Spectrophotometric Analysis

Overview:

Spectrophotometry can be used in as an analytic tool in two significant ways. The light absorbed by a particular species will produce a pattern called a spectrum. This pattern as a graph of amount of light absorbed (Absorbance) vs. wavelength can be used to identify a particular molecule. Spectroscopy can also be used to determine the amount of a particular species that is present in a sample as the amount of light absorbed will be related to the number of light absorbing molecules in the sample. In this series of experiments, you will investigate each of these relationships using samples of food dyes, then you will use visible spectroscopy to investigate the purity of the aspirin you synthesized at IUP. You will also use three other common techniques to assess the purity of your aspirin: Thin Layer Chromatography, Titration, and Melting Point.

Goals of these experiments:

Spectrophotometry of Food Dyes

• Prepare a standard food dye solution.
• Prepare a set of diluted dye solutions.
• Obtain a UV-Visible Spectrum of the dye solutions.
• Determine the concentration of an unknown dye solution using “Beer’s Law.”
• Obtain the spectrum of a mixed dye sample.
• ID the components of black dye.

Analysis of Aspirin

• Produce a complex of Fe$^{3+}$ and salicylic acid that absorbs visible light.
• Use spectroscopy and Beer’s Law to determine the purity of synthesized aspirin.
• Use TLC, Titration and Melting Point to determine the purity of synthesized aspirin.
Spectrophotometry of Food Dyes

(Anne Kondo, Wendy Elcesser and John Woolcock: 1/21/05)

Pre-laboratory Assignment

NAME:_______________________

Before coming to lab, read the experiment and complete these exercises in the space provided.

1. Refer to a color wheel and a wavelength table of the visible spectrum to determine:

   (a) The color that is complementary to violet

   (b) The color observed by your eye if all the violet light was absorbed by a sample

   (c) The color of light corresponding to 500 nm

   (d) The color observed by your eye if light corresponding to an absorbance maximum at 500 nm was absorbed by a sample

   (e) The absorption spectrum of chlorophyll A has absorbance maxima at 663 nm and 420 nm. What colors correspond to each of these wavelengths? What color will be transmitted or reflected from chlorophyll A and seen by your eyes?

2. Determine the energy of light with a wavelength of 500 nm.

3. A sample absorbs 72% of the light passing through it at a certain wavelength. What percent is transmitted? Calculate the absorbance, A, of the sample.
4. How would you expect the absorbance of a 25% grape Kool-aid sample to compare to a 50% grape Kool-aid sample? Explain.

5. Why would visible spectroscopy NOT be an appropriate analytical technique to use when determining Na⁺ content in sea-water?

6. What is the purpose of using a “blank” before making spectrophotometer measurements?

7. Why should you keep the outside of the cuvette clean?
Background: Why do some solutions have a color?

In this experiment group, solutions of dyes and transition metal complex will be examined. Both have the common feature of having a color. Many solutions in chemistry appear to be colorless. For example, salt-water (NaCl in water) is a colorless solution. Note that the word "clear" applies to solutions that light can pass through - thus, we can have clear, colorless solutions, and clear, colored solutions.

When light shines on a group of molecules or ions, these species can absorb light of various wavelengths resulting in electron transitions in those absorbing species. In the case of solutions with a color, the energy of these transitions corresponds to wavelengths of light in the visible region of the electromagnetic spectrum.

![Electromagnetic Spectrum Diagram](image)

It is possible that a species will interact with only light outside of the visible region of the spectrum. In the case, no color will be observed, as the human eye is only sensitive to the visible region of the spectrum.

Since the visible spectrum is a combination of all colors (primary colors red, yellow, and blue which can be combined to produce secondary colors orange, green, and violet), light from a typical bulb appears “white”. An "artist's" color wheel is a useful way show to these relationships. If you add the colors on opposite sides of the wheel together, white light is obtained. We only detect colors when one or more of the wavelengths in the visible spectrum have been absorbed, and thus removed, by interaction with some chemical species. When the wavelengths of one or more colors are absorbed, it is the colors on the opposite side of the color wheel that are transmitted. We call opposite colors on the color wheel "complementary colors".
When light passes through a solution containing molecules with particular structures, as in the case of the dyes or transition metal complexes as is the case in the aspirin and iron (III) ion, we see the color of the wavelength of the light that is transmitted.

