Group: Spec 1
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Spectroscopy Lab

Chemistry Concepts:

1. Spectroscopy & Absorption Spectra: Using the Vernier spectrometer to identify λ max of a solution, which signifies the color a compound absorbs. Use Beer-Lambert's law to calculate the concentration of the initial solution.
2. Serial Dilutions: Use a volumetric flask to dilute a solution composed.
3. Real-life application: Berries & Anthocyanin: Test the absorbance of anthocyanin to explain the red color of fruits such as strawberries and blackberries which are household items.
4. Absorbance & Concentration: Utilize a spectrometer to show the linear relationship between absorbance and concentration.

Experimental Objectives:

1. Collect calibration curve data with a Vernier spectrovis spectrometer.
2. Determine the concentration of anthocyanins in an unknown solution.
3. Identify an unknown solution given a concentration and by finding the absorbance at λ max.

Materials and Instrumentation:

Materials for Part I:

1. Berries that contain Pelargonidin (a type of anthocyanin) (found in both strawberry and raspberries) SDS: https://cdn.caymanchem.com/cdn/msds/19753m.pdf
2. Isopropyl Alcohol (80 mL) - https://www.sigmaaldrich.com/US/en/sds/SIAL/W292912?userType=undefined
3. 150 mL Beaker
4. Glass Stir Rod
5. Gravity Filtration Apparatus (funnel and Erlenmeyer flask)
6. Filter Paper
7. 50 mL Beakers
8. 100 mL Beaker
9. Deionized (DI) Water (50 mL)
10. 10.00 mL Volumetric Flask
11. 10.0 mL Pipette

12. Cuvette(s) for Spectrometer
13. Vernier Spectrovis Spectrometer
14. LabQuest Device and adapter
15. Kimwipes (for wiping cuvettes)
16. PPE (Gloves & Safety Goggles, lab coat)
17. Waste Containers (for proper disposal of solutions)

General Procedure:

Part I:

1. **Safety Precautions:**
    - Obtain and wear goggles.
    - Be cautious to avoid ingestion or skin contact with any stock solution. Inform your instructor immediately in case of spills or accidents.
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2. Weigh out **10 grams** of the berry sample (strawberry or raspberry) and transfer to a **150 mL beaker**.
3. Add **80 mL of isopropanol** to the beaker and stir gently to break down the berries.
4. Set up a **gravity filtration apparatus** and filter the solution to obtain the anthocyanin-rich extract.
5. Transfer the extract into a **clean 50 mL beaker**
6. **Preparation of Standard Solutions:**
    - Add approximately 10 mL of the anthocyanin stock solution (extracted from strawberries) to a 50 mL beaker.
    - Fill another 100 mL beaker with about 50 mL of deionized (DI) water.
    - Label five clean, dry beakers 1–5 for your standard concentrations.
    - Use a two-fold serial dilution technique to create four diluted standard solutions. The first standard solution will be the original stock solution.

- As a group, calculate the concentration of each diluted solution (#2–5) and document the calculations with a photo uploaded to LabArchives.
- Insert a photo of the beakers containing your standard solutions into LabArchives.

7. **Spectrometer Calibration:**
    - Connect the spectrometer to the LabQuest and choose New from the File menu.
    - Calibrate the spectrometer:
        - Place a blank cuvette (containing DI water) in the spectrometer.
        - Select Calibrate from the Sensors menu.
        - The message will change to Warmup complete when ready.
        - Select Finish Calibration, then press OK when the message Calibration completed appears.
8. **Determining the Optimal Wavelength for Anthocyanin Absorption:**
    - Remove the blank cuvette and insert a cuvette containing the stock anthocyanin solution.
    - Start data collection. A full spectrum graph of the solution will be displayed.
    - Stop data collection and determine the wavelength of maximum absorption (λmax) for anthocyanin.
    - Insert a photo of your spectrum graph into LabArchives and note the maximum absorbance wavelength value.
    - Tap the meter button (top left tab), select mode, and choose "events with entry" to record data.
9. **Collecting Absorbance-Concentration Data for Standard Solutions:**
    - Start data collection by pressing the play button at the bottom left of the screen.
    - Empty the cuvette, rinse it twice with ~1 mL of the solution from beaker 1, and then fill it 3/4 full.

