbance at 505 nm and Red Dye 40 has an absorbance at 425 nm.

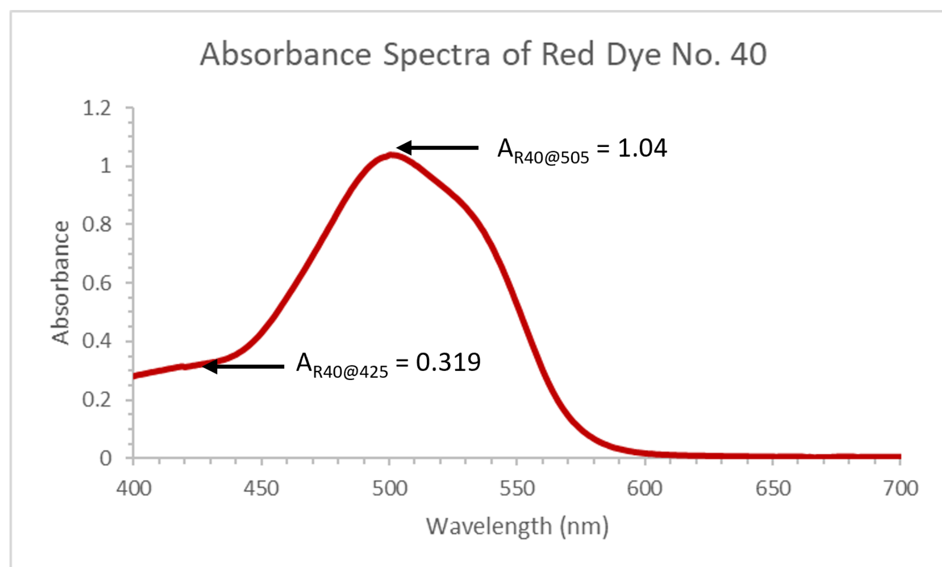

Figure 9.2: Absorbance spectra of Red Dye No. 40 highlighting important analysis locations. The raw data is available as a supplementary resource.

If both dyes are present, then an absorbance at 425 nm (A_{425}) comes from two sources: yellow dye ($A_{Y5@425}$) and red dye($A_{R40@425}$). The absorbance at 505 nm (A_{505}) will also come from two sources: red dye ($A_{R40@505}$) and yellow dye ($A_{Y5@505}$).

$$A_{425} = A_{Y5@425} + A_{R40@425}$$

$$A_{505} = A_{Y5@505} + A_{R40@505}$$

By looking at the absorbance spectra of the pure dyes, we can see the relationship between the absorbance peak and the interfering wavelength. Figure 9.2 shows the absorbance spectra for FD&C Red No. 40. We can see that the absorbance at the peak of 505 nm is about 1.04 while the absorbance at 425 nm is about 0.319. When correcting for the interference between two dyes, what we need is the ratio of the absorbances at those two wavelengths. The ratio of the absorbances is 0.319/1.04 = 0.31.