For more background on transition metals:

http://www.chemguide.co.uk/inorganic/complexions/colour.html

for more background on dyes and color:

http://www.chemguide.co.uk/analysis/uvvisible/theory.html
Spectroscopy

A UV-visible spectrum shows a plot of the absorbance of light by the sample versus the wavelength of light in nm. We expect to see peaks (called absorption bands) at wavelengths corresponding to the complementary color of the absorbing species. It is also possible to plot % transmittance for the sample. In contrast to absorbance, transmittance is a measure of the light that passes through a sample. The intensity of the absorption band is measured by the percent of the incident light that passes through the sample:

\[(1) \% \text{ Transmittance} = \left( \frac{I}{I_0} \right) \times 100\% \]

where:

- I = intensity of transmitted light
- I₀ = intensity of incident light

Absorbance and transmittance are related by:

\[(2) \text{ Absorbance} = -\log\left( \frac{I}{I_0} \right) = 1 - \log(\%\text{Transmittance}) \]

Because light absorption is a function of the concentration of the absorbing molecules, a more precise way of reporting intensity of absorption is by use of the Beer-Lambert Law:

\[(3) \text{ Absorbance} = \varepsilon cl \]

where:

- ε = molar absorptivity, a constant dependent on the absorbing species
- c = molar concentration of absorbing species
- l = length of sample cell (cm)

Equation 3 is known as the Beer-Lambert Law, or sometimes simply as "Beer's Law".

In these experiments, we will be most interested in the fact that absorbance is directly related to concentration.
Procedure: Experiment 1

Part I: Formation of Groups

For group discussion and data analysis, this experiment is best done in groups of 2-3.

Part II: Sample Preparation and Spectra of Standard Solutions of Food Dye of One Color

You will be assigned red, yellow, or blue food dye. Prepare a stock solution by mixing 1.0 ml of the dye concentrate with water in a graduated cylinder to make 100 ml of the solution. Be sure the solution is well mixed. Label this solution as “Stock Dye Solution”. Be sure to obtain the concentration of the dye concentrate.

Using a pipet and 10.0 mL graduated cylinder, prepare four standard dye solutions using the stock you prepared and water. Once mixed, transfer each standard to a test-tube and label them appropriately. (You will have many solutions today, and labeling is key!) Use table 1 below as a guide. The total volume of each solution is 10.00 ml.

Table 1: Composition of Standard Solutions

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Volume stock (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.00</td>
</tr>
<tr>
<td>B</td>
<td>3.00</td>
</tr>
<tr>
<td>C</td>
<td>2.00</td>
</tr>
<tr>
<td>D</td>
<td>1.00</td>
</tr>
<tr>
<td>E</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Obtain an “unknown dye” solution (sample E) that corresponds to your dye color. This sample may require dilution as it is probably too concentrated for the spectrophotometer to accurately determine an absorbance. You can dilute the sample using a small syringe.

Obtain six plastic "cuvettes" (one is to be used as a "blank", four for the standards, and one for the unknown) and obtain the spectrum of your samples. A "blank" is a cuvette that contains any solvents in which you are not interested. In this experiment, you diluted dye in water. It is the dye that is of
interest, and so the blank cuvette should contain just water. The spectrophotometer will automatically subtract any absorbance by the blank from the absorbance of your sample, so your final signal is just the dye.) You will need to set up the spectrophotometer according to the instructions provided. Check the report tutorial to determine what information you need to obtain from your spectra. Print the spectra obtained by your group. All spectra need to be appropriately labeled. Save sample B and your stock solution for later use.

