Chemistry Techniques and Explorations

An Introductory Chemistry Laboratory Manual

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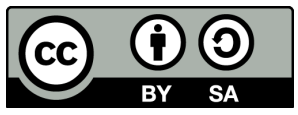

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About the Author

Dr. Daniel R. Albert is an Associate Professor of Chemistry at Millersville University of Pennsylvania. Dr. Albert is an experimental physical chemist who is engaged in research on understanding the impact of gas-liquid interactions on environmental processes. He is also engaged in chemical education research and develops inexpensive instrumentation for targeted applications and educational purposes.

Dr. Daniel R. Albert

Author Acknowledgements

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Goal of Laboratory Manual

The goal of this laboratory manual is to provide opportunities for introductory chemistry students to learn about and practice important techniques that will be utilized throughout their career. These opportunities will allow students to develop as practicing scientists. Another important aspect of becoming a practicing scientist is to develop new experiments in the laboratory. The exploration laboratory experiments provide freedom and time for students to use previously acquired skills toward answering a new question. Experiments identify whether the focus is technique or exploration. It is important to provide flexibility and time for student discussion during the exploration laboratories. All labs are designed for a two-hour laboratory session with the exploration laboratories extending over a two-week period.

Table of Contents

Safety and Record Keeping

Learning to work safely in a laboratory setting while also keeping accurate and appropriate records are key skills that we are developing during this course. Safely working in a laboratory means that we minimize risks for ourselves, other people in the laboratory and building, and for the environment.

Figure 1.1: "Dr. Adah Elizabeth Verder mouth pipetting" by National Institutes of Health (NIH) is marked with Public Domain Mark 1.0.

In first learning about safety in the laboratory we often encounter lists of guidelines for what we should and should not do in the laboratory. These guidelines are to help us build a baseline for understanding and mitigating risk in teaching laboratories with well-established experiments. One of the safety rules is to not “Pipet by Mouth.” What do you think the purpose of this safety rule is?

Safety Rules

Here are some examples of standard safety rules for teaching laboratories. To aid in understanding these rules, you will be discussing with others in the laboratory what you think their purposes are.

- **Never leave chemical containers open.**
- **Label all containers with what is inside of them.**
- **Long hair and loose clothing must be secured.**
- **Closed toed shoes must be worn in the laboratory.**
- **Do not wear laboratory gloves into the hallway.**
- **Laboratory goggles must always be worn.**
- **Know the location of fire extinguishers and emergency exit routes.**
- **Do not work alone in the laboratory.**

RAMP Approach to Safety

Enhancing your understanding of laboratory safety requires preparing for new situations and equipping yourself with ways to ensure safe practices. A commonly used acronym to aide in working safely in the laboratory is RAMP.

R – Recognize

A – Assess

M – Minimize

P – Prepare

Recognize

In order to operate safely, we need to identify potential risks. If we never recognize the risks associated with an experiment then there is no way we can be adequately prepared. Think about the Titanic. The people in charge of safety never fully recognized the risks of hitting an iceberg and therefore were wholly unprepared when that situation arose. So how do you recognize a risk when you haven't performed an experiment before?

In introductory laboratories we will help you recognize new risks that exist when performing new techniques or using new reagents. Chemical labeling under the Global Harmonized System (GHS) and National Fire Protection Agency (NFPA) diamonds are both used to help people working with chemical reagents recognize the associated risks. Look at the GHS symbols and try to guess what risks they are identifying. Check your accuracy by looking at the OSHA Hazard Communication Standard Pictograms.

Figure 1.2: "GHS HAZCOM Safety Labels" by Mpelletier1 is licensed under CC BY-SA 3.0.

Assess

Once you have recognized the risks associated with particular actions in the laboratory, the next step is to assess how working with different materials can be potentially hazardous. GHS symbols and NFPA diamonds are a first step in assessing risks for chemicals, but they usually do not provide enough information to fully assess the risk. Remember: NFPA diamonds and GHS symbols are present to help recognize a potentially risky situation.

Assessing a risk requires us to gather more information. Gathering and interpreting quality information are universally beneficial skills and are often called Information Literacy.

In the chemistry lab there are certain well-established tools to aid in assessing risk. Among the primary tools used are Safety Data Sheets (SDS) or Material Safety Data Sheets (MSDS) that outline the risks and cautions associated with a given materials. Companies that sell chemical reagents are required to provide this information for their products. Places that use hazardous materials are also required to make Safety Data Sheets available to those who encounter those materials.

A Chemical Hazard Risk Evaluation Matrix can be helpful in thinking through the various risks of carrying out a laboratory experiment. Take a look at this Chemical Hazard Risk Evaluation Matrix from SUNY-Stony Brook

Minimize

Once we have established the risks associated with performing an action in the laboratory, the next step is to reduce those risks as much as reasonably possible. We won't ever be able to fully eliminate risks, but we can take measures to decrease the likelihood of serious injuries and accidents. The common steps to minimize risks are as follows:

Replace Hazardous Substances for Less Hazardous Substances.

In the 20th century benzene was commonly used in organic chemistry synthesis but we eventually learned that benzene was quite toxic and carcinogenic. This knowledge led to benzene being replaced with other, less harmful substances.

Use Spaces and Devices Engineered To Minimize Hazards.

Performing chemical experiments in chemical laboratories equipped with good ventilation or fume hoods can decrease the risk of inhaling a particular chemical. Refrigerators used to store chemicals look externally similar to what you would find in your kitchen, but they are specifically designed to be explosion proof. The risk of your milk exploding is really low, but the risk of stored chemical in a laboratory exploding is much greater!

Use Appropriate Personal Protective Equipment (PPE).

Safety goggles help protect your eyes from being exposed to hazardous chemicals. Wearing closed-toed shoes protect your feet from drops and spills. Gloves can protect your hands from being exposed to hazardous substances. These are examples of PPE that minimize risk.

Prepare

While we can minimize risk, we can never eliminate all hazards. Preparing for accidents can aid us in responding appropriately when an accident occurs. By preparing, we not only increase our chances of solving a problem, but we also help ensure that we do not worsen a problem when responding to an accident.

We have emergency measures in place in the laboratory. One example is an eye-wash station that is used if someone gets a hazardous substance in their eye. You might think that this station is redundant because we should all be wearing safety eyewear. You are correct. It is redundant, and that is the point. While we expect that we will not need to use the eyewash because of our minimization of risk with PPE, we are still prepared in case an accident does occur. Other examples of these measures include first aid kits for minor cuts and scrapes, a safety shower for large spills on people, spill kits to clean up spills on surfaces, and a fire extinguisher for small fires.

Laboratory Notebook

The laboratory notebook is where all information about laboratory experiments should be stored. The idea is that the laboratory notebook is a permanent record of all preparation, all observations and data in the laboratory, and all analysis of data after an experiment. The main purpose is to record all that you have done so that you and others can look at the notebook and replicate experiments. Laboratory notebooks are routinely used in court cases to determine if samples were handled properly and who gets awarded patents for new technology.

Guidelines for Maintaining a Laboratory Notebook

- **Table of Contents is kept current with page numbers.**
- **Pages are numbered consecutively and no blank spaces are left in the notebook.**
- **All entries are made in permanent ink at the time of the observation.**
- **Errors are corrected by crossing out with a single line, initialing the correction, and dating when the correction was made.**
- **Procedures for the experiments are well documented.**
- **Notebook is signed and dated to document when data entry begins and ends.**
- **Data and observations are recorded in an organized fashion.**
- **Data are recorded with appropriate units and significant figures.**
- **Notebook is signed by a witness at the end of data collection.**
- **Sample calculations and details of data analysis are provided.**
- **Summary of findings is recorded after the experiment.**

References

American Chemical Society, “Guidelines for Chemical Laboratory Safety in Academic Institutions.” 2016, https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/acs-safety-guidelines-academic.pdf Accessed: February 26, 2023.

Separating Substances, Measuring Mass, and Analyzing Data - Technique Laboratory

Introduction for Measurement of Masses

Balances are used to measure masses of objects. Chemists have used balances for centuries to gain key insights into chemical processes and reactions. The balance has long been one of the most important tools in the chemistry laboratory!

Use of Balance

The tare button is the one important button on the balance. By pressing the tare button you "zero" the balance. Zeroing the balance means that whatever mass is currently on top of the balance is set to zero mass.

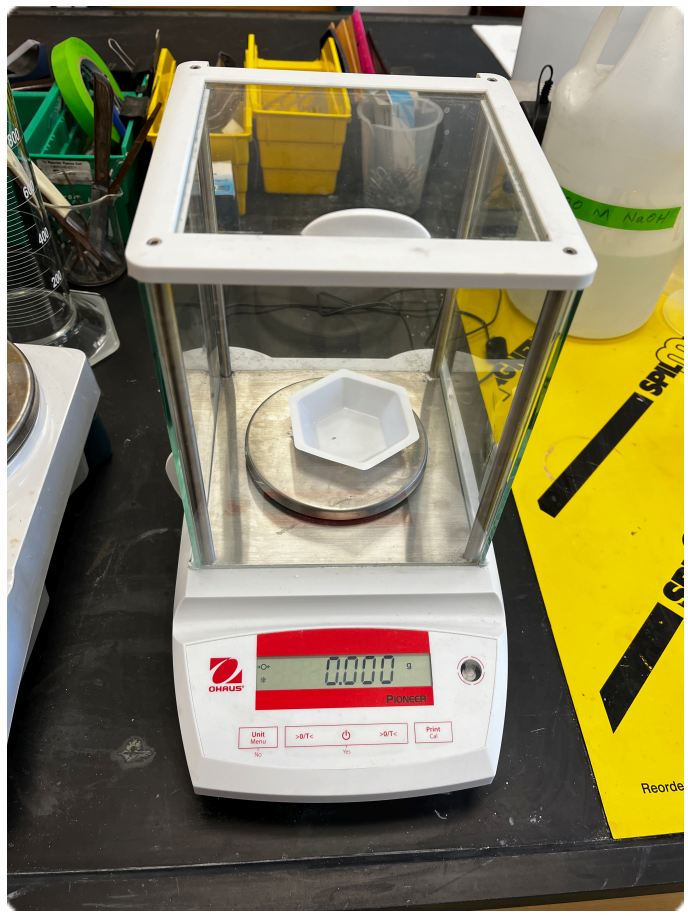

Figure 2.1: Electronic Laboratory Balance

Tips for measuring masses using a top-loading balance:

1. Never put the object being massed directly on the balance. Always use a piece of weighing paper or a container (beaker/flask) to hold the material being massed.
2. Close the balance door/lid to avoid drafts from air currents that will cause readings to fluctuate.
3. All objects being massed must be at room temperature. This avoids buoyancy correction problems due to warm air being less dense than cold air and prevents air currents that will cause readings to fluctuate.
4. Write down all the digits from the balance. If you are asked to mass 2 grams of material, it is perfectly fine if the balance reads 1.956 grams, but you must write down the actual value (1.956 grams) and not just write 2 grams in your laboratory notebook.
5. Clean up any spills immediately using a brush.

