Spectrophotometric Analysis of Commercial Aspirin

The concentration of acetylsalicylic acid (ASA) is determined spectrophotometrically by the percent transmittance (%T) of visible light at a given wavelength

\[ \%T = \left( \frac{I_t}{I_o} \right) \times 100\% \]

where \( I_t \) = Intensity of the beam transmitted by the solution, \( I_o \) = Intensity of the light beam before it is passes through the sample solution.

The absorbance of the analyte in solution is related to the transmittance (T) as follows:

\[
A = -\log \left( \frac{I_t}{I_o} \right) \\
A = -\log T \\
A = -\log \left( \frac{\%T}{100} \right) \\
A = -\log (\%T) + 2 \\
A = 2 - \log (\%T)
\]

Beer’s Law says that

\[ A = \varepsilon \cdot l \cdot c \]

where:

\( \varepsilon = \) Molar absorptivity of the particular absorbing species in \((L /\text{moles cm})\) \\
\( l = \) Pathlength of the light through the solution in cm \\
\( c = \) Concentration of the absorbing species in moles/L

From these equations, we can determine the concentration \( c \), in mole/L, for the absorbing species if the percent transmission (\%T) can be determined from the spectrometer.

To determine the concentration of the absorbing species in solution, you need to construct a standard Beer’s Law Plot. This is done by preparing a series of known concentrations of the analyte and reading the percent transmission of each standard solution at the maximum absorbing wavelength (\( \lambda_{\text{max}} \)) in the visible region. The resulting plot of absorbance at \( \lambda_{\text{max}} \) versus concentration is a straight line plot known as the Beer’s Law plot.
The plot of absorbance versus concentration gives a straight line plot passing through the origin (0,0). The slope (k) of the line can be obtained from a linear regression or the least-squares fit method and is equal to the $k = A / c$, where

$k$ (slope of the Beer’s Law plot) = $\varepsilon \times l$

$\varepsilon$ = molar absorptivity (L/moles cm)

$l$ = pathlength of the light (cm)

If the absorbance of the unknown solution is measured, then the concentration of the ASA complex can be calculated by the formula

$$c = \frac{A}{k}$$

The analyte of interest in this experiment, acetylsalicylic acid (ASA) complex ion, is formed by hydrolyzing the aspirin sample (ASA) in NaOH solution and complexing it with Fe$^{3+}$ ion in acid solution to bring out the color as shown in the chemical reaction. The complex displays a maximum absorption at a wavelength ($\lambda$) of 530 nm, and has a crimson red color.
In this experiment, we will determine how much active ingredient, acetylsalicylic acid (ASA), in mass %, is contained in commercially available aspirin tablets using a visible spectrometer.

**Prelab Questions**

Construct a Beer’s Law plot for the following experimental data using computer software like Excel or by plotting the points on precision graph paper.

<table>
<thead>
<tr>
<th>Concentration of Cobalt (III) Complex (Standard solution, M)</th>
<th>Percent Transmission</th>
<th>Absorbance at 530 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.00 x 10^{-4}</td>
<td>76.91</td>
<td></td>
</tr>
<tr>
<td>6.00 x 10^{-4}</td>
<td>59.16</td>
<td></td>
</tr>
<tr>
<td>1.20 x 10^{-3}</td>
<td>35.08</td>
<td></td>
</tr>
<tr>
<td>1.50 x 10^{-3}</td>
<td>27.10</td>
<td></td>
</tr>
<tr>
<td>1.80 x 10^{-3}</td>
<td>20.65</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>42.57</td>
<td></td>
</tr>
</tbody>
</table>

1. Calculate the absorbance of each solution using:
   \[ A = -\log \left( \frac{I}{I_0} \right) = -\log T = -\log \left( \frac{\%T}{100} \right) = -\log (\%T) + 2 = 2 - \log (\%T) \]

2. Plot concentration on the x-axis versus absorbance on the y-axis for the five standard solutions. Use computer software or precision graph paper.

3. Calculate the slope (k) from the Beer’s Law plot generated in Step 2. Use linear regression to find the best slope, if you are using computer software. The straight line should pass through the origin (0,0), otherwise a large error may occur.
4. Determine the molar concentration of the unknown cobalt (III) solution, using:
   \[ c = \frac{A}{k} \]

**Materials**

- Ocean Optics Flame Spectrometer and direct attach light source-cuvette holder (or standalone light source, cuvette holder and optical fibers)
- Computer with OceanView software installed
- Reagent grade ASA (acetylsalicylic acid)
- Aspirin
- 1.0 M NaOH solution
- 0.020 M FeCl₃/KCl/HCl solution (pH=1.6)
- DI water
- Weigh paper or weigh boats
- Two 125 mL Erlenmeyer flasks
- Two 250 mL volumetric flasks with stoppers
- Seven 50 mL volumetric flasks with stoppers
- 10 mL graduated cylinder
- Pipettes with suction bulbs
- Large test tubes with stoppers
- Glass funnel
- Hot plate
- Cuvettes

**Safety**

Wear safety goggles and other appropriate safety gear (gloves or apron) during the experiment. Take all proper safety precautions when handling the solutions.

