1—Spectrophotometric Analysis of Commercial Aspirin

Name: _____

Date: _____

Lab Day/Time: _____

Lab Partner: _____

Objectives

- Learn about the absorption of light by molecules
- Learn the basic components of a spectrophotometer
- Learn to prepare standards diluting a stock solution
- Prepare a Beer's Law curve from standard solutions
- Use a Beer's Law curve to calculate the concentration of an unknown substance
- Gain experience pipetting, a technique you learned in CHEM 131L
- Gain experience weighing small samples

Pre-Laboratory Requirements

- Read Chapter 7.1 – 7.3 in Silberberg
- Watch the instructional videos titled "Mass Balance/Analytical Balance" and "Pipetting"
- Pre-Lab Questions (if required by your instructor)
- Laboratory Notebook—prepared before lab (if required by your instructor)

Safety Notes

- Eye protection must be worn at all times
- Sodium hydroxide is caustic and should not come in contact with your skin or clothing. Wear gloves when handling this chemical. A lab coat or lab apron is recommended.

Discussion

Light is a form of electromagnetic radiation. We are most familiar with the visible portion of the electromagnetic spectrum because this is the region of light to which our eyes are sensitive. Visible light, however, is only a small segment of the entire electromagnetic spectrum (see Silberberg, pg. 218).

Electromagnetic radiation is a form of energy that may be represented as a wave or as a particle. The wave model for light is more useful for predicting the behavior of light in our day to day activities, but at an atomic scale light is better described by particles called photons.

The wave model for light describes electromagnetic energy in terms of wavelength, frequency and intensity (see Figure 1). One wavelength is represented by the time from one peak to the next in any wave front (also, one trough to the next). As the wavelength of the radiation becomes shorter, a larger number of waves per unit of time

(i.e., the frequency), becomes greater. Wavelength is often expressed in meters, or nanometers for visible light, and frequency is usually expressed in hertz (Hz). One Hz is one cycle per second. The energy of light is calculated from frequency using equation 1:

$$E = \mathbf{h}\upsilon \qquad \text{equation 1}$$

where E is the energy in joules, **h** is Planck's constant (6.626×10^{-34} J·s), and υ is the frequency in Hz.

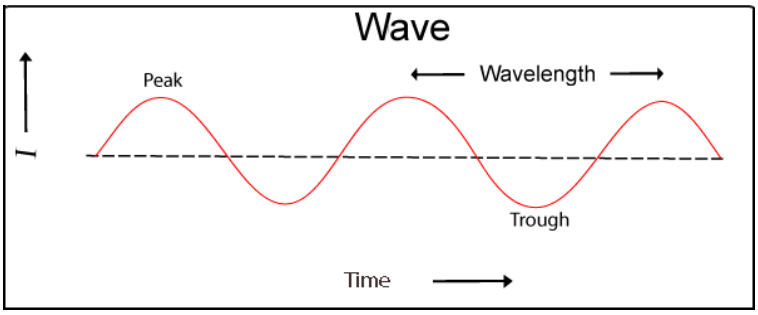


Figure1. The wave model for light.

The symbol for wavelength is the Greek letter lambda, λ, and the symbol for frequency is the Greek letter nu, υ. The speed of light in a vacuum is 3.0×10^8 m/s. Since the speed of light is a constant, we can use it to calculate wavelength if we know the frequency of radiation (see sample Problem 7.1 in Silberberg). The relationship is shown in equation 2:

$$\lambda\upsilon = c \qquad \text{equation 2}$$

where λ is the wavelength of radiation, υ is the frequency of radiation, and c is the speed of light (3.0×10^8 m/s). White light is composed of all the wavelengths within the visible region of the spectrum (see Figure 7.3 in Silberberg). When white light falls on an object, some of the incoming radiation may be absorbed. Wavelengths that are not adsorbed will be transmitted from the object. In the example below (Figure 2), the white egg does not adsorb any of the incoming radiation, and therefore appears white. The apple adsorbs blue and green radiation from white light, transmitting red light. Therefore, the apple appears red.

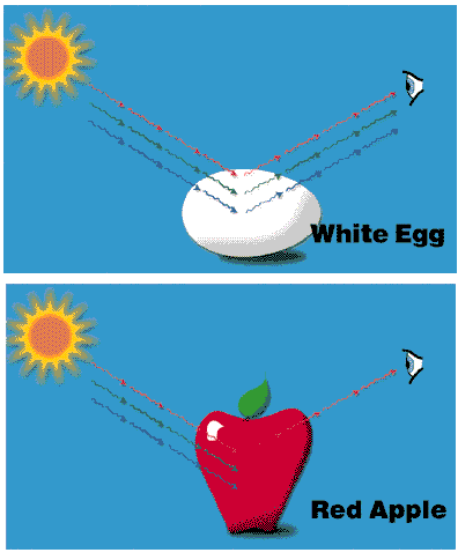


Figure 2. The white egg in this illustration does not absorb red, green or blue light, and appears white when illuminated with white light. The apple absorbs green and blue light, and transmits red light. The apple therefore appears red.