- Wipe the outside of the cuvette with a tissue and place it in the spectrometer.
- Once the value stabilizes, select Keep and enter the calculated concentration (in mol/L) for this sample. Select OK to save the data.
- Discard the cuvette contents as instructed. Repeat the rinsing and measurement process for beakers 2–5.
- Stop data collection when all standard solutions have been measured.

10. Analyzing Data and Generating a Standard Curve:

- To examine the collected data, tap any data point on the displayed graph. The absorbance and concentration values will be shown on the right side of the graph.
- Display a graph of absorbance vs. concentration with a linear regression curve:
    - Choose Graph Options from the Graph menu.
    - Select Autoscale from 0 and press OK.
    - Choose Curve Fit from the Analyze menu.
    - Select Linear as the Fit Equation.
    - The equation y = mx + b will be displayed, where x is concentration, y is absorbance, m is the slope, and b is the y-intercept.
    - Check the correlation coefficient (r^2) value to assess the accuracy of the linear fit (a value near 1.00 indicates a strong fit).
    - Select OK and insert a photo of your graph into LabArchives.

11. Determining the Absorbance of an Unknown Sample:

- Tap the Meter tab.
- Obtain about 5 mL of an unknown anthocyanin solution in a clean, dry beaker. Record the sample number in your data table.
- Rinse the cuvette twice with the unknown solution and fill it 3/4 full.

- Wipe the outside of the cuvette and place it in the spectrometer.
- Monitor the absorbance value. Once stabilized, record it in your data table.

12. Repeat for the Raspberry Stock Solution

- Repeat Steps 2-11 with the raspberry stock solution.
- There should now be two calibration curves, one for strawberry and one for raspberry.

Part II:

1. Measure the absorbance of an unknown berry extract (the concentration is known though). Use the calibration curve and Beer's Law to identify whether the solution was made using strawberries or raspberries (test the equations found in part I)

2. Another cuvette contains a berry extract where the identity is known, but the concentration is not. To find concentration, determine the absorbance value of the unknown solution.
    a. Tap the Meter tab.
    b. Obtain about 5 mL of the *unknown* in another clean, dry, beaker. Record the number of the unknown in your data table.
    c. Rinse the cuvette twice with the unknown solution and fill it about 3/4 full. Wipe the outside of the cuvette and place it into the device.
    d. Monitor the absorbance value. When this value has stabilized, record it in your data table.

Waste Disposal/Safety Plan:

- **PPE:** Use gloves and safety goggles when handling solutions.
- Gloves are put in the marked container in the front desk
- Only water goes down the drain
- Any other non-water liquids go in the container under the hood
- Paper towels can go in the trash can
- Plastic single-use equipment (eg. pipettes) go in the marked container in the front desk

Minimizing Risk:

- Avoid open flames, replacing the flame test with colorimetric analysis.
- All chemicals, with or without inhaling hazards, are recommended to be placed under the fume hood.
- Keep work area clean and organized to prevent any sort of incidents inside the lab.
- Triple rinse all contaminated glassware and dispose of all waste according to waste disposal plan.

Data Tables:

Part I:

Table 1: Concentration and Absorbance of the Strawberry Stock Solution

Sample #	**Concentration (mol/L)**	**mL Previous Solution**	**Final Volume (mL)**	**Absorbance**
Sample 1 (No Dilution)				
Sample 2				
Sample 3				
Sample 4				
Sample 5				

Part II:

Data Table 2: Identifying the Berry Used in an Unknown Solution

Unknown Sample #	Absorbance at λmax (AU)	Berry Identified (Strawberry/Raspberry)	Based on Calibration Equation
			y = mx + b (from Part 1)

Data Table 3: Determining the Concentration of an Unknown Solution

Unknown Sample #	Absorbance at λmax (AU)	Calibration Equation Used	Calculated Concentration (M)
		y = mx + b	________ M

****New reference**

Dangles, Olivier. Anthocyanins as Natural Food Colorings: The Chemistry Behind and Challenges Still Ahead. Journal of Agricultural and Food Chemistry. 2024. https://pubs.acs.org/doi/10.1021/acs.jafc.4c01050 (accessed April 1, 2025).

- This article provides information about anthocyanins, the chemical compounds that give berries red, purple, and blue coloring. The article gives examples of the chemical spectroscopy of anthocyanins. It discusses the chemical properties and high reactivity as well as the drawbacks of the compounds.