Measure Directly

Measuring a mass directly is just like stepping on a scale to determine your weight. This approach is convenient to use when some uncertainty is acceptable for the measurement and when it is easy to separate the material you are massing from its container. The basic procedure for massing directly is as follows:

1. Place something on the balance (weighing paper or a beaker).
2. Tare the balance so it reads zero when the weighing paper or beaker is on the balance or record the mass of the weighing paper/beaker.
3. Place the material to be massed on the weighing paper or beaker and record the mass.

Mass by Difference

Measuring a mass by difference is how I would determine the weight of babies in my house. I would step on the scale while holding the baby to determine my weight and that of the baby. Then I would step on the scale again without the baby. The difference between the two weights is the weight of the baby.

(Mass of Dr. A Holding a Baby) − (Mass of Dr. A) = (Mass of Baby)

Measuring by difference always infers a measurement based on two other measurements. It is used when you want an accurate mass and when it is difficult to separate an object at the end of an experiment.

As an example, say you wanted to measure how many grams of peanut butter are left in a jar. You could try to scrape all the peanut butter out and measure the mass directly, but it would be much easier to determine the mass by difference. If you know the mass of the empty peanut butter jar, then all you need to determine the weight of the peanut butter is the mass of the jar with the peanut butter in it. Then you can take the difference between the masses to determine the mass of peanut butter remaining.

When to Tare?

When taking initial masses of objects, it is often beneficial to think through to the end of the experiment to decide if you will be taking a mass by difference at the end or determining a mass directly. This dictates if you should tare the balance with your container on it or include the mass of the container. For example, if you

are asked to directly mass 5 grams of solid into a beaker you could either tare the balance without the beaker or tare the balance with the beaker present.

If you will take a mass of something in the beaker at a later date, then you should tare the balance before putting the beaker on it. This would lead to recordings in your notebook of something like the following:

Mass of Empty Beaker: 101.568 grams

Mass of Beaker with Solid: 106.237 grams

Mass of Solid: $106.237\ g - 101.568\ g = 4.669\ g$

Alternatively, if you will not take the mass of an object in the beaker later, then you could tare the balance after adding the beaker. This would lead to recordings in your notebook of something like the following:

Mass of Solid: 4.669 grams

It certainly feels easier to just have the one value written down but be careful that you won't need the mass of the beaker later. If you are unsure about whether you will need the mass of the beaker later, then it is best to record its mass and follow the first approach. Although you will need to write down two more numbers and do a little subtraction, it could save you in the end.

Separating a Heterogeneous Mixture and Determining Masses

You will be provided with a mixture that contains sand (SiO_2), table salt (NaCl), and benzoic acid (C_6H_5COOH). Your goal is to separate the mixture into its components and determine the percent composition of each component in the mixture.

Here is some basic information about the properties of sand, table salt, and benzoic acid. We will use these properties to separate the three different solids from each other. The table below lists how many grams of each substance will dissolve in 100 grams of water at various temperatures in degrees Celsius.

Aqueous Solubility (g/100 g water) at Various Temperatures

Substance	0 °C	20 °C	40 °C	80 °C	100 °C
SiO_2	0	0	0	0	0
NaCl	35.6	35.8	36.5	38.1	39.2
C_6H_5COOH	0.17	0.42	1.1	2.7	7.1

In separating the mixture, we will use a few different techniques.

Heating With Bunsen Burner

Bunsen burners are effective ways to heat a sample. There are two adjustable parameters on a Bunsen burner: the gas flow rate and the mixing ratio with air.

To ignite the Bunsen burner, first turn on the gas and then use a striker to ignite the gas. Once the Bunsen burner is lit, the gas flow and mixing ratio are adjusted to produce an efficient flame. An efficient flame has a sharp inner blue cone of combustion.

If your Bunsen burner does not light, first check to make sure you have gas flow. If you are properly turning on the gas and have flow, then check to see if the striker is producing sparks when you use it. Test the striker a few times to make sure you can consistently produce sparks. If you have gas flow and are producing sparks, but the Bunsen burner still won’t light, then you need to adjust the air mixing ratio. Turn the air mixing valve to minimize the amount of air that enters the Bunsen burner. This should allow you to light the burner but then you will need to increase the amount of air to produce a more intense flame.

Safety Considerations for a Bunsen Burner

Bunsen burners can pose serious risks in the laboratory. Probably one of the most obvious risks is that you will use the Bunsen burner to heat objects, and those hot objects can burn you if not handled appropriately. Remember that hot glassware looks identical to cold glassware, so be aware of the temperature of a laboratory tool before handling it.

You also risk igniting other objects. Make sure that paper and other flammable materials are not near the Bunsen burner when lit. This is especially true for flammable liquids. Make sure you are aware of the SDS requirements for the materials you are using on a Bunsen burner. Flammable liquids should never be used in the proximity of a Bunsen burner.

Vacuum Filtration

Filtration is a technique that enables us to separate solids from liquids in the laboratory. The solids become trapped because they are too large to pass through the piece of filter paper, while the liquid travels through the paper.

Vacuum filtration uses the same idea while also pulling a vacuum inside the filter flask to increase the flow rate of liquid through the filter paper.

After filtration is completed, all the solid is stuck to the filter paper. It is difficult if not impossible to separate completely the filter paper from the solid at this point.

If you are trying to quantify the mass of the solid, which type of mass measurement technique should you use? Massing by difference is needed when using filter paper.

The solid on the filter paper is usually washed multiple times before being allowed to dry on the filter paper.

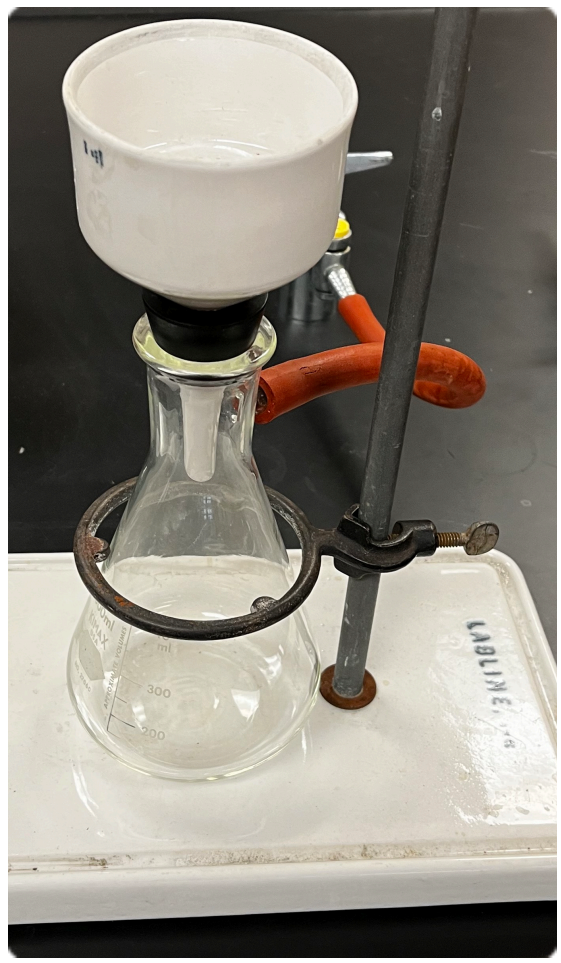

Figure 2.2: Vacuum filtration apparatus. Red tubing is connected to vacuum line. Filter paper is placed in white funnel.

Decanting

To decant means to pour off the liquid from a solid.
When you decant, it is important to prevent the solid from being transferred to the new container. It is also helpful to rinse the solid after decanting to make sure the separation is complete.

Experiment

1. You will analyze about 5 grams of the mixture in this laboratory. Make sure to think about what masses you need to record during this process. Remember we are trying to quantify what percentage of the mass is SiO_2, what percentage of the mass is NaCl, and what percentage of the mass is C_6H_5COOH.

2. To the total mixture, add about 50 mL of distilled water and boil the water and slurry mixture while stirring. What components will dissolve in the water? What components will NOT dissolve in the water? Use the solubility table to make this determination. After the water has boiled, decant the liquid into a

new beaker. Think about if you want to take the mass of the new beaker before pouring the liquid into it. Rinse the solid with more boiling distilled water and add that to the new beaker as well.

3. Once the liquid in the new beaker is cool to the touch, place that beaker in an ice bath for 15 minutes to cool it down to zero degrees Celsius. After the beaker is cooled in the ice bath you should see a new solid formed. What component is the solid that forms in the ice bath? Use the solubility properties to determine what components are in the solution and what components are in the solid at this time.

4. Use vacuum filtration to separate the liquid from the solid. Before performing vacuum filtration, make sure to think about what information you will need to determine the mass of the solid and what you will use to wash the solid (either boiling water or ice water).