Colors are often used to identify objects. The spectrum of an object is a graph of the absorbance of that object plotted against the wavelength of light. At the molecular level, spectra are used to identify unknown molecules. The peaks in the spectrum can be used to identify unique components within a molecular structure. Figure 7.11 in your textbook is a spectrum for chlorophyll. Notice that the chlorophyll spectrum has intense adsorption peaks in the blue and the red regions of the spectrum. Why do you think chlorophyll is green?

Spectrophotometers are used to measure the amount of light absorbed or transmitted by a sample. The instrument disperses white light into its component wavelengths by passing light through either a prism or a grating. The intensity of light at any wavelength can be measured with a detector, such as a photocell, a photomultiplier tube, or a solid-state device called a charge-coupled device (CCD). The optical path of the Spectronic 200 used in today's experiment is shown below in Figure 3:

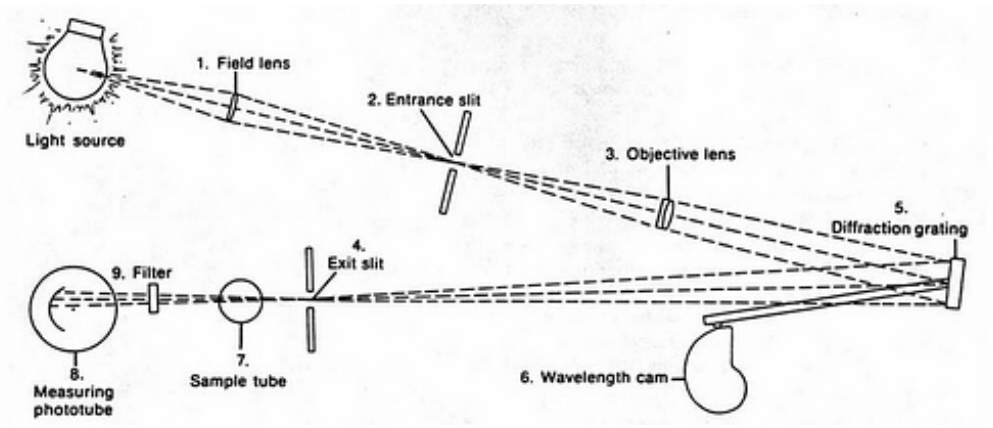


Figure 3. Optical path of the Spectronic 200 spectrophotometer (Courtesy of Fisher Scientific).

Absorbance is the ratio of the negative logarithm of light intensity transmitted from a sample divided by the intensity of incoming light. This is expressed mathematically in equation 3:

$$-log_{10}\frac{I}{I_o} = A \qquad \text{equation 3}$$

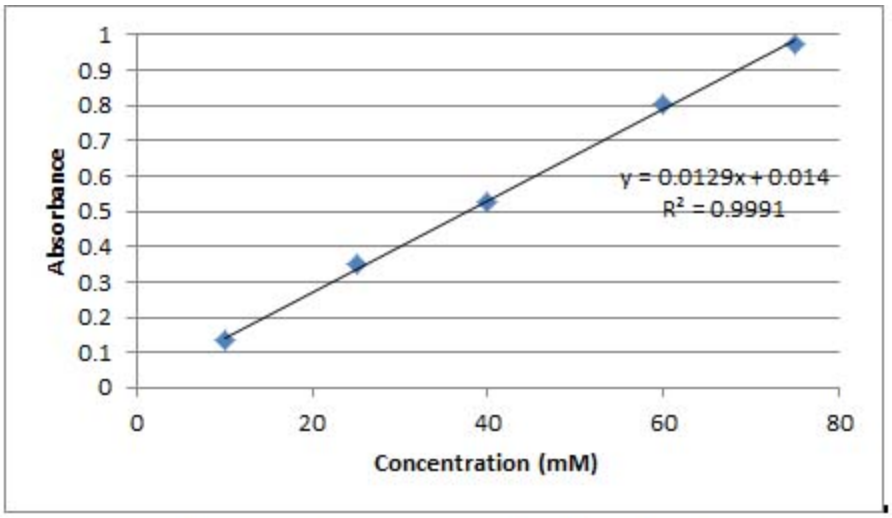
This relationship can be used to measure the amount of material present by measuring the intensity of the absorption peak at a specific wavelength. In today's experiment we will measure the intensity of the colored complex that forms when iron (III) is mixed with aspirin to determine the amount of pure aspirin (acetylsalicylic acid) in commercial aspirin tablets.