**Part III: Beer’s Law Plot and Determination of an Unknown Concentration**

Select an analytical wavelength for your particular dye and obtain the value of the absorbance at that wavelength for samples A-D and the unknown, E. Plot absorbance vs. concentration for the dye. Use the plot and appropriate calculations to determine the concentration of the unknown dye sample.

**Part IV. Sample Preparation and Spectra of Dye Mixtures**

Using a different color dye stock solution from another group, prepare a solution by adding 3.00 ml (using a pipet) of this stock to a graduated cylinder and then adding water to achieve a total volume of 10.00 ml. Label this solution “F”. Prepare a mixture of the two dye solutions by adding 3.00 ml of sample B + 3.00 ml of sample F + enough water to achieve a total of 10.00 ml. Label this solution “G”. Obtain spectra of solutions B, F, and G. Also note the color of each of these solutions.

**Part V. Black Dye Problem**

Design and conduct an experiment to determine if the two dye colors for which you obtained spectra are present in black dye.
Spectrophotometry of Food Dyes Report Tutorial

(AEK/JCW: 1/28/2013)

Report Submitted by ______________________

Date Submitted ______________________

Purpose: In this experiment you will investigate how the color of a dye affects the absorption of light (as a function of the wavelength of the light), how the concentration of a dye affects the amount of light absorbed at a given wavelength, how mixing two dyes affects the amount of light absorbed as a function of wavelength, and if it is possible to identify dyes in a mixture.

Procedure Notes:

In the space below,

• make a list of the students in your group and

• describe in one or two sentences the major roles each one played in collecting the data for this experiment.

• describe the procedure you developed for Part V of the experiment.
Data and Results For Part II: Sample Preparation and Spectra of Standard Solutions of Food Dye of One Color

Color of dye assigned (dye #1): _______________

**TABLE I**: Maximum Absorbance and Analytical Wavelength for the Dilute Dye Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Maximum Absorbance</th>
<th>Analytical Wavelength* (nm)</th>
<th>Solution concentration (g dye/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The analytical wavelength, $\lambda_{\text{max}}$, is the wavelength where the absorbance is a maximum.

**Part II Analysis:**

(a) The solution of the dye appears ______________________ when it is more diluted.

(b) Does lambda max change significantly upon dilution with water? Explain.

(c) Describe the variation in the absorbance readings for the solutions A, B, C and D at $\lambda_{\text{max}}$. What effect does dilution have on the value of the absorbance at $\lambda_{\text{max}}$?
Data and Results for Part III: Beer’s Law Plot and Determination of an Unknown Concentration

Document all work done to determine the concentration of the unknown dye sample.
Part IV: Sample Preparation and Spectra of Dye Mixtures

Obtain samples of dye # 2 from another group.

Color of dye #1:__________     Color of dye #2 :__________     Color of mixture:________

TABLE II: Absorbance and Analytical Wavelength for Dye #1, Dye #2 and the Dye Mixture.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Maximum Absorbance</th>
<th>Analytical Wavelength* (nm)</th>
<th>Other significant absorbances and their wavelengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G (Mixture of B and F)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Part IV Analysis

(a) Examine the absorbance values for the solutions B, F and G. In particular, look at the absorbance readings of each solution at the $\lambda_{\text{max}}$ values for each individual dye. Do the absorbance values change as a result of mixing the samples? Explain.

(b) Compare wavelength values for the individual dyes and the mixture. What effect did mixing the dye solutions have on the value(s) of $\lambda_{\text{max}}$ of the mixture compared to the $\lambda_{\text{max}}$ of the individual colors?

(c) Using the wavelengths of light, explain why you observe the color you do for the mixture.
Data and Results for Part V

Explain how your spectra help you answer the question "are the two dye colors for which you obtained spectra present in black dye?"
Conclusion

1. **BRIEFLY** summarize what you did in this experiment using a bulleted list.

2. For the samples listed, complete the table and establish the relationship (if any) between the observed color and the color (wavelength) of light absorbed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color observed</th>
<th>Color of light absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. In Parts II, you examined the effect of dilution on absorbance, and used your standard solutions to apply Beer's Law in Part III. Use your Beer’s Law plot to discuss whether or not your data supports Beer’s Law.
4. In Part IV, when you mixed your dye #1 solution with different colored dye solution, the observed color changed. Summarize how this changed the spectrum, relative to the spectra of the individual dyes. Summarize what remained unchanged.