5. We now have two solids and one liquid. The solid on the filter paper can be dried by continually pulling the vacuum. The solid in the beaker can be dried by gently heating over a flame. The component that is still dissolved in water can be isolated by transferring to a beaker and gently heating to remove the water.

6. When heating to remove the water, use “boiling stones,” so the solution does not splatter. Add a few boiling stones to the solution. Think about whether you will need to know the mass of these stones to determine the mass of the last component.

Safety Considerations

Practice appropriate fire safety protocols for using Bunsen burners and working with hot glassware. Examine SDS sheets for NaCl, SiO_2, and benzoic acid (C_6H_5COOH) to note special safety considerations for working with these materials.

Waste Disposal

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

Introduction for Treatment of Data

A key component of scientific analysis is the repeatability of experiments. We want to get consistent results when trials are repeated and also understand how much variability exists in the measurements. Accomplishing both repeatability and gaining an understanding of how much measurements will vary from one experiment to the next are key to the scientific process.

Accuracy and Precision

Whenever we take measurements we are generally concerned with the quality of those measurements. We define the quality of the measurement using precision and accuracy measures. In your Introductory Chemistry courses at Millersville University, we will define the precision of a measurement using Relative Average Deviation and accuracy using Relative Error.

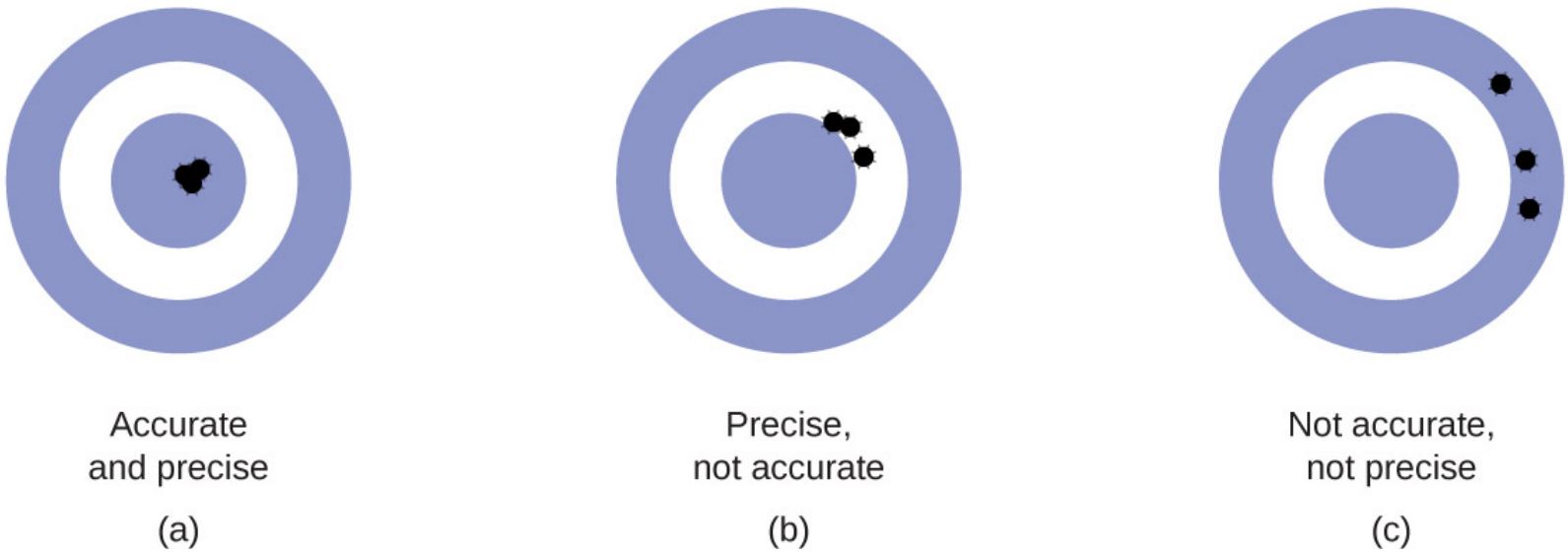

Figure 2.3: 1.6: Measurement Uncertainty, Accuracy, and Precision is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax

Accuracy is a measure of how close we are to the "true" value. Sometimes we know the true value, and sometimes we do not know the true value.

Precision is a measure of the repeatability of our measurements. An experiment with high precision means that all the results are close to each other. An experiment with low precision has not very repeatable results.

Average Value

Since we want to understand the repeatability of experiments, we will often repeat an experiment many times. This repetition allows us to see the consistency or lack of consistency in our results. A key result of those many measurements is the average value. An average is found by adding the results from all the trials and dividing by the total number of trials.

Overall results are typically reported as an average value. In addition to the average value, we also want to report the repeatability of the measurements. One way to report repeatability is by using Relative Average Deviation.

Relative Average Deviation

Relative Average Deviation (RAD) is expressed as a percentage and essentially tells us how much repeated measures fluctuate. A RAD of 1% would mean we expect each trial to differ by about 1% from each other. This is effectively the uncertainty associated with our measurement. A smaller RAD means your measurements have higher precision. Here we will use a four-number data set (97, 99, 100, and 103) to demonstrate how to calculate RAD:

Step 1: Calculate the average of all your trials:

$$\frac{97 + 99 + 100 + 103}{4} = 99.8$$

Step 2: Take the absolute value of difference between each trial and the average:

$$|97 - 99.8| = 2.8$$

$$|99 - 99.8| = 0.8$$

$$|100 - 99.8| = 0.2$$

$$|103 - 99.8| = 3.2$$

Step 3: Calculate the average of the four deviations from the average found in Step 2:

$$\frac{2.8 + 0.8 + 0.2 + 3.2}{4} = 1.8$$

Step 4: Calculate the RAD by taking the average of deviations in Step 3, dividing it by the average from Step 1 and multiplying it by 100 to get into a percentage.

$$\frac{1.8}{99.8} \times 100\% = 1.8\%$$

Percent Error

Relative Error is expressed as a percentage and essentially tells us how far our measured value is from a true value. It is calculated by taking the absolute value of the difference between a measured value and a true value. If we measure the density of an aluminum bar to be 2.63 g/mL, but the true density of aluminum is 2.70 g/mL then our Relative Error is 3%:

$$\frac{|2.63 - 2.70|}{2.70} \times 100\% = 2.6\%$$

Pre-Lab Questions

1. What are the safety considerations for using a Bunsen burner?

2. In the SDS sheets for SiO_2, NaCl, and C_6H_5COOH, what are the main hazards associated with these substances? What precautions do you think should be taken when working with these substances?

3. Look at the different steps of the experiment. In what phase is each of the three different components at each step of the procedure? In step 2 of the experiment, what type of mixture will you have before decanting and after decanting?

4. Think about the masses you will need to take during the experiment. Set-up a table for recording data in your laboratory notebook where you will record all those masses. Think about whether you will be measuring directly or measuring by difference for each of the components. If measuring by difference, between what masses will you be taking the difference?

5. What physical property of the mixture components are you using to separate them? What are the laboratory techniques used to separate each of the different components from the various mixtures?

Post-Lab Questions

1. What are the masses of each component?

2. What is the percent mass of each component in the mixture?

3. Collect data about the percent masses from four other groups in the laboratory. Use that data to calculate the average percent mass of each component and the relative average deviation of the mass percent of each component.

4. Your instructor will provide you with the true mass percents of each component. Calculate the percent error of your group average mass percents.

5. Which mass percent was the most and which was the least precise? Which mass percent was the most and which was the least accurate?

6. Explain experimentally why you think the mass percents of certain components were more or less precise or accurate.

References

Dr. Aimee Miller, *CHEM 103 Laboratory Manual*, Millersville University.

Measuring Volumes - Technique Laboratory

Motivation

Figure 3.1: "Oxfam engineer tests the water quality" by Oxfam East Africa is licensed under CC BY 2.0.

Many chemical reactions take place in the solution phase with the reacting chemicals dissolved in a liquid solvent. In environmental and biological applications, that solvent is routinely water. In the synthesis of organic compounds for medicines, fabrics, plastics, etc., the solvent is typically something other than water. No matter your primary experimental scientific interests (biology, chemistry, earth sciences, medicine, etc.), you will likely work with liquid samples.

A convenient and often used measurement for quantifying the amount of a liquid is its volume. This is true for everyday products we use like gasoline, water, or milk, and it is true in the chemistry laboratory, too. In this lab we will explore different techniques and tools for measuring liquid volumes, explore their accuracy and precision, and determine when the different tools will be appropriate.

Cleaning Glassware for Volumetric Measurements

Clean glassware is critical to achieving reliable results in the laboratory. For volumetric measuring devices you are using, it is best to clean them with water first and then to clean with the liquid that you will be using. The primary idea is that a little bit of the liquid always gets left behind on surfaces, so the best thing to do is ensure that the liquid left behind is the liquid you are using in the measurement.

When rinsing glassware for cleaning, you should do multiple small volume cleanings instead of one large volume cleaning. Rinsing a beaker three different times with 10 mL of water each time is much more effective than rinsing once with 30 mL of water. When rinsing make sure to coat all of the walls of the glassware with the rinse liquid.

Volume Measuring Devices

Below are descriptions of some of the common tools in the chemistry laboratory for measuring volumes and tips and tricks for using them.

Beakers And Erlenmeyer Flasks

Beakers and Erlenmeyer flasks are convenient ways to work with liquids in the laboratory because it is easy to add and remove liquids to them. Beakers and Erlenmeyer flasks come in many different sizes (total volumes) and often have markings along the side that indicate additional volumes. For example, a 100 mL beaker might have markings every 10 mL and a main marking at 100 mL.

It is tempting to use beakers and Erlenmeyer flasks to measure a volume because they are always readily available, but the markings on these tools are like the picture below, a “mL inspired volume.”

Figure 3.2: Erlenmeyer Flask: "Erlenmeyer Flasks 1" by biologycorner is licensed under CC BY-NC 2.0.

