SPECTROSCOPIC ANALYSIS OF DYES – MORE THAN PRETTY COLORS

MATERIALS:  
FD&C food stock solution (Red), 100 mL beaker, volumetric pipets (one each of 1, 2, 5, 10 mL), five 100 mL volumetric flasks, Spectronic 20, 6 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.
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 page E37B2-5 before 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 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.

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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.
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. This is the **stock solution**. You will use this solution to prepare Standards A – D. (Pre-rinse pipets as necessary.) 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 25 mL of the stock Red dye solution.
2. Use a volumetric pipet to transfer 10.00 mL of the stock solution to a 100.00 mL volumetric flask. Let the pipet tip drain freely, touching it to the side of the flask. Do not blow out the liquid remaining in the tip. Add distilled water to the mark, 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 Solution A (in units of molarity, report to 3 significant figures):

3. Use a pipet to transfer 7.00 mL of the stock solution to a 100.00 mL volumetric flask (use the 5.00 mL and 2.00 mL pipets). 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**.

   Calculate the concentration of Solution B:

4. Use a pipet to transfer 5.00 mL of the stock solution to a 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**.

   Calculate the concentration of Solution C:

5. Use a pipet to transfer 2.00 mL of the stock solution to a 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**.

   Calculate the concentration of Solution D:

6. Transfer each of your prepared standard solutions and the unknown to 5 separate cuvettes, pre-rinsing each cuvette (the round cuvettes are used here). Each cuvette only needs to be about 2/3 full with sample. Make sure you know which solution is contained in each cuvette. Place the cuvettes in a test tube rack.
7. Fill one cuvette with distilled water after pre-rinsing the cuvette.
8. As an estimate, compare the color intensity of your unknown with the standards. Using a white background behind your solutions should help distinguish intensities. Estimate a concentration for the unknown based on this comparison. Record your guess on the data table.
9. Make sure to wipe off the outside of each cuvette with a soft tissue to remove fingerprints or liquid.
Part B. Absorbance Measurements

1. Set the Spectronic 20 to the $\lambda_{\text{max}}$ value for the Red dye shown in the prelab by turning the Wavelength knob. Record the wavelength used. Do not change the wavelength after this is set.
2. Set the Spect20 to the %Transmittance Mode by pressing the Mode button.
3. With nothing in the sample compartment and the lid closed, set 0% T with the left knob (labeled 0% T).
4. Place the distilled water cuvette (the blank) into the sample compartment, close the lid and set 100% T with the right knob (labeled 100%T).
5. After removing the blank and with the lid closed, the signal should go back to 0%T. The Spect20 has been calibrated. Spect20’s require recalibration with a blank whenever the wavelength has changed or the knobs have been moved.
6. Set the Spect20 to the Absorbance Mode. With no sample, the display will be flashing. In Absorbance Mode, the sample absorbance reading will be displayed directly on the monitor. There are no units for absorbance.
7. Measure and record the absorbance of each of your solutions.
8. 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?
9. 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 Spect20.
10. 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 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>Your Guess ( \rightarrow )</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 standards. 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.

   \( \varepsilon = \) ________________________________

3. Given the absorbance for the unknown, solve for the concentration of the unknown. 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. Explain the purpose of a calibration curve in a quantitative analysis.
Complete these questions before lab.

1. a. Read the lab procedure and calculate the concentrations of Red dye Standards A-D (which you will prepare in lab).
   Show your work on page E37B2-2, and record your final values here (to 3 sig figs, and with units).
   - Concentration of Standard A = _______________________________
   - Concentration of Standard B = _______________________________
   - Concentration of Standard C = _______________________________
   - Concentration of Standard D = _______________________________
   Note: these concentrations are quite small. Not much dye is needed to see a color.

   b. Inspect the absorbance spectrum for red dye No 3 shown below. What is the approximate value of the $\lambda_{\text{max}}$, that is, the wavelength at which the dye absorbs most strongly? Include units.
   $\lambda_{\text{max}} = _______________________________

   c. How do you predict the absorbances of each of the 4 standards 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 standards will compare at 650 nm? Will they be the same or different? Explain.

2. Examine the Beer’s law plot on the right and use the information provided to obtain the extinction coefficient, $\varepsilon$, for salicylic acid. Assume a pathlength of 1.0 cm. Provide the correct units for $\varepsilon$. 

\[ y = 856.09x + 0.0169 \]
\[ R^2 = 0.9967 \]