Analysis of Aspirin by Visible Spectroscopy

Introduction

The analysis of solution content by spectroscopic methods is a fast and easy quantitative method that produces reliable results when conducted properly. In previous decades, chemicals analysis was conducted using tedious chemical separations followed by mass measurements. That were often not very accurate due to impurities or partial separations. (Think back to the Separation of Mixtures lab in Chem 211L.) The advent of a variety of spectroscopic methods has provide a modern scientists with the ability to accurately measure the content of solutions without the need for arduous separations. This ability is of particular use to those working in the pharmaceutical industries. Quality control of the content of drugs is vital to assure both the efficacy and safety of their products. The pharmaceutical industry hires a large number of chemists every year to work in their research facilities. The ability to use spectroscopy to analyze solutions is therefore a key skill every chemist should have. In this laboratory, you will apply spectroscopy to the analysis of a common drug, acetylsalicylic acid, also known as aspirin.

Relevance: Why Measure Individual Pills?

Aspirin is a drug used to relieve pain, lower fever, and reduce swelling. However, almost no medication taken in pill form is 100% active ingredient. When a pill is manufactured, it contains the active ingredients along with preservatives, stabilizing agents, coloring agents, flavoring agents etc. A pill of aspirin may weigh ~400 mg but only conation 325 mg of active ingredient (acetylsalicylic acid). It is critical to know if a pill contains significantly more or less of the intended dose, as an overdose can result in serious medical problems. Your goal in today's lab is to determine the mass of active ingredient in an aspirin tablet and compare it to the amount listed on the bottle. Acetylsalicylic acid is colorless in solution. However, it can be converted to a colored compound easily by reacting with iron. In the first step, the acetylsalicylic acid reacts with a base (sodium hydroxide) to create the salicylate ion. In the second step, the salicylate ion reacts with Fe^{3+} to form a highly colored complex ion, shown below.

$3C_7H_5O_3^- + Fe^{3+} \rightarrow (C_7H_5O_3)_3Fe$

The highly colored iron complex that forms absorbs at 530 nm, which is in the visible region of the light spectrum. The reaction will occur as soon as you add the aspirin to the NaOH and iron solution, both of which will be in excess. You will be measuring the complex ion that forms from this reaction.

Spectroscopy

Spectroscopy is any technique that uses light to measure chemical concentrations. For example, in today's experiment the aspirin concentration in a sample will be measured by Ultraviolet-Visible (UV-Vis) spectroscopy. This means that light in the ultraviolet and visible regions of the light spectrum will be used.

The color-treated aspirin solution sample is placed in a cuvette (a small, transparent container), which is then placed in the UV-Vis instrument. Inside the UV-Vis spectrometer, light is passing through the sample cell at the selected wavelength (Figure 2.1). The instrument will measure the amount of light before it passes through the sample (P_0) as compared to the amount of light after it passes through the sample (P). This fraction is termed the Transmittance (T), **$T = P/P_0$**.

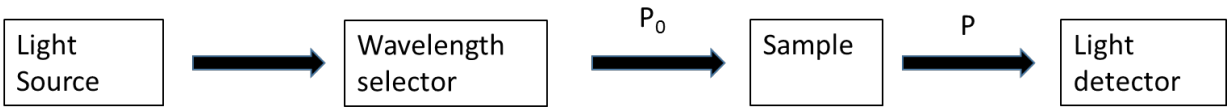

Figure 2.1

In today's experiment, we will be looking at the Absorbance (A) of light in the sample. This value is calculated by equation **A = -log T.** The computer software will convert from Transmittance to Absorbance automatically for you, so you will "read off" absorbance values.

Absorbance is important because it is directly proportional to the concentration of the light absorbing species in the sample. For today's experiment, the absorbing species is the active ingredient in an aspirin tablet. Essentially, the greater the absorbance the greater the concentration of active ingredient.

Beer's law describes the ratio of absorbance to concentration:

$A = \varepsilon bc$ Beer's Law

A = absorbance
ε = molar absorptivity constant (M^{-1} cm^{-1})
b = path length (cm)
c = Concentration (M)

The Experiment

You will use a cuvette that has a path length of 1.0 cm. The molar absorptivity is specific to each compound at a specific wavelength of light. In order to quantify molar absorptivity you will first create what is call a standardization curve using solutions with *known* concentrations. The standard solution allow you to create a linear trendline equation were the slope will equal the molar absorptivity (ε). The second part of the experiment will be to use this calculated value of ε to find the concentration of an unknown solution.