The Beer-Lambert Law (often shortened to Beer's Law) relates the absorbance of a sample to the concentration of a species in solution and is the relationship used when making quantitative measurements. Mathematically, Beer's law is expressed as shown in equation 4:

$$A = \boldsymbol{\epsilon} lc \qquad \text{equation 4}$$

where A is the measured absorbance of the solution, $\boldsymbol{\epsilon}$ is the molar absorptivity of the substance, *l i*s the path width for the cell, and c is the concentration. By measuring the absorbance of several solutions of known concentration, we are able to prepare a graph that can be used to determine concentrations of unknowns.

A graph of absorbance vs concentration is called a Beer's Law curve in honor of the chemist who first discovered the relationship between absorbance and concentration. Figure 4 is Beer's Law curve for the absorbance of an iron-salicylate complex (the substance prepared in today's experiment) plotted against different concentrations.

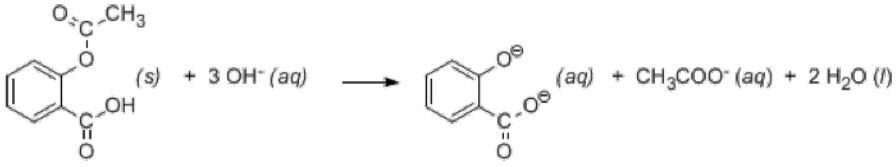


Absorbance vs Concentration of Fe(III)-salicylate complex.

Figure 4. Beer's Law plot of Fe(III)-salicylate complex. *Note: The graph you generate in lab may not look exactly like the example above.*

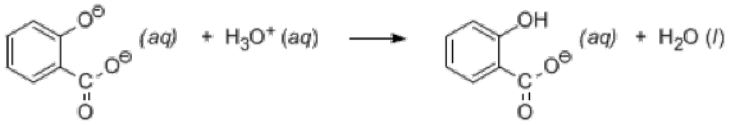
Acetylsalicylic acid, commonly known as aspirin, absorbs light in the UV region of the electromagnetic spectrum. The Spectronic 200 operates in the visible region. Therefore, we must perform a series of chemical reactions to convert acetylsalicylic acid to a colored complex, as shown in Figure 5. In reaction 1, a base (e.g., sodium hydroxide) hydrolyzes acetylsalicylic acid to yield salicylate dianion. In reaction 2, acidification converts the dianion to a monoanion, which complexes with iron(III) in reaction 3 to produce a violet-colored complex.

Reaction 1:
(*procedural step #3*)



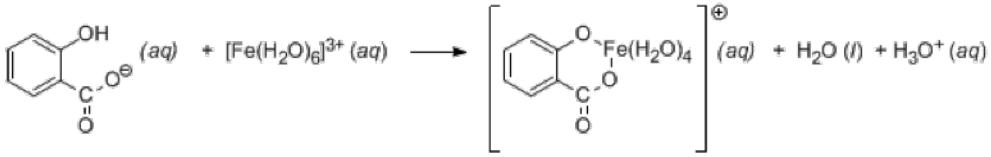
Acetylsalicylic acid (Aspirin) — Dianion

Reaction 2:
(*procedural step #6*)



Dianion — Monoanion

Reaction 3:
(*procedural step #6*)



Monoanion — Violet colored aspirin-iron complex

Procedure

Part I. Preparation of Standards and of the Aspirin solutions

1. Weigh out approximately 0.16 g of acetylsalicylic acid and record the *exact* mass on your report sheet or in your notebook.
2. Transfer this sample into a 125 mL Erlenmeyer flask.
3. Add 5 mL of 1 M sodium hydroxide and *carefully* heat the mixture until all solid dissolves.
4. Allow this solution to cool, and then transfer it into a 100.0 mL volumetric flask with the use of a glass funnel. Rinse the flask with DI water to insure complete transfer.
5. Dilute the solution with deionized water to the 100.0 mL mark on the flask (*Note: Be sure to label this solution as your stock solution).* Invert the flask several times to insure the sample is thoroughly mixed.
6. Using a 1 mL graduated pipet, transfer a 0.5 mL sample of this stock solution into a 10.0 mL volumetric flask and dilute this solution to the 10.0 mL mark with 0.02 M iron(III) chloride that is buffered to pH 1.6.
7. Place this solution in a test tube labeled solution A.
8. In a similar fashion, prepare solutions labeled B, C, D, and E by using 0.40, 0.30, 0.20, and 0.10 mL aliquots of the sodium salicylate solution, diluting to 10.0 mL with iron(III) chloride solution.
9. Obtain one commercial aspirin tablet and break it into two approximately equal pieces. If the tablet crumbles when split, crush the entire tablet and divide the crushed tablet in two equal halves. Record the exact mass of each piece (or crushed halve) on your report sheet or in your notebook.
10. Transfer the two aspirin pieces into two 125 mL Erlenmeyer flasks, and label the flasks Sample 1 and Sample 2.
11. Add 5 mL of 1 M sodium hydroxide and *carefully* heat the mixture until all solid dissolves.
12. Allow these solutions to cool, and then transfer then into two 100.0 mL volumetric flasks, using a glass funnel to insure a quantitative transfer.
13. Dilute these solutions to the 100.0 mL mark on the flask, and label these flasks Sample 1 and Sample 2.
14. Invert the volumetric flasks several times to insure the samples are thoroughly mixed.
15. Using a 1 mL graduated pipet, transfer a 0.3 mL sample of each solution into two 10.0 mL volumetric flasks and dilute to the 10.0 mL mark with 0.02 M iron(III) chloride. Label these flasks Sample 1 and Sample 2.
16. Transfer solutions to test tubes, and label the test tubes Sample 1 and Sample 2.

