Experiment 37B-2

SPECTROSCOPIC ANALYSIS OF DYES – MORE THAN PRETTY COLORS

PART 2

MATERIALS: FD&C food stock solution (Red), 100 mL beaker, 50 mL buret, buret funnel, 4 100 mL volumetric flasks, Spectronic 200, 5 test tubes, cuvettes, unknown.

PURPOSE: The purpose of this experiment is to understand how light interacts with a dye solution and to use this knowledge to determine the amount of dye in an unknown.

LEARNING OBJECTIVES: By the end of this experiment, the student should be able to demonstrate the following proficiencies:

1. Prepare diluted solutions and calculate their concentrations.
2. Understand what an absorbance spectrum is and select $\lambda_{\text{max}}$.
3. Relate the color observed of a solution to its $\lambda_{\text{max}}$ value (and color absorbed).
4. Understand Beer’s Law and what factors affect it.
5. Create a calibration curve using standard solutions.
6. Determine a concentration of an unknown using a calibration curve.

PRE-LAB: Complete the pre-lab questions on pages E37B2-5 and E37B2-6 before lab. Bring your laptop to lab.

DISCUSSION: Colorimetric analysis, or colorimetry, is an important analytical technique in chemistry. It allows an analyst to quantify the amount of a substance of interest (analyte) in a solution based on color properties. Colorimetric tests are not just used in the chemistry lab. For instance, it is important to quantify the amount of chlorine present in a swimming pool. Pools are chlorinated to disinfect the water. If chlorine levels are too low, harmful microorganisms can thrive. If the level of chlorine is too high, the water can be irritating. Chlorine test strips are commercially available. By comparing the test strip to a set of known standards, it is possible to quantify the chlorine in the pool and adjust the pool chemistry as needed.

While sometimes it is sufficient to determine only whether an analyte is present or the approximate amount of analyte present, it is important to be able to measure a precise amount of analyte present in a sample. The quantitative relationship between absorbance and concentration is one method to determine the amount of analyte present. This relationship takes the form of Beer’s law. Beer’s law states that

$$A = \varepsilon l c$$

where $A$ is the absorbance of the solution, $\varepsilon$ is the molar absorptivity of the analyte, $l$ is the path length of the spectrophotometric cell, and $c$ is the concentration of the analyte in the solution. By constructing a Beer’s law plot of absorbance vs. concentration, the value of $\varepsilon l$ can be determined; it is the slope of the line. Because the path length of the spectrophotometric cell can be measured, it is possible to calculate the molar absorptivity, $\varepsilon$, of the solution.

The molar absorptivity ($\varepsilon$) is an important proportionality constant. If you measure the absorbance of a solution of unknown concentration, then you can determine its concentration by rearranging Beer’s Law and solving for concentration. To do this, the value of molar absorptivity must be known. Molar absorptivity depends on wavelength, so it is important to conduct all of your analyses at the same wavelength. Beer’s law plots are frequently used in colorimetric analysis because they provide the simple relationship between absorbance and concentration.

To compare two solutions, it is not necessary to measure the entire absorbance spectrum. Rather, absorbance (or %T) can be measured at a specific wavelength. Usually, $\lambda_{\text{max}}$ is chosen because it produces the maximum response from

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1 This lab is based on “Spectroscopic Analysis of Food Dyes” by Barbara A. Reisner, Joycette Santos-Santori, Dawn Rickey, and Melonie Teichert.
the spectrophotometer. In other words, it is the wavelength at which absorbance is the most sensitive to concentration changes. This allows you to work with less concentrated solutions.

In this part of the experiment, you will measure the absorbance of four “standard solutions” of known concentration and construct a Beer’s Law plot from your data. Finally, you will determine the amount of FD&C Red No. 3 dye contained in a sample of a powdered drink mix or a prepared sample.

PROCEDURE:

Work with a partner.