Figure 3.3: Beaker: "Siphon beaker" by niallkennedy is licensed under CC BY-NC 2.0.

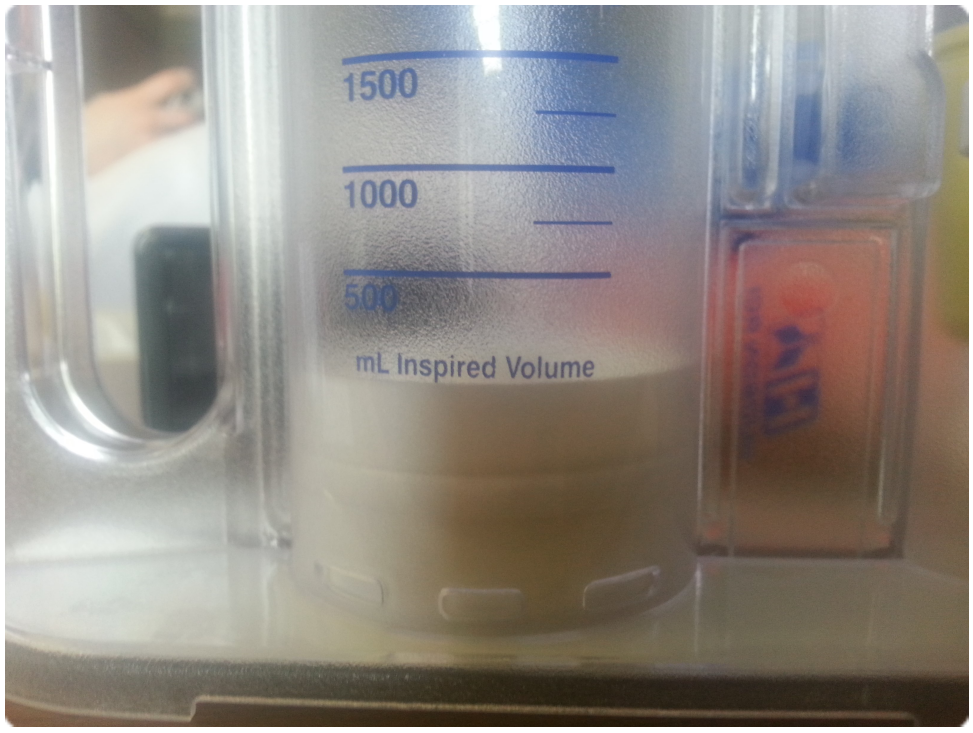

Figure 3.4: Volume measuring container at a hospital.

If you care at all about having a known or consistent volume, avoid the temptation of using the markings on beakers and Erlenmeyer flasks for measurements of volume.

Graduated Cylinders

Graduated cylinders are tubes with marks that indicate specific volumes. Graduated cylinders come in a variety of sizes, and the uncertainty associated with the graduated cylinder depends on the size of the cylinder and the markings. Advantages of graduated cylinders are that they are relatively quick and easy to use and that they also allow for the measurement of any volume within the range of the cylinder. For example, the 100 mL graduated cylinder pictured below could be used to record any volume between 10 and 100 mL.

Figure 3.5: Graduated Cylinder: "Laboratory Equipment - Graduated Cylinder" by eltpics is licensed under CC BY-NC 2.0.

To use a graduated cylinder, you simply fill the cylinder with a liquid and then read the liquid level using the marks on the side of the cylinder. You will notice that the liquid surface is not flat at the top but instead curved as the water adheres to the glass walls of the cylinder. The curved surface of the liquid is called the meniscus. The liquid level is read at the bottom of the meniscus in order to have a consistent measurement standard. Notice that in figure 3.6 the edges of the meniscus are between 19 and 20 mL but the bottom of the meniscus is between 20 and 21 mL.

When you are reading a graduated cylinder, it is critical that you take the time to look closely at the markings and understand the spacing. Not all graduated cylinders are the same, so you can't assume a certain marking pattern. In the picture above 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 graduated cylinder in the picture above, I see 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.

Figure 3.6: "Buret" by photobunny is licensed under CC BY-NC-ND 2.0.

My thought process for reading the cylinder in figure 3.6 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, and then finally I make my determination of 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 come along and have a slightly different estimation of the last digit, maybe they think that 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.

An important error to avoid in volume measurement readings is parallax error. This error occurs because the bottom of the meniscus is not in the exact same location as the markings. The bottom of the meniscus is in the center of the measuring device and the markings are on the walls of the device. You will get an incorrect reading that has parallax error if your eye level is above or below the meniscus. Always read volumes with your eye at the same height as the meniscus to ensure you don't have parallax error. This will often mean you need to lift up the measuring device or reposition your body in order to be at eye level with the meniscus.

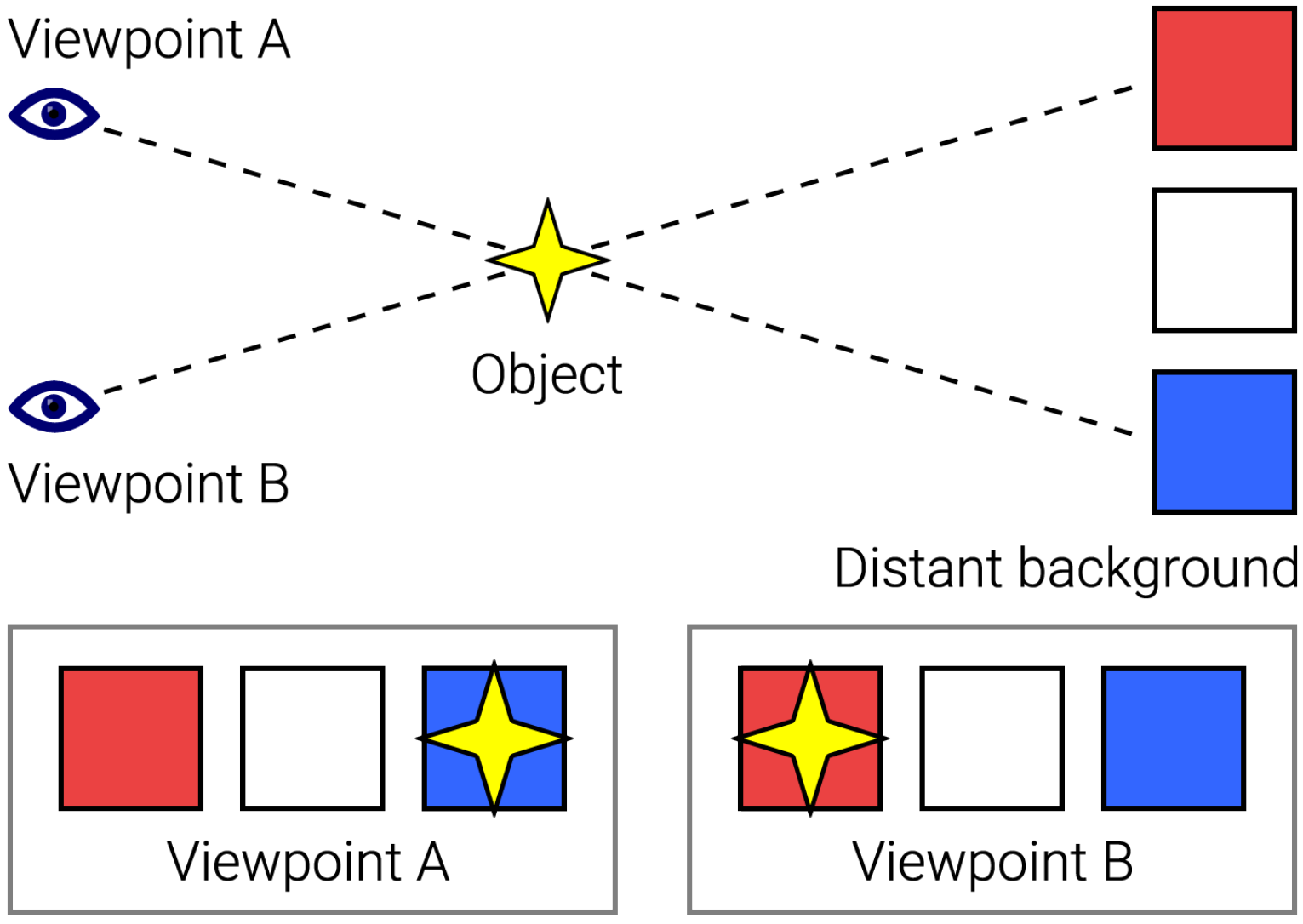

Figure 3.7: Parallax Example.png by JustinWick is licensed under CC BY-SA 3.0.

Reminders for graduated cylinders:

1. Pay attention to carefully interpret the markings.
2. Avoid parallax error by having the meniscus at eye level.
3. Estimate the last digit to appropriately reflect the uncertainty of the measurement.

Volumetric Pipets

Unlike graduated cylinders, volumetric pipets are designed to deliver only a specific volume of liquid. If 10 mL of liquid is needed, a 10 mL volumetric pipet is used, but if 25 mL of liquid is needed, then a completely different 25 mL volumetric pipet is used. A volumetric pipet is a specialist in that it can deliver only the one specific volume that it was designed to deliver. A volumetric pipet has only one mark on it. That mark is the line for the volume of liquid the pipet was designed to deliver.

Figure 3.8: Various sizes of volumetric pipets.

A pipet bulb is used to draw liquid into the pipet from a separate container (typically an Erlenmeyer flask or beaker). To use a volumetric pipet:

1. Place the tip of the pipet into the liquid.

2. Take the pipet bulb not attached to the pipet and squeeze it.

3. Attach the squeezed bulb to the top of the pipet.

4. Slowly release the bulb while making sure to keep the tip of the pipet submerged in the liquid. If the tip of the pipet becomes dislodged from the liquid, air will be introduced into the pipet and potentially make a big mess to clean-up.

5. Continue to allow the liquid to rise in the pipet until it rises above the mark but has not reached the pipet bulb. It is important to keep liquid from entering the pipet bulb.