5. Did mixing the dye solutions in Part III result in a chemical change or a physical change? Provide evidence to support your answer.

6. Does the black dye contain either or both of the food dye colors that you analyzed? Provide evidence to support your conclusion.
Analysis of Aspirin

Background

**Melting point** is a physical property inherent to a substance’s identity. *Point* is actually a misnomer, substances actually melt over a range. (The purer the substance, the smaller the melting range) The broader range of an impure sample (a mixture) results from the creation of a solution upon melting. A mixture’s melting point is a **colligative property**, and is, therefore, dependent on the ratio of chemicals present. For this experiment, assume ASA (Acetylsalicylic Acid) is a solvent and any impurities play the role of solute. (The more impurities present in the sample, the lower the melting point)

Traditionally, this phenomenon is described as *freezing point depression*, but in this experiment the solvent, ASA, is a solid at room temperature and is heated to melt... not cooled to freeze. In this experiment, you will measure the melting point of your crude product and a crushed aspirin tablet. These can be compared to the literature value.

A solution of ASA is colorless and, therefore, does not absorb light in the visible region between 400 and 700 nm. After treatment with a strong base, a hydrolyzed and deprotonated ASA fragment (the conjugate base of salicylic acid, SA) is able to bind with Fe³⁺ ions in acidic solution. A solution of the resulting cationic Fe³⁺-SA complex is deeply colored.

When white light (light containing all wavelengths from 400 to 700 nm) passes through a Fe³⁺- SA solution, wavelengths corresponding to green light are strongly absorbed and the solution appears purple. This absorption is a result of the excitation of valence electrons to higher energy electron orbitals. Therefore, the intensity of light at the absorbed wavelengths is reduced passing through solution; the amount of reduction is dependent on the concentration of the absorbing species and the distance the light travels through the solution (path length). This linear dependence is known as the Beer-Lambert Law (or "Beer's Law").

*Conversion of ASA to Fe³⁺ - SA*

![Conversion of ASA to Fe³⁺ - SA](image)
Finally, the number of impurities in different samples will be determined by thin layer chromatography. Silica will serve as the stationary phase and an organic solvent will be the mobile phase. The different compounds in aspirin move up the TLC plate at different rates based on the competing intermolecular interactions of the polar silica plate and the less polar solvent. More polar compounds adsorb more strongly to the silica and do not move as far up the TLC plate. Less polar components will favor the solvent and move higher. To The TLC of the ASA you synthesize in lab will be compared with the TLCs of the salicylic acid starting material, and an aspirin tablet.

**Procedure I: Melting Point Determination**

1) Prepare all samples by pulverizing them with a mortar and pestle.

2) Pack the sample into a melting point tube to a depth of about 1 cm.

3) Warm the tube and sample in a mineral oil bath and record the temperature when the sample melts.

**Procedure II: TLC**

Thin Layer Chromatography (TLC): *Chromatography* is an analytical method of separating a mixture into its molecular components. In thin layer chromatography (TLC), a glass or plastic plate with either silica or alumina adsorbed onto them is used. A capillary tube is used to apply a small sample (usually in the ng-µg scale) of the mixture onto the plate. The TLC plate is then placed in a developing jar containing a small amount of eluent (solvent). The solvent climbs up the silica or alumina on the plate and moves the pigment molecules of the mixture along at different rates. The greater the attraction between the molecules and the absorbing medium (coated on the plate), the slower the molecules ascend the coating. The greater the solubility of the components in the solvent, the greater the distance the molecules move.

1) Place a piece of 5.5 cm filter paper into a TLC developing jar. Pour ~5 mL of ethyl acetate into the jar (should be 0.5 cm from bottom of jar) and close with a cap. (Take only the ethyl acetate you need and do not return unused solvent to the bottles in the hood.)