Procedure

Part A: Setting up your spectrometer

Make sure the instrument is displaying units of Absorption

1) Go to "Sensors" → "Change Units" → "USB: Spectrometer" → "Absorption"
2) Collect a cuvette and rinse with DI water.
3) Rinse the cuvette again with a small amount of iron (III) chloride solution.
4) Fill the cuvette 2/3 full with the iron (III) chloride solution. Be sure to wipe the cuvette with a Kimwipe or paper towel to remove and finger prints!

Running the Blank $FeCl_3$ Solution

5) Go to "Experiment" → "Calibrate" → "Spectrometer 1"
    a. Allow lamp to warm up (90 sec)
    b. Add cuvette with only $FeCl_3$ solution
    c. Click "Finish Calibration" then OK

Running a blank on a spectrometer before a measurement is critical. The reason is similar to why you zero a balance before a mass measurement. In this experiment, the iron (III) chloride solution has some absorption at around 530 nm. By running a blank we are able to subtract this absorption so when you run a sample the reported absorption is only due to the acetylsalicylic acid species. Without a blank the absorption would be higher than it should be.

Part B: Running the standard concentrations

Five standards have been prepared for your use in the experiment. Typically, you would be preparing these standards yourself however in the interest of time and minimizing waste accumulation we have prepared them for you. You will still be responsible for calculating the concentration of your standards based on the preparation instructions we followed.

Approximately 0.400g of pure acetylsalicylic acid (aspirin) was dissolved in 10 mL of 1.0M NaOH and then diluted to exactly 250.0mL in a volumetric flask. This solution is labeled as **Aspirin Stock Solution**. **CHECK THE CONCENTRATION OF THE STOCK SOLUTION IN LAB!**

Standard 1: was created by taking 5.00 mL of Aspirin Stock Solution and diluting to 100.0 mL using the $FeCl_3$ solution in a volumetric flask

Standard 2: was created by taking 4.00 mL of Aspirin Stock Solution and diluting to 100.0 mL using the $FeCl_3$ solution in a volumetric flask

Standard 3: was created by taking 3.00 mL of Aspirin Stock Solution and diluting to 100.0 mL using the $FeCl_3$ solution in a volumetric flask

Standard 4: was created by taking 2.00 mL of Aspirin Stock Solution and diluting to 100.0 mL using the $FeCl_3$ solution in a volumetric flask

Standard 5: was created by taking 1.00 mL of Aspirin Stock Solution and diluting to 100.0 mL using the $FeCl_3$ solution in a volumetric flask

Measuring Standards

1) Fill the cuvette 2/3 full with your first standard solution, clean the surface of the cuvette, as you did in Part A.
2) Place the cuvette into the spectrometer, click collect (green arrow), wait 5-10 seconds, then click Stop.
3) Record the absorbance value at 530.2 nm by selecting a wavelength with the stylus and adjusting using the buttons on the x-axis or by selecting tabulated data on the top of the screen and scrolling to the 530.2 nm wavelength.
4) Load spectrometer with the next standard concentration and repeat the collection process. Be sure to hit erase and continue at the start of each run!

Part C: Analysis of a commercial aspirin tablet

1) Obtain a commercial aspirin tablet form your TA.
2) Crush tablet and transfer into a 125 ML Erlenmeyer flask.
3) Add 10 mL of 1.0 M NaOH.
4) Heat and stir until the tablet has dissolved.
5) Transfer the solution to a 250 mL volumetric flask (use DI water to ensure a complete transfer) then dilute with distilled water to the 250 mark. Make sure the solution is thoroughly mixed.
6) Now transfer 5.0 mL of solution from the 250 mL flask into a 100 mL volumetric flask (use $FeCl_3$ solution to ensure a complete transfer), then dilute with $FeCl_3$ solution to the 100 mark. Make sure the solution is thoroughly mixed.
7) Measure and record the absorbance of this diluted solution at 530.2nm in the same way as you measured the absorbance or the standards.