6. Remove the pipet bulb and place a finger (thumb or pointer finger work best) on top of the pipet to keep the liquid level constant in the pipet.

7. Slowly release pressure on your finger so that the liquid level slowly drops.

8. Get the bottom of the meniscus to the mark (make sure you read it at eye level to avoid parallax error!). This can take some practice. If you go past the line, you will need to use the pipet bulb again to draw more liquid into the pipet.

9. Take the pipet out of the liquid and wipe off the outside of the pipet.

10. Place the tip of the pipet into a new container, release the pressure from your finger, and allow the liquid to flow down the wall of the container.

11. You will notice that there is a small amount of liquid remaining in the tip of the pipet. Leave that small amount of liquid in the pipet. The small amount of liquid is supposed to be there as the pipets are designed as TD (to deliver) pipets and calibrated knowing that the small amount of liquid gets left behind.

Volumetric Flasks

Volumetric pipets are designed to deliver a specific volume, whereas volumetric flasks are designed to contain a specific volume. Like volumetric pipets, volumetric flasks are designed for only one specific volume, so if you want a different volume, then you need to use a different volumetric flask.

When filling a volumetric flask, you need to be careful when the liquid approaches the line because you will need to start completely over once it passes the line. It is helpful to use a dropper to control the last bit of liquid added to ensure that it is not filled above the line.

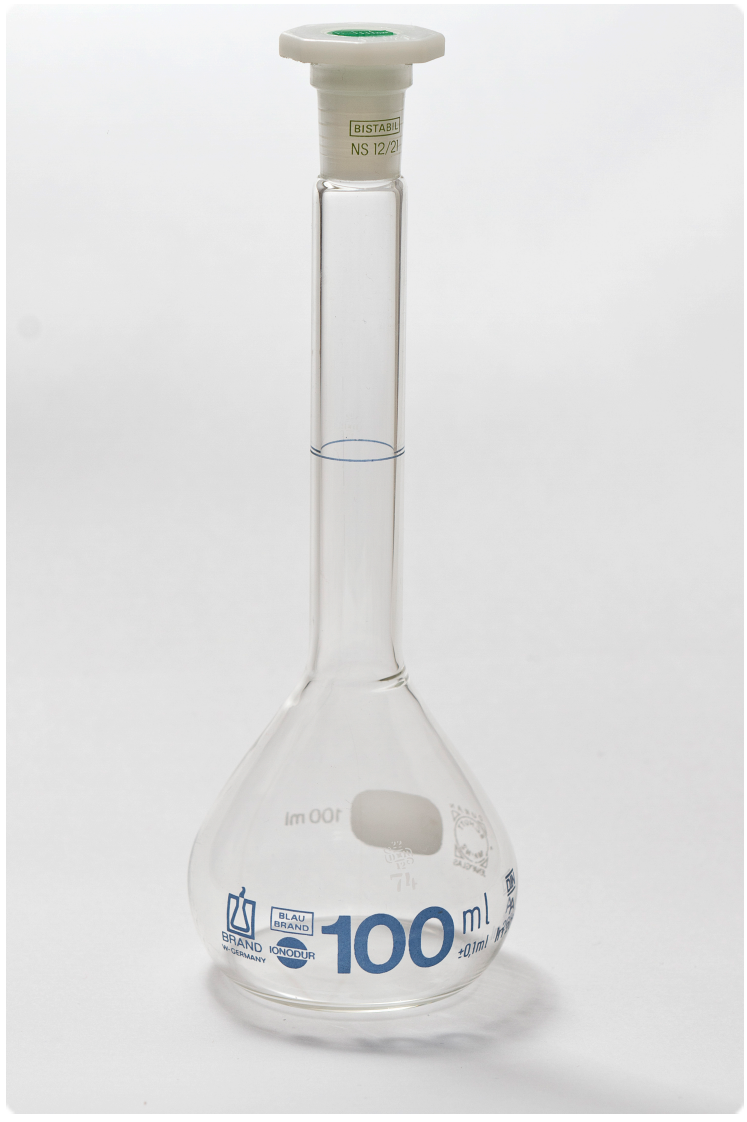

Figure 3.9: Volumetric Flask: "File:Brand volumetric flask 100ml.jpg" by Lucasbosch is licensed under CC BY-SA 3.0.

Practice Technique for Graduated Cylinder, Volumetric Pipet, and Volumetric Flask

Before we start comparing the different measuring tools, we will practice and demonstrate to our lab partners that we can appropriately apply these techniques.

For the graduated cylinder add a random amount of liquid and record the volume. Have everyone at your lab bench do the same without communicating. Now read the volumes on all the graduated cylinders. Compare your recorded results with the other people in your lab group to ensure you have consistent results. Have the lab bench sign off in your notebook that you properly can use this skill.

Add exactly 25 mL of liquid to your graduated cylinder. Have a lab partner check that you have appropriately added 25 mL. Have the lab bench sign off in your notebook that you properly can use this skill.

Demonstrate the appropriate use of a volumetric pipet to your lab bench. Have the lab bench sign off in your notebook that you properly can use this skill.

Demonstrate the appropriate use of a volumetric flask to your lab bench. Have the lab bench sign off in your notebook that you properly can use this skill.

Determine Accuracy and Precision of Measurement Devices

Goal: Determine the accuracy (relative error) and precision (relative average deviation) for measuring 25 mL of water using four different tools: beaker, graduated cylinder, volumetric pipet, and volumetric flask.

A common way to calibrate volume-measuring devices is to measure the mass of a liquid that has a well-established density. Water is commonly used since it is readily available, and the density is well-established. Here is the basic procedure for determining a volume using density:

1. Record the mass of a dry, empty container. Beakers or Erlenmeyer flasks work well.

2. Measure an amount of liquid using a volume measuring device and pour the liquid into the container of known mass from step 1. For example, measure 25 mL of water with a graduated cylinder and pour the water into a beaker.

3. Record the mass of the container holding the water.

4. Determine the mass of the water that was poured into the container with the massing-by-difference approach you learned about previously.

5. Use the known density of the water to calculate the actual volume of water that was delivered.

Volumes and densities change as the temperature changes, but mass does NOT change with temperature. You will need to record the temperature in the room and look up the true density of water at that temperature using the US Department of the Interior water density table.

Here is an example of what a record of this could look like in your notebook and the accompanying calculations.

Mass of 150 mL beaker: 240.345 g

Recorded Volume on graduated cylinder: 25.0 mL

Mass of 150 mL beaker containing water: 265.234 g

Mass of water delivered: $265.234\ g - 240.345\ g = 24.889\ g$

Temperature: 20.9 degrees Celsius

Water Density: 0.9980 g/mL

Actual Volume Delivered: $24.889\ g \times \frac{1\ \text{mL}}{0.9980\ g} = 24.94\ \text{mL}$

In this case I measured 25.0 mL with the graduated cylinder, but the mass tells me that I actually poured 24.94 mL of water into the container.

For each of the four tools, complete four trials of measuring 25 mL. There will be sixteen total trials. The container does not need to be emptied between each trial for a tool.

Waste Disposal

Only distilled water is used in this lab. All waste generated can be disposed of in the sink.

Safety Considerations

Different types of glassware are utilized in this laboratory. Take special care to handle volumetric pipets gently as the neck of the pipet can easily break if a large force is applied.

Pre-Lab Questions

1. What described tools should not be used to measure volumes?

2. Which tools are continuous (can measure any volume) and which tools are discrete (can only measure certain volumes)?

3. What is a meniscus in the chemistry lab and how is it important in volume measurement tools?

4. You have a 15.3 gram mass of liquid water. What is the volume of the water at 21 degrees Celsius? What is the volume of water at 93.3 degrees Celsius?

5. Explain, using a particulate level diagram, what is happening with the total volume and the water molecules in the solution as you change the temperature from 21 to 93.3 degrees Celsius.

Post-Lab Calculations and Questions

1. Calculate the average volume delivered for each tool. You should have four averages.

2. Calculate the relative average deviation (RAD) for each tool. You should have four RAD values.

3. Calculate the percent error for each tool. You should have four percent errors.

4. Rank the tools in order of their accuracy.

5. Rank the tools in order of their precision.

6. Rank the tools in order of their ease of use.

7. Are any tools unacceptable to use? Explain why.

8. For future labs, we will typically use either a graduated cylinder or a volumetric pipet to deliver a specific volume. What are the advantages and disadvantages of a graduated cylinder compared with a volumetric pipet?

9. When do you think it would be appropriate to use graduated cylinders compared with volumetric pipets?

Reaction Types and Qualitative Analysis - Technique Laboratory

Introduction

A wide variety of chemical reactions can be classified into a few types of chemical reactions with different driving forces for what causes the reaction to occur. In this laboratory we will look at a variety of different types of chemical reactions and see how we can use some of those chemical reaction properties to identify different compounds. Our previous labs have all been quantitative, meaning we took measurements to determine precise values. This lab is a qualitative lab, where we don't need to worry about precise values and amounts. In a qualitative lab, keen observations are key to being successful. Practicing scientists use combinations of qualitative and quantitative observations to solve problems. Building both quantitative and qualitative laboratory skills and knowing when to utilize each skill set will help you grow as a scientist.

Observing Chemical Reactions

We can often see chemical reactions occurring by making specific observations that utilize our everyday senses.

Observation 1: Color Changes

The formation of new chemical during a reaction can often produce new colors. Changes in color associated with chemical reactions are most often observed when a transition metal is involved in the reaction.

Observation 2: Formation of Gases and Smell

Gas evolution is often the driving force for a chemical reaction to occur. We can see gas evolution reactions by seeing the bubbling of a gas. Even if we cannot see a gas being formed, we can still check for properties of a gas being formed.