2) Prepare the TLC plate by lightly drawing a line on the plate 1 cm from the bottom with a pencil. Be careful not to scratch the silica surface. This is the origin line.

3) Place 5 drops of ethyl acetate into a small beaker or vial. Dissolve as much of the crude aspirin sample into the ethyl acetate as possible. If the ethyl acetate evaporates before the next step or you put in too much solid, add the minimal number of drops required to redissolve all solid. (Note: The objective of this step is to create a very concentrated solution to put onto the TLC plate, but a lot of solution is not needed and you don’t want to waste sample.)

4) Repeat the above step the aspirin tablet and pure SA.
5) Your instructor will demonstrate how to use TLC spotter. Use a new TLC spotter for each sample to transfer it drop by drop to the origin line on the plate. The spots should be evenly spaced and the right size so that samples will not run together. (Make the spots as small as possible. Use a UV lamp to see if the spots are of the right size (~1mm diameter).)

Note: Dispose of TLC spotters in the Broken Glass Containers. Do not throw them away in the trash-cans. This is a health hazard for the janitorial staff.

6) Let the TLC plate dry for 1 minute, then place in the TLC chamber and cap. Make sure your TLC plate does not touch the filter paper. The purpose of the filter paper is to ensure that the atmosphere inside the chamber is saturated. If the filter paper touches the side of the TLC plate, the spots will be drawn sideways.

7) Solvent will begin to move up the plate. Once the ‘solvent front’ is within 1 cm of the top of the plate, remove the plate from the chamber and mark the solvent front line with a pencil. This distance from starting line to the final solvent front is needed to determine the $R_f$ value of each component. Note: $R_f$ value cannot be determined if the solvent front could not be seen. Do not let your solvent front travel to the top of the TLC plate.

8) Circle any visible spots on the TLC plate with a pencil, then use a UV lamp to see spots not otherwise visible. Measure the distance from the origin to the solvent front and from the origin to each spot. Sketch or take a digital picture of the plate to include with your report. Record data neatly in a table and use the following equation to determine the $R_f$ values of the various spots on the TLC plate:

$$R_f = \frac{\text{Distance from the start to the center of the TLC spot (cm)}}{\text{Distance from the start to the solvent front (cm)}}$$

9) Liquids should be discarded in the containers in the hood labeled “Organic Waste”. Used TLC plates should be discarded in the containers in the hood labeled “Solid Waste”.

10) Take a picture of the developed TLC plate for your report.
Procedure III: Spectrophotometric Analysis

You will obtain the spectra for three samples: the crude aspirin, aspirin tablet and salicylic acid.

Sample preparation:

1) Measure out 0.20 g of salicylic acid.

2) Combine the salicylic acid, 10 ml of ethanol and 150 ml of water in a beaker.

3) Transfer the solution to a 250 ml volumetric flask and add enough water to reach the 250 ml mark.

4) Remove 10 ml of this solution and place it into a 100 ml volumetric flask.

5) Add 0.025 M Fe$^{3+}$ to reach a total volume of 100 ml.

6) Repeat the procedure (1-5) using the aspirin tablet and crude aspirin.

7) Transfer the sample to a cuvette and obtain the visible spectrum. Print the spectrum for each sample.
**Analyzing the spectra:**

1) Determine the $\lambda_{\text{max}}$ for each sample and the absorbance value for the same wavelength on each spectrum. (Note, this should be very near the $\lambda_{\text{max}}$)

2) Determine the molarity of the salicylic acid that you prepared and used to obtain the spectrum.

3) Compare the other two spectra to the one for the salicylic acid and calculate the concentration of salicylic acid in those samples.

4) Use your results for #3 to determine the mass of salicylic acid per gram of sample for the aspirin tablet and for the crude aspirin.

Follow up Questions:

1) Did you make aspirin in the synthesis at IUP? Support your answer with data.

2) How pure was your crude aspirin sample?

Report:

- Your data and results.
- A conclusion that addresses the follow-up questions.