Part A. Preparation of Standard Solutions

You will start with a solution of Red Dye No. 3 with a concentration of $1.36 \times 10^{-4}$ M (used in week 1). This is the stock solution. You will use this solution to prepare Standard Solutions A – D. Both partners should be preparing the solutions to save time. Part of your lab grade will be based on the accuracy of determining the concentration of Red Dye in your unknown so work accurately and precisely.

1. In a small dry beaker, obtain about 40 mL of the stock Red dye solution (not the unknown).
2. Pre-rinse a 50 mL buret with a small amount of the Red dye stock solution, discarding the rinses. Fill the buret and make sure the buret tip has no air bubbles in it. If your buret is filled to about the 15 mL line, that should be enough.
3. Record the initial buret reading with the correct number of significant figures and units. A light background may help in seeing the lines on the buret.
   
   Initial buret reading = _________________________________

4. Using the buret, transfer about 9 mL of the stock solution into a 100.00 mL volumetric flask. Let the buret tip drain freely, touching it to the side of the flask upon the last drop. It’s not important that you get exactly 9.00 mL but that you know the amount you added (based on the buret readings; try to get around 9 mL).
5. Record the final buret reading and volume added.

   Final buret reading = ______________________

   Total Volume of stock solution added to flask = ______________________

6. Add distilled water to the mark of the volumetric flask, using a dropper to reach the fill line. Stopper the flask and invert it at least 5 times to mix the solution. Label this Solution A.

   Calculate the concentration of Standard Solution A (in units of Molarity, report to 3 significant figures):

7. Repeat this process adding about 7 mL of the stock solution to another 100.00 mL volumetric flask. Add distilled water to the mark, using a dropper to reach the fill line. Stopper the flask and invert it at least 5 times. Label this Solution B.

   Initial buret reading = ______________________

   Final buret reading = ______________________

   Total Volume of stock solution added to flask = ______________________

   Calculate the concentration of Standard Solution B:
8. Repeat this process adding about 5 mL of the stock solution to another 100.00 mL volumetric flask. Add distilled water to the mark, using a dropper to reach the fill line. Stopper the flask and invert it at least 5 times. Label this Solution C.

   Initial buret reading = _________________    Final buret reading = _________________

   Total Volume of stock solution added to flask = ____________________

   Calculate the concentration of Standard Solution C:

9. Repeat this process adding about 2 mL of the stock solution to another 100.00 mL volumetric flask. Add distilled water to the mark, using a dropper to reach the fill line. Stopper the flask and invert it at least 5 times. Label this Solution D.

   Initial buret reading = _________________    Final buret reading = _________________

   Total Volume of stock solution added to flask = ____________________

   Calculate the concentration of Standard Solution D:

10. All your standard solutions (A-D) should now be prepared (fully mixed and homogeneous).
11. Obtain the Red Dye Unknown from your instructor. Fill a test tube about half to 2/3 full. Place it in a test tube rack.
12. Transfer each of your prepared standard solutions to 4 separate test tubes. Make sure you know which solution is contained in each test tube. Place the test tubes in a test tube rack.
13. As an estimate, compare the color intensity of your unknown with the standards. Using a white background behind your solutions should help distinguish intensities. Guess at a concentration for the unknown based on this comparison. Record your guess on the data table.

Part B. Absorbance Measurements
1. Using the directions by the instrument, calibrate the Spectronic 200 to 0% transmittance and to 100% transmittance with distilled water as your blank.
2. You will now configure the Spectronic 200 to measure the absorbance readings of a sample. Set the Spectronic 200 to the λ_{max} value for the Red dye (from Week 1) by turning the wavelength knob. Record the wavelength used. Do not change the wavelength after this is set.
3. Set the Spectronic 200 to absorbance mode.
4. Measure and record the absorbance of each of your solutions starting with the most dilute solutions first. Rinse the cuvette between samples and make sure the cuvette is clear of fingerprints.
5. Make sure your data make sense before discarding your solutions. Should absorbance increase or decrease with increasing concentration? Does the absorbance for your unknown fall within the range of the absorbances of the standards? Does the value make sense?
6. Clean up all glassware and return them to their proper locations. Dye solutions can be disposed down the drain with water. Make sure to rinse out the cuvettes well. Turn off the Spect200.
7. Start working on the calculations.
DATA AND ANALYSIS
Experiment 37B-2