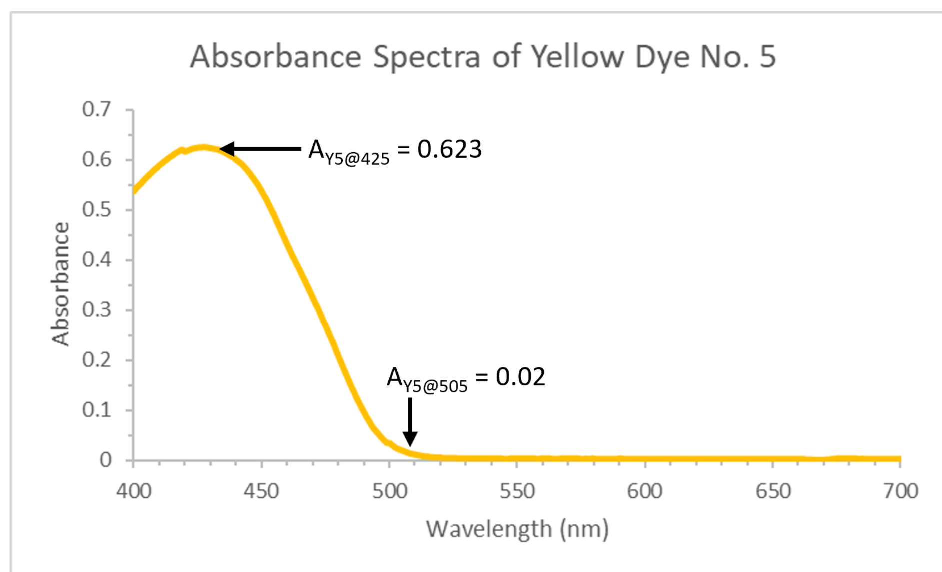

Figure 9.3: Absorbance spectra of Yellow Dye No. 5 highlighting important analysis locations. The raw data is available as a supplementary resource.

The red dye absorption value at 425 nm is 31% of what the absorption is at the peak (505 nm). If we do a similar analysis, the yellow dye absorption at 505 nm is about 3% of what the absorption is at the peak (425 nm).

This gives us these equations:

$$A_{R40@425} = (0.31) \cdot A_{R40@505}$$

$$(0.03) \cdot A_{Y5@425} = A_{Y5@505}$$

With some algebra, this allows us to determine the absorbances from each individual dye at their peak wavelengths by measuring the absorbances at both wavelengths. For the absorbance for yellow dye at the peak of 425 nm that would give

$$A_{Y5@425} = \frac{A_{425} - 0.31 \cdot A_{505}}{1 - (0.03) \cdot (0.31)}$$

For the absorbance for red dye at the peak of 505 nm that would give

$$A_{R40@505} = \frac{A_{505} - 0.03 \cdot A_{425}}{1 - (0.03) \cdot (0.31)}$$

These two values, $A_{Y5@425}$ and $A_{R40@505}$, are the absorbance values at those wavelengths that come from the specific dye and would be used in determining

concentrations of an unknown using the calibration curves of pure dyes as you did in the first UV-Vis lab.

You will need to determine the exact values of the ratios (3 and 31% in this example) using your data, but the forms of the equations are identical, and you just need to swap in whatever you find as the ratios in place of 0.03 and 0.31.

To determine masses of dyes in the drink mixes, you will also need to use the molar masses of the dyes. The molar mass of FD&C Red No. 40 is 496.42 g/mol. The molar mass of FD&C Yellow No. 5 is 534.39 g/mol. The molar mass of FD&C Blue No. 1 is 792.85 g/mol.

Safety Considerations

Wear eye protection and wash hands regularly to avoid harmful materials having contact with your eyes.

Disposal of Waste

All waste and excess reagents should be disposed of in the labeled waste container in the room.

Pre-Lab Questions

1. Predict the wavelengths of maximum absorbance of red dyes, blue dyes, and yellow dyes based on your knowledge of complementary colors.
2. If a 100.0 mL solution has a concentration of 1.0×10^{-5} M of Blue Dye No.1, how many milligrams of the dye are in the solution?
3. For a certain packet of Kool-Aid, the serving size is 8 grams of the drink mix. In the question above, if you used 2 grams of drink mix to make the 100.0 mL solution, how many milligrams of Blue Dye No. 1 would you consume in a single serving?
4. Use the equations above, correcting for interference between Yellow No. 5 and Red No. 40 dyes. You measure a drink mix that contains Yellow No. 5 and Red No. 40. The absorbance at 425 nm is 0.65. The absorbance at 505 nm is 0.43. What are the corrected absorbance values that you would use to determine the individual dye concentrations when you use the calibration curves made for the pure dyes?

Post-Lab Questions

1. What dyes are present in your powdered drink mix?

2. What are the concentrations of the dyes in the drink mix you measured?

3. How many milligrams of each dye are in an entire packet of the drink mix?

4. If you drink one serving of the drink, how many milligrams of dye would you ingest?

5. Ingredients on a food label are listed in order from highest amount to lowest amount. Are your results consistent with the food label on your drink mix packet?

References

Sigman SB and Wheeler DE. “The Quantitative Determination of Food Dyes in Powdered Drink Mixes. A High School or General Science Experiment.” *Journal of Chemical Education*, 2004, 81: 1475–1478. DOI: 10.1021/ed081p1475.

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