**Hydrolyzing the ASA and Aspirin Samples**

Work in pairs to prepare the standard acetylsalicylic acid (ASA) solution and aspirin sample solution simultaneously.

1. Weigh to the nearest mg (±0.001 g) on a piece of weigh paper or a weigh boat approximately 0.4 g of reagent grade ASA. Transfer the sample to a 125 mL
Erlenmeyer flask labeled “Flask #1.” Record the exact mass of ASA and weigh boat or weigh paper on your Data Sheet 1.

2. Record your unknown aspirin brand name and weigh each aspirin tablet to the nearest milligram (±0.001 g). Record this mass on Data Sheet 2. Repeat the above procedure with the aspirin sample and label the Erlenmeyer flask “Flask #2.”

3. Measure 10 mL of 1.0 M NaOH solution in a clean, dry, graduated cylinder. Add the NaOH to the ASA in the 125 mL Erlenmeyer Flask #1.

4. Repeat for Flask #2.

5. Heat the mixtures (Flask #1 and Flask #2) to a mild boil for five minutes on a hot plate to hydrolyze the ASA. Be careful to avoid splattering and do not let the solution dry up to prevent loss of contents. Rinse the inside of walls of the flasks with a small amount of DI water to ensure complete chemical reaction of ASA.

6. Quantitatively transfer the solution of sodium salicylate in Flask #1 to a 250 mL volumetric flask through a glass funnel. Thoroughly rinse the flask and funnel with DI water so that the rinse water flows into the volumetric flask. Add DI water to the solution in the flask until the bottom of the meniscus touches the index mark of the flask neck. Stopper the flask. While firmly holding the stopper with your forefinger, invert the flask 10 times to thoroughly mix the solution. Repeat the process for Flask #2 containing the aspirin sample.

7. Transfer the solutions into 250 mL Erlenmeyer flasks with rubber stoppers and label “Standard” for the sample from Flask #1 and “Aspirin” for the sample from Flask #2. Store these flasks in your drawer until needed.

NOTE: Aspirin solutions may have milky appearance due to starch fillers. Some buffering agents such as aluminum hydroxide will not dissolve completely in base. In such cases, allow the undissolved material (precipitate) to settle to the bottom of the flask. If a precipitate is present, use your pipette to remove solution from the top portion of the liquid so that you will not draw any precipitate into your pipette.

8. Check with your instructor whether you may proceed beyond this point.

Preparing Standard and Aspirin Solutions

1. Clean your pipette with small portions of the solution you are trying to measure by drawing in solution with the suction bulb and discarding it.
2. Pipette a 2.40 mL portion of the standard solution into a clean 50 mL volumetric flask. Allow the solution to drain and gently allow the tip of the pipette to touch the side of the flask. Add 0.020 M FeCl₃/KCl/HCl solution (pH=1.6) to the 50 mL volumetric flask until the bottom of the meniscus touches the index mark on the flask.

NOTE: You should see the solution turn red at this point. If no color appears after adding FeCl₃ solution, notify the instructor.

3. Stopper the flask. While firmly holding the stopper, with your forefinger, invert the flask 10 times to thoroughly mix the solution. Label this flask “Standard Solution A.”

4. Repeat Steps 2 and 3 to prepare Standard Solutions B, C, D and E by diluting 2.00, 1.60, 1.00, 0.40 mL portions of the sodium salicylate standard solution with the FeCl₃/KCl/HCl solution.

5. Transfer the Standard Solutions to large test tubes with stoppers and label them clearly.

6. Prepare two unknown aspirin solutions. Use a clean, well-rinsed pipette, transfer 1.20 mL of the aspirin stock solution into a 50 mL volumetric flask and dilute it to the mark with 0.020 M FeCl₃/KCl/HCl solution. Mix thoroughly as described in Step 3. Make a second unknown aspirin sample using 1.60 mL of the aspirin stock solution. Transfer the unknown aspirin solutions to large test tubes with stoppers and label them clearly.

**Measuring the % Transmittance using a Spectrometer**

1. Connect the spectrometer to your computer using the USB cable and turn on your light source. If you are using a direct attach light source, start the OceanView software and click the Strobe/Lamp Enable box in the Acquisition Group Window to turn the lamp on.

2. Allow the spectrometer and light source to warm up for at least 15 minutes before proceeding.

3. If you have not already done so, start OceanView. Click the Start button, then select All Programs | Ocean Optics | OceanView | OceanView or use the Desktop shortcut created when you installed the software.

4. Select the Spectroscopy Application Wizards option on the Welcome Screen.
5. Select the **Transmission** option in the **Spectroscopy Wizards** window to start the Transmission Wizard.

6. Select the **Active Acquisition (Recommended)** option and follow the steps through the Wizard to optimize the acquisition parameters for the spectrometer and acquire background and reference spectra for your transmission measurement.