Testing a Gas With a Lit Wooden Splint

A wooden splint (popsicle stick) can be lit with a match. If we place the lit splint over a reaction that is occurring, we can see if a produced gas has any impact on the lit splint. If the gas supports combustion (oxygen gas is a good example of this), then the flame will glow brighter. If the gas is combustible (methane and hydrogen are good example of this), then a quick "pop" is often heard. If the gas

is neither combustible nor supporting of combustion (carbon dioxide is a good example of this), then the flame will extinguish on the splint.

Wafting To Smell a Gas

Many gases have characteristic smells, so even if we don't see the bubbling for the formation of a gas, we can still detect its production via smell.

We never want to inhale a larger amount of a new compound produced in a reaction. To avoid inhaling large amounts, we use a technique called "wafting." To waft you gently wave your hand over the top of a container and lift your hand to your nose. This keeps the concentration that reaches your nose low to avoid harmful impacts.

Observation 3: Formation of a Solid

Another common driving force for chemical reactions in solution is the formation of a solid precipitate. We can detect the formation of a solid by seeing if a solution gets cloudy (hard to see through) when we mix two liquids. A cloudy solution means that solid particles are suspended in the liquid, signifying that a solid was formed.

Clear, Cloudy, and Colorless

In the chemistry lab we use terminology that you use in your everyday life, but sometimes it has a slightly different meaning in the chemistry lab.

In your everyday life you probably use clear and colorless as interchangeable terms. But in chemistry they refer to two completely different ideas. In the chemistry laboratory clear is the opposite of cloudy. When we say a solution is clear, that means that there are not any suspended solids or gases in the solution. Cloudy means there are suspended particles in the solution. A colorless solution means that the solution has no color, and the opposite is when a solution does have color.

In the chemistry lab we can have a clear red solution. Clear means it is free of suspended particles and red gives the color. A clear and colorless solution both has no particles and no color. We can also have a cloudy colorless solution or a cloudy blue solution.

Observation 4: Heat Change Associated With a Reaction

Many chemical reactions occur with large amounts of heat being consumed or produced. We can sense these changes by determining if the temperatures of the materials change when they are mixed together. Simply holding a beaker in your hands will allow for the detection of these changes.

Oxidation-Reduction Reactions: Movement of Electrons

Oxidation-reduction reactions involve the transfer of electrons from one atom to a different atom. Many types of chemical reactions are oxidation-reduction reactions; combination reactions, many decomposition reactions, combustion reactions, and single-displacement reactions are all classified as oxidation-reduction reactions. The key feature to identify is the changing oxidation numbers of different atoms when these reactions occur. When transition metals are involved, we will typically see color changes occurring when the transition metal undergoes a change in oxidation state.

Red-Ox Reaction 1: Oxidation States of Manganese

Here we will look at the reaction of potassium permanganate ($KMnO_4$) with iron(II) chloride ($FeCl_2$) under acidic conditions.

In a medium test-tube, mix about 3 mL of the potassium permanganate solution with 2 mL of HCl solution and 1 mL of iron(II) chloride solution. Record your observations. Manganese compounds in aqueous solutions tend to have different colors based on the oxidation state of the manganese. When manganese is in the +2 oxidation state, aqueous solutions are a pale pink color. When manganese is in the +4 oxidation state, it tends to form insoluble brown compounds. When manganese is in the +7 oxidation state, aqueous solutions have a rich purple color.

Oxidation-reduction reactions involve the transfer of electrons. Is manganese losing or gaining electrons in this process? Some other atom in the reaction must have the opposite behavior with its oxidation state. What other atom do you think is changing its oxidation state? Is it being oxidized or reduced?

Write the reduction half-reaction, oxidation half-reaction, and the balanced Red-Ox reaction for this process.

Red-Ox Reaction 2: Zinc With Hydrochloric Acid

In a small test tube and a small amount of zinc metal to 3 M hydrochloric acid. Zinc is typically found in the +2 oxidation state in ionic compounds.

Record your observations.

Write the reduction half-reaction, oxidation half-reaction, overall balanced chemical reaction, and net ionic equation that occurs and identify all the oxidation states of the atoms.

Red-Ox Reaction 3: Zinc With Copper(II) Chloride Solution

In a small test tube add a small amount of zinc metal to the copper(II) chloride solution.

Record your observations.

Write the reduction half-reaction, oxidation half-reaction, overall balanced chemical reaction, and net ionic equation that occurs and identify all of the oxidation states of the atoms.

Acid-Base Reactions: Movement of H^+

Bronstead-Lowry Acid-Base reactions involve the transfer of hydrogen ions (H^+) from one chemical species to another. We can often determine if a liquid is acidic or basic by seeing if it performs an acid-base reaction with an indicator.

Testing With Litmus Papers and Phenolphthalein

Litmus and phenolphthalein are two different compounds that change color depending on whether hydrogen ions (H^+) are added or removed from them. Litmus turns red when an H^+ is added to it and turns blue when an H^+ is removed from it. We can classify solutions as basic if they remove a hydrogen ion from litmus and acidic if they add a hydrogen ion to litmus.

Most litmus paper is sold in two types (Red and Blue). Red litmus paper will turn blue if a solution is placed on it that is basic. Blue litmus paper will turn red if a solution that is acidic is placed on it.When testing with litmus paper, it is important to dip a stir rod into the solution and then touch the stir rod to the litmus paper. This keeps the liquid free from contamination and allows for multiple tests with a single piece of litmus paper.

Phenolphthalein is often used as an indicator in acid-base reactions. Phenolphthalein is colorless when the solution is acidic, but when the solution is

basic, phenolphthalein loses an H^+ and then changes to a pink color. To test solutions with phenolphthalein, only a few drops (2 to 3) of the indicator are added to a solution.

We will test each of the following 1M solutions with red and blue litmus and with phenolphthalein.

NaCl, HCl, NaOH, Ammonium Chloride, and Sodium Carbonate

Classify all these solutions as acidic, basic, or neutral based on your observations.

Acid-Base Reaction 1: HCl With Sodium Carbonate

Mix equal volumes of the HCl and sodium carbonate solutions you tested above. Make observations based on your senses to determine if a reaction occurred.

Record all observations and write the balanced chemical reaction along with the full and net ionic equations. Was the reaction exothermic or endothermic?

Acid-Base Reaction 2: HCl With NaOH

Mix equal volumes of the HCl and sodium hydroxide solutions you tested above. Make observations based on your senses to determine if a reaction occurred.

Record all observations and write the balanced chemical reaction along with the full and net ionic equations. Was the reaction exothermic or endothermic?

Acid-Base Reaction 3: Ammonium Chloride With NaOH

Mix equal volumes of the ammonium chloride and sodium hydroxide solutions you tested above. Make observations based on your senses to determine if a reaction occurred.

Record all observations and write the balanced chemical reaction along with the full and net ionic equations. Was the reaction exothermic or endothermic?

Precipitation Reactions: Solid Formation

Precipitation reactions occur when two soluble ionic compounds react to form an insoluble solid (precipitate). These reactions can be predicted by knowing solubility rules. The driving force for these reactions is the formation of an insoluble solid.

We will test the following solutions with both silver nitrate and barium nitrate. The silver and barium tests are a good way to determine what anions are present in a solution of an ionic compound.

Sodium Chloride, Sodium Acetate, Sodium Sulfate, Sodium Carbonate, Sodium Nitrate, and Sodium Chromate.

Figure 4.1: Lead nitrate and sodium iodide are mixed to give a lead iodide precipitate. This Wikimedia image is shared under a CC BY-3.0 license.

Precipitation Reactions 1: Silver Nitrate

Record your observations when adding three drops of silver nitrate solution to a small amount of each of these solutions in a small test tube.

Write balanced full and net ionic equations for each ch emical reaction that occurs.

Precipitation Reactions 3: Barium Chloride

Record your observations when adding three drops of silver nitrate solution to a small amount of each of these solutions in a small test tube.

Write balanced full and net ionic equations for each chemical reaction that occurs.

Flame Tests

Flame tests are often used to identify cations in soluble ionic compounds. Think about the vibrant colors you see in fireworks. The same process occurring to produce color in fireworks is what we will see with flame tests. Many cations give off characteristic colors when placed in the outer cone of a flame.

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.

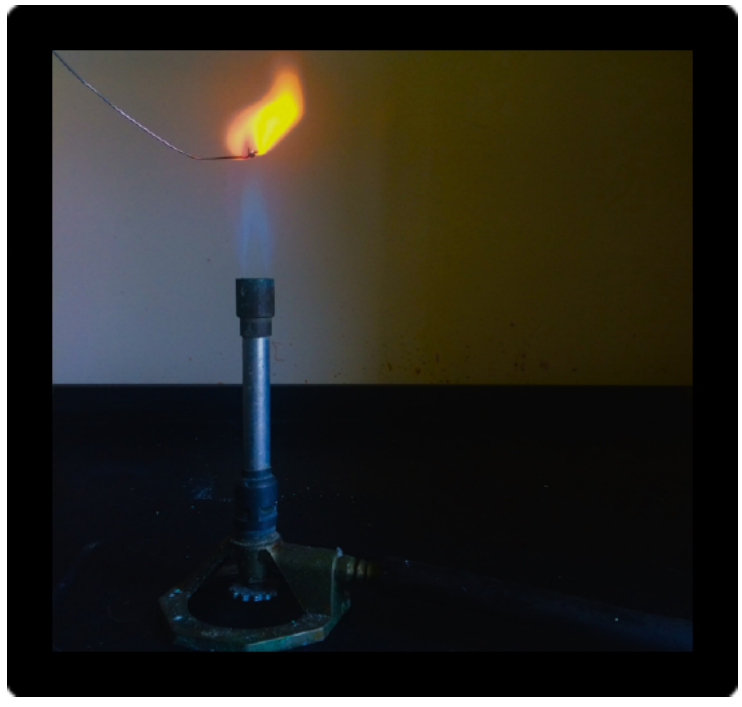

Figure 4.2: Flame Tests is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by LibreTexts.

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.