Data Analysis

1) Calculate the molarity of the acetylsalicylic acid in each of the five stock solutions.
2) Using Excel, plot a scatter plot of the absorbance value with the corresponding concentrations for the five standard solutions. (y-axis = absorbance, X-axis = concentration). See Figure 2.2.
3) Add a linear trendline with the y-intercept set to zero. This line should follow from Beer's Law: **$A = \varepsilon bc$;** y = absorbance, x = c, and the slope = εb. Since b = 1 cm, you should be able to calculate the molar absorptivity constant.
4) With your calculated ε, you can determine the concentration of the diluted sample of the commercial tablet, using Beer's Law once again.
5) Knowing the concentration will allow you to back calculate to the mass (mg) of acetylsalicylic acid in your original tablet.

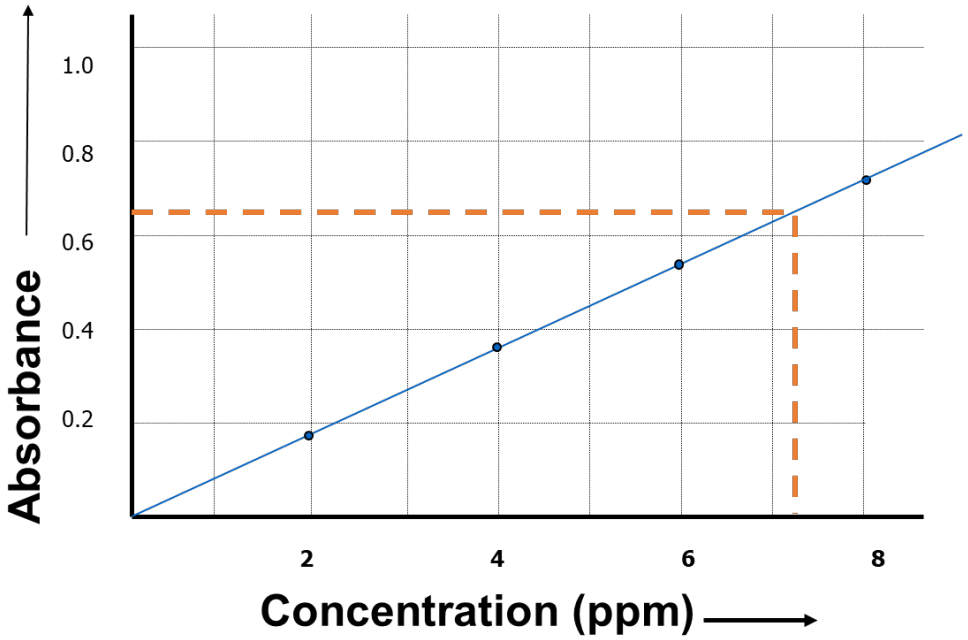

Figure 2.2

Creating a scatter plot and trendline in Excel

The exact method will vary slightly depending on which version of Excel you are using and if you using a PC or a Mac. Generally you select the data you want to plot, click insert, under the Charts section choose a scatter plot with no lines. Then add a linear trendline, by right-clicking on the data, where you have has set the intercept to zero and displaying Equation and R-squared values on the chart.

Using the standardization curve equation, you found along with the absorbance of your unknown determine the concentration of the diluted aspirin solution. From this concentration, you are able to back calculate to the mass of Acetylsalicylic acid in the original tablet.

Report Sheet

Please ensure that you document all of the values in **black** on your lab notebook page! These are numbers that you will enter into LabFlow's Data Report Sheet, and will be used to calculate the values in **red**.

Part I: The Stock Solution

1. Molarity of the Stock Solution (M)	

Part II and III: The Standards	**Absorbance**	**Calculated Conc. (M)**
Standard Solution 1		
Standard Solution 2		
Standard Solution 3		
Standard Solution 4		
Standard Solution 5		

[2] Construct a calibration graph of Concentration Vs Absorbance with a trendline equation. (This graph will need to uploaded to the Data Report, either as a jpg or as the entire excel document. Please remember to add a title, labelled axes, and a best fit line with equation for full credit.)

[3] Molar Absorptivity	

Part IV: Analysis of a Tablet		
Absorbance of Diluted Commercial Tablet		
[4] Calc. Conc. (M) of Diluted Commercial Tablet (100mL)		
[5] Calc. Conc. (M) of initial Commercial Tablet (250mL)		
[5] Moles of Aspirin in Tablet (mol)		
[5] Mass of Aspirin in Tablet in milligrams (mg)		