7. Fill a cuvette ~⅔ full with the solvent (FeCl₃/KCl/HCl solution) to serve as a blank. Place the cuvette in the cuvette holder and set your acquisition parameters in the **Set Acquisition Parameters** window.

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a. Click the **Automatic button** to automatically adjust the Integration Time to the optimum value.

8. Set **Scans to Average** to 10 and **Boxcar Width** to 5 to reduce measurement noise and improve the measurement. You can set the **Scans to Average** to a higher value but this will slow down your acquisitions (total measurement time equals number of averages times the integration time).

9. Place a checkmark in the **Nonlinearity Correction** box if this feature is available for your spectrometer.

10. Click **Next**.

11. Click the **Store Reference** (yellow light bulb) to store a reference spectrum. Click **Next**.

12. For direct attach light sources, click the **Strobe/Lamp Enable** box to close the shutter of the light source for your Background measurement. For other light sources, block the light from entering the spectrometer (do not turn your light source off). Click the **Store Background** (gray light bulb) button to store a background spectrum.

13. If you are using a direct attach light source, click the **Strobe/Lamp Enable** box to open the shutter. Click **Finish**. You are now ready to generate transmission spectra.

14. The Transmission spectra will be displayed in the **TransmissionView window**. You can adjust the graph appearance using the graph tools above the transmission graph. Click the **Scale Graph Height to Fill Window** button to zoom in on the absorbance spectra.

15. Click anywhere on the graph to place a cursor on the graph. Position the cursor at 530 nm and record the percent transmittance (%T) of the standard solutions A, B, C, D and E and of the two unknown aspirin solutions. Record the %T values on Data Sheets 1 and 2.

NOTE: Your aspirin sample solutions should have % Transmittance between 30% and 70%. If not, make appropriate dilutions or additions so that they fall within the range. Remember to record the dilution or addition factor used to prepare your aspirin sample if you decide to adjust your concentration.
16. You can save your absorbance spectrum by clicking the Configure Graph Saving button to configure the File Writer.
   a. Select the directory where you want to save the file.
   b. Enter a filename for your spectrum.
   c. Click Apply and then Exit to close the dialog box.

17. Click the Save Graph to Files button to save your absorbance spectrum.

18. You can overlay your spectrum by clicking the Convert Active Spectrum to Overlay button to create an overlay of the spectrum in your graph view.
Data Sheet 1

Preparing Standard Solutions:

**Weighing boat or paper, g**

**Mass of ASA + weighing boat or paper, g**

**Mass of ASA, g**

**Concentration of ASA complex in solution, M**

<table>
<thead>
<tr>
<th>Solution</th>
<th>Concentration, M</th>
<th>% Transmittance</th>
<th>Absorbance</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>

Slope of the Beer’s Law plot = ________________________________ 

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Data Sheet 2

Analyzing Commercial Aspirin Tablets:

Sample ID __________________________

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th>Trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent Transmittance, %T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absorbance of solution, A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration of aspirin in solution, M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass of ASA in tablet, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent ASA in tablet, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean percent of ASA, %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Calculations**

1. Find the slope \((k)\) from your Beer’s Law plot.

2. Convert the percent transmittance \((%T)\) to the equivalent absorbance \((A)\) using:

\[
A = -\log \left( \frac{I_t}{I_o} \right) = -\log T = -\log \left( \frac{%T}{100} \right) = -\log (%T) + 2 = 2 - \log (%T)
\]

3. From the calculated absorbance and the Beer’s Law plot slope \((k)\), determine the concentration of ASA in your aspirin solution using:

\[
c = \frac{A}{k}
\]

4. Calculate the mass of ASA in each tablet, using:

\[
\text{Mass of ASA in grams} = [\text{ASA}] \times (180.2 \text{ g/mol ASA})(0.0500 \text{ L})(250 \text{ mL}/1.60 \text{ mL})*
\]

* Use your experimental values of [ASA] in mole/L and the volume of the “original” aspirin solution in mL.

5. Find the percent ASA in each tablet, using:

\[
\% \text{ASA in tablet} = \left( \frac{\text{mass ASA in tablet, g}}{\text{mass of tablet, g}} \right) \times 100\%
\]

6. Calculate the mean percent ASA in your commercial brand of aspirin, using:

\[
\text{Mean \% ASA per tablet} = \frac{[\text{ASA (trial #1)}] + [\% \text{ASA (trial#2)}]}{2}
\]
Post-Lab Questions

1. Explain why the Fe$^{3+}$/H$^+$ solution was used as a reference solution. Suggest a procedure you could follow to determine whether it was necessary to use the solution as a reference or whether de-ionized water would have been satisfactory.

2. Most commercial aspirins claim to contain 5.0 grains of ASA per tablet, where a grain is an old apothecary unit of measurement for mass, equal to 65 mg. Compare your calculated value of ASA per tablet with respect to the advertised value (i.e., 5.0 grains) and determine the percent error.

3. Compare the results of the class data, if available, for different commercial brands of aspirin. Is there any difference between the different brands of aspirin? Support your answer using simple statistics.