N.L. Mandel, B. Le, R. Ward, S.J. R. Hansen, and J.C. Ulichny. “Titrating Consumer Acids to Uncover Student Understanding: A Laboratory Investigation Leading to Data-Driven Instructional Interventions." *Journal of Chemical Education*, 2022, *99*(6), 2378-2384. DOI: 10.1021/acs.jchemed.1c01207.

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.

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

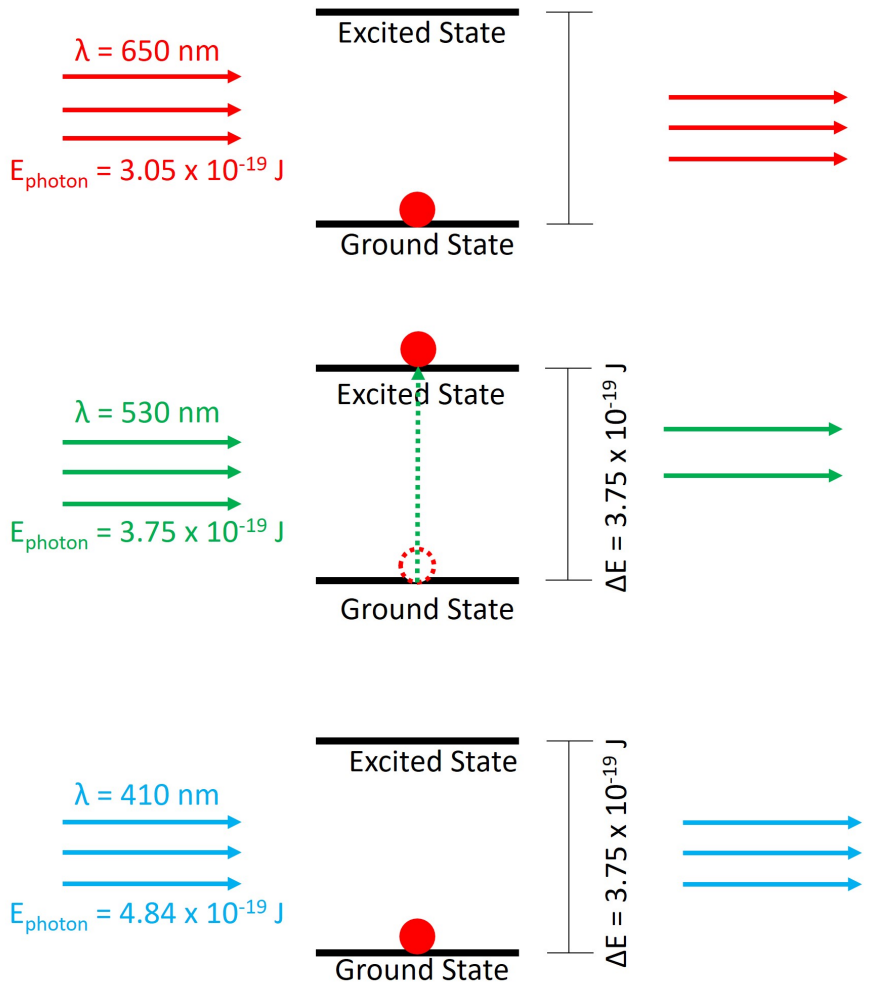

Figure 8.2: Energy level diagram that shows how different wavelengths of light interact with a molecule.

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.

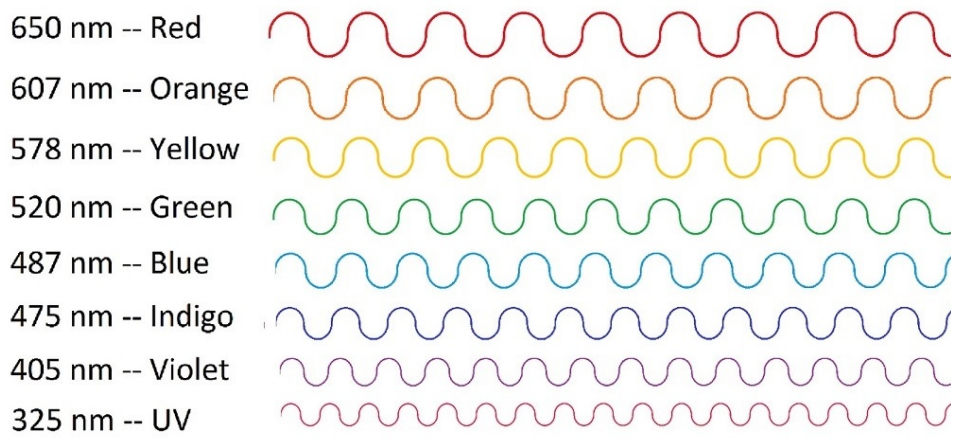

Figure 8.3: Derived from "File:Light wave harmonic diagram.svg" by Rubber Duck (✇ • ✍) is licensed under CC BY-SA 3.0.

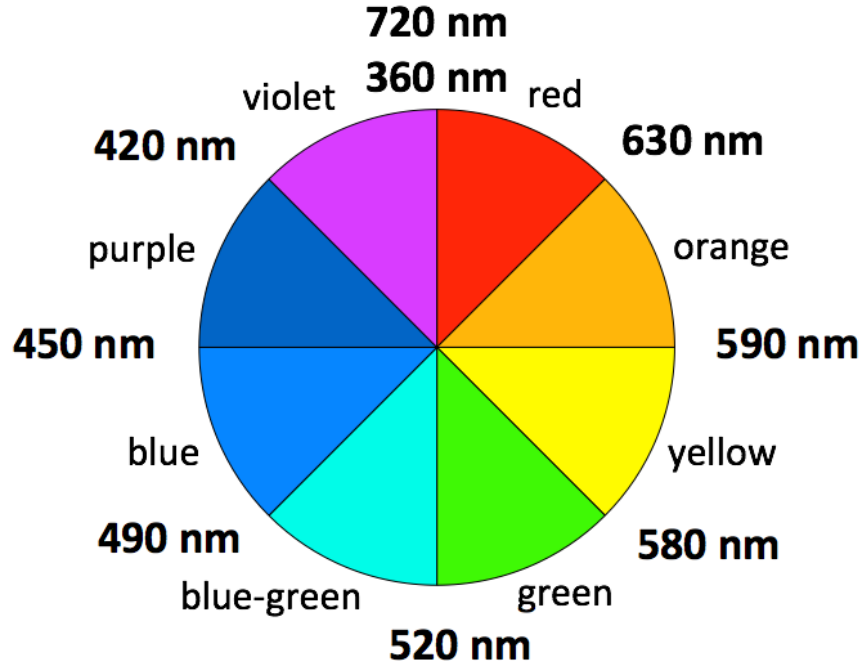

Figure 8.4: "File:Color wheel wavelengths.png" by Tem5psu is licensed under CC BY-SA 4.0.

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.

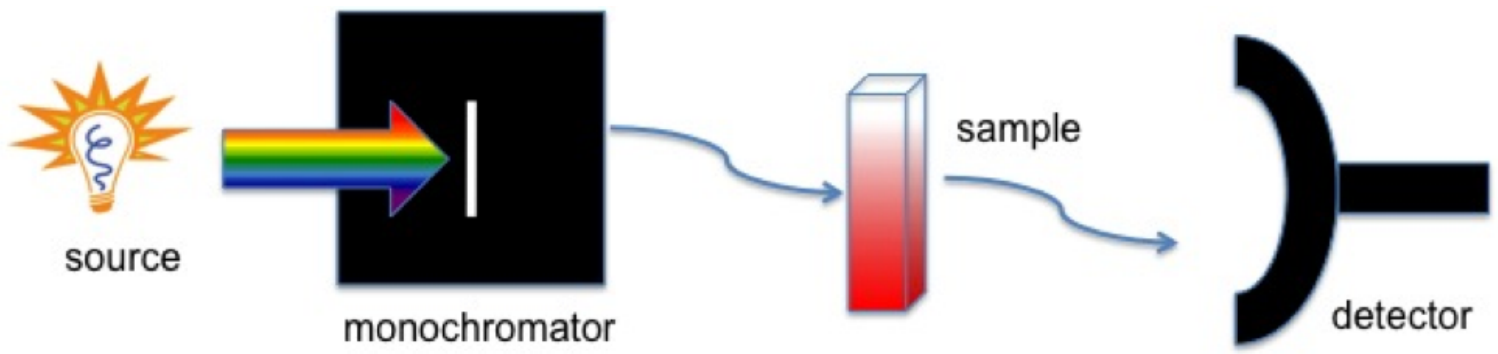

Figure 8.5: 4.4: UV-Visible Spectroscopy is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by Pavan M. V. Raja & Andrew R. Barron (OpenStax CNX).

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.

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.