\( \lambda \) used = ______________________________

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Concentration (M)</th>
<th>Absorbance at ( \lambda ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Solution A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Solution B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Solution C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Solution D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>Your Guess</td>
<td></td>
</tr>
</tbody>
</table>

1. Using Microsoft Excel, construct a Beer’s Law plot of Absorbance (y) vs Concentration (x) using the 4 standard solutions. Label the axes. This Beer’s Law plot is also known as a CALIBRATION CURVE.

2. Construct a trendline through the data. Include the equation of the line and \( R^2 \) value on the plot.

   Equation of the line \( \rightarrow \) __________________________________________________________

   If the pathlength \( l \) of the cuvette is 1.00 cm, what is the value for the molar absorptivity \( \varepsilon \) for this Red dye at \( \lambda_{\text{max}} \)? Report the value with units.

   \( \varepsilon = \) __________________________________________________________

   Submit your properly formatted spreadsheet and graph with your report.

3. Given the absorbance for the unknown, solve for the concentration of the unknown (in M). Use your equation of the line above (not the Beer’s Law equation which assumes a zero y-intercept). Show your work below.

4. How close was your guess to the calculated concentration of the unknown?

5. Given your concentration of the unknown from #3, what if you used this same solution and measured its absorbance in a larger cuvette (i.e., a pathlength \( l \) of 2.00 cm instead of 1.00 cm). How would a larger cuvette change the absorbance you measure at \( \lambda \) (increase, decrease, stay the same)? Explain your answer.

6. Explain the purpose of a calibration curve in a quantitative analysis.
Complete these questions before lab.

1. a. You will be preparing diluted standard solutions from a stock solution of known concentration. Suppose you had a Stock Red Dye solution with a concentration of $4.00 \times 10^{-4}$ M.

If you added 10.00 mL of the stock solution to a 100.00 mL flask and diluted it to the line, what would be the concentration of this standard solution (call it A)? Report with 3 significant figures. (Hint: this is a dilution.)

Concentration of Standard Solution A = _______________________________

If you added 7.00 mL of the stock solution to a 100.00 mL flask and diluted it to the line, what would be the concentration of this standard solution (call it B)?

Concentration of Standard Solution B = _______________________________

If you added 5.00 mL of the stock solution to a 100.00 mL flask and diluted it to the line, what would be the concentration of this standard solution (call it C)?

Concentration of Standard Solution C = _______________________________

If you added 2.00 mL of the stock solution to a 100.00 mL flask and diluted it to the line, what would be the concentration of this standard solution (call it D)?

Concentration of Standard Solution D = _______________________________

b. What was $\lambda_{\text{max}}$ for Red dye No. 3 (from Week 1)? Include units. 

$\lambda_{\text{max}}$ = _______________________________

c. How do you predict the absorbances of each of the 4 standard solutions above (Solutions A-D) will compare at $\lambda_{\text{max}}$ for the Red dye? Will they be the same or different? Explain.

d. How do you predict the absorbances of each of the 4 standard solutions above will compare at 650 nm? Will they be the same or different? Explain. (Hint: review your absorbance spectrum for Red from week 1 to see how Red dye solutions behave at 650 nm.)
2. Use a **molecular-level** model to answer the question: “Which absorbs more light, a concentrated Blue solution or a dilute Blue solution, and why?” Draw pictures and **clearly explain your thinking in words.** (Distinguish between this model for a Blue solution and your previous refined model for a Red solution.)

3. On the graph below, sketch the expected function of absorbance versus concentration at $\lambda_{\text{max}}$. (Is it a straight line, curved, periodic? Is it increasing or decreasing or flat?)
   
   The function in the graph below is described by what law (you will be using in this lab)? ______________