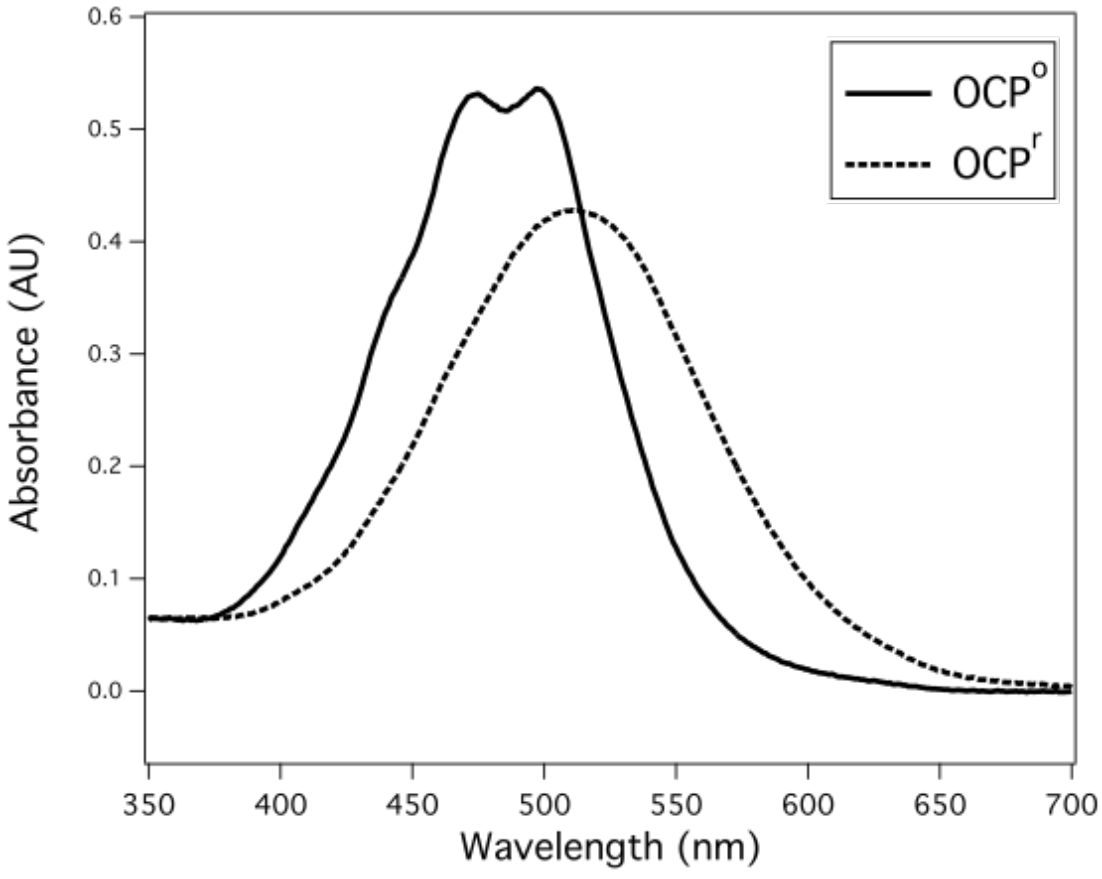

Figure 8.6: "File:Orange Carotenoid Protein spectra of orange vs red form.svg" by Ryan Leverenz and Cheryl Kerfeld is licensed under CC BY-SA 4.0.

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.

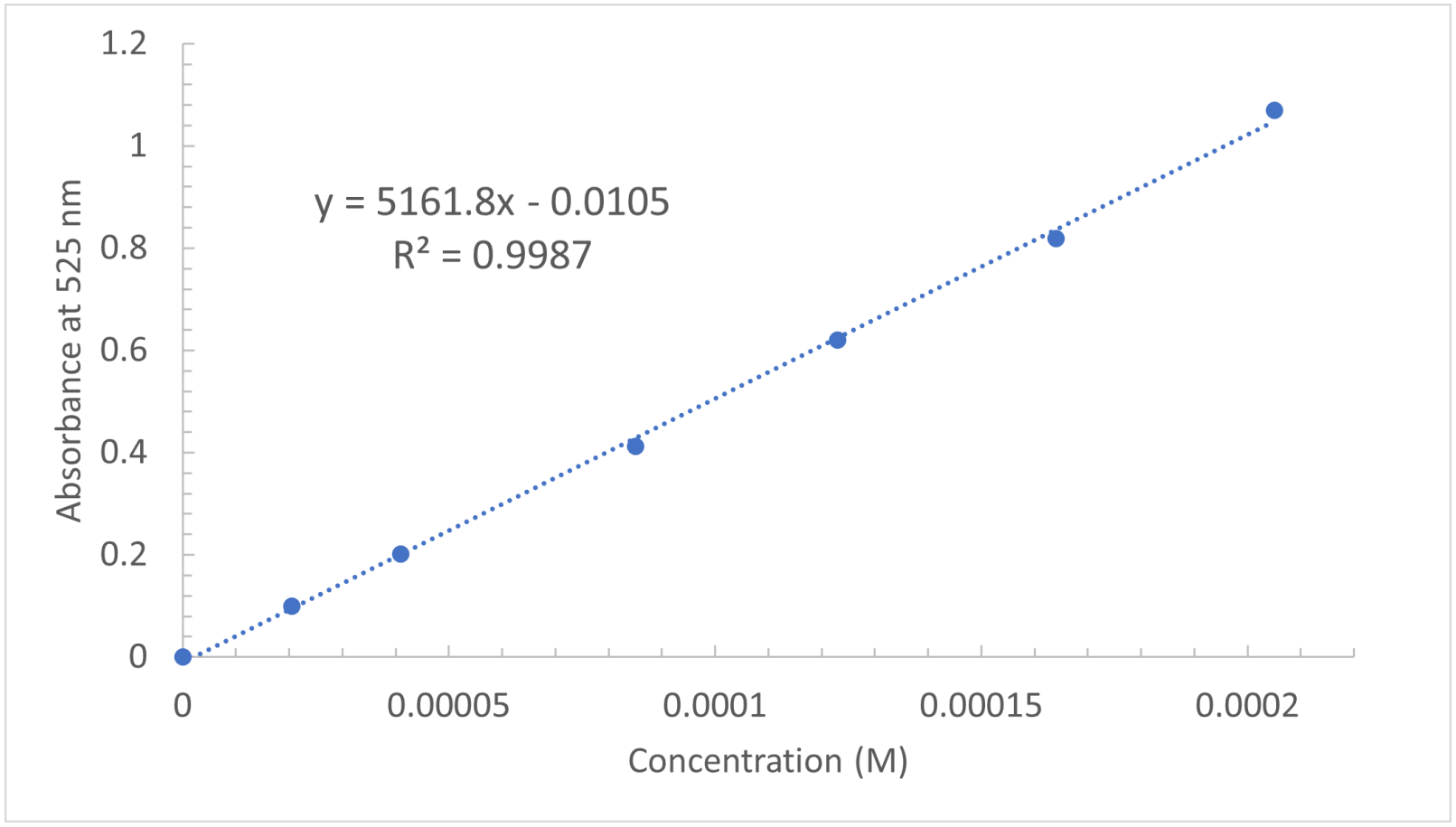

Figure 8.7: Example calibration curve for the red form of orange carotenoid protein. The line of best fit for the data is $y = 5161.8x - 0.0105$ with $R^2 = 0.9987$. The raw data is available as a supplementary resource.

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.

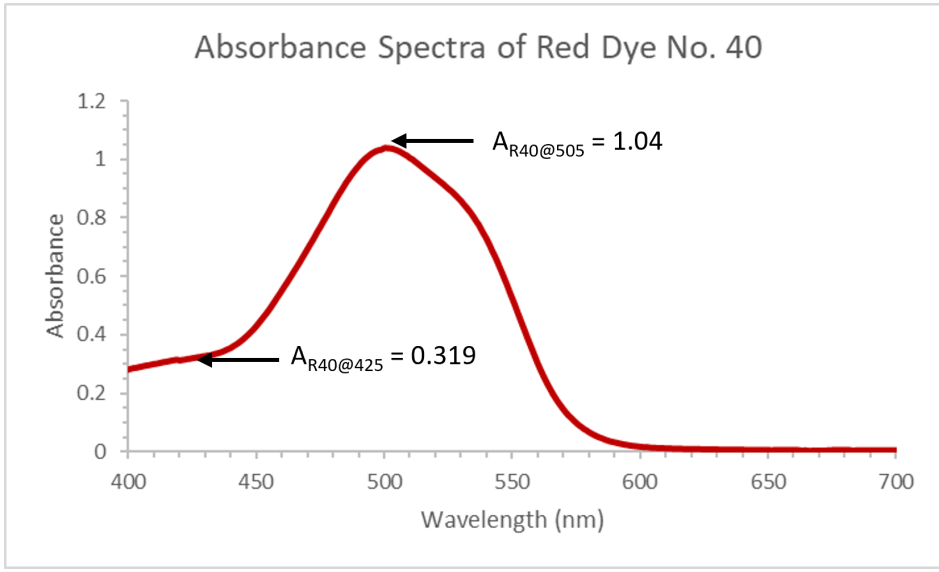

Figure 9.2: Absorbance spectra of Red Dye No. 40 highlighting important analysis locations. The raw data is available as a supplementary resource.

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.

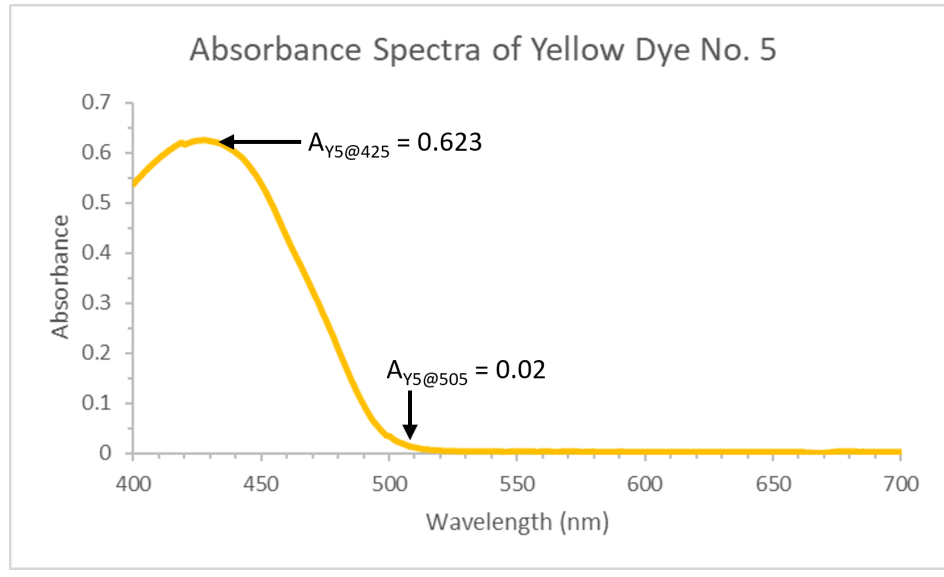

Figure 9.3: Absorbance spectra of Yellow Dye No. 5 highlighting important analysis locations. The raw data is available as a supplementary resource.

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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