Data

Mass of acetylsalicylic acid, (±0.001 g) _____

Moles of acetylsalicylic acid (mol) _____

Concentration of acetylic acid in 100.0 ml volumetric flask (Stock solution) _____

Mass of aspirin sample 1 (±0.001 g) _____

Mass of aspirin sample 2 (±0.001 g) _____

Part II. Measure Absorbance of Standards and Aspirin Samples

1. With the assistance of your instructor, zero the instrument with the iron (III) chloride solution.
2. Record the absorbance for standard solutions A, B, C, D and E.
3. Record the absorbance for Sample 1 and Sample 2.

Solution	Concentration	Absorbance
A	_____	_____
B	_____	_____
C	_____	_____
D	_____	_____
E	_____	_____
Sample 1	_____	_____
Sample 2	_____	_____

Calculations

For this example we assume 0.400 g of acetylsalicylic acid (aspirin, $C_9H_8O_4$) is treated as outlined in the procedure (you should use the actual mass recorded on your report sheet). The concentration of complex in the stock solution can be found as follows:

The molar mass of acetylsalicylic acid ($C_9H_8O_4$) = 180.2 g/mol

$$0.400g\ acetylsalicylic\ acid\ x\ \frac{1\ mol}{180.2\ g} = 2.22x10^{-3}\ \text{mol}\ C_9H_8O_4$$

The concentration of the acetylsalicylic acid stock solution then is:

$$2.22x10^{-3}\ \text{mol}\ C_9H_8O_4 \div 0.1000\ \text{L} = 2.22x10^{-2}\ \text{M}$$

This stock solution is used to prepare standards for the Beer's Law curve by diluting aliquots of the stock solution into 10.00 mL volumetric flasks, and diluting to volume with iron(III) chloride solution. The concentration of $C_9H_8O_4$ in the standards is calculated with the relationship $M_1V_1 = M_2V_2$, where M_1 is the concentration of the stock solution, V_1 is the volume of the stock solution transferred, V_2 is the volume of the diluted solution (10.00 mL for all standards in this experiment), and M_2 is the new concentration.

For example, the concentration of $C_9H_8O_4$ in the solution A would be calculated as follows:

$$M_1V_1 = M_2V_2 = 2.22x10^{-2}M\ x\ 0.50\ mL = M_2\ x\ 10.00\ ml$$

$$M_2 = \frac{0.50\ mL\ x\ 2.22x10^{-2}M}{10.00\ mL} = 1.11x10^{-3}\ M$$

You can plot your absorbance data for standards A, B, C, D and E on the graph paper included with this experiment, or you can plot the data with Excel as an X-Y scatter plot. The concentration data should be plotted on the x-axis and the absorbance data on the y-axis. If you use Excel to plot the data, add a trend line after the graph has been generated. Your graph should look like the one in Figure 4 but your numbers may be different.

Use the graph you just prepared to find the concentration of aspirin in Samples 1 and 2. Use the trend line to find the concentration that would give the absorbance recorded for Sample 1, and write this number on the data sheet. Find the concentration for Sample 2, using the same procedure, and write this number on the data sheet. You can also use the equation from the trend line to find concentrations by entering you absorbance value for Y in the equation, and solving for X.

The concentration obtained from the Beer's Law plot represents the concentration of acetylsalicylic acid in the 10.0 mL volumetric flask. To find the mass of acetylsalicylic acid in your original sample you must correct for the dilutions that were done preparing the sample. This is simply the reverse of the process just described.

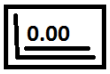
Calculation of percent aspirin in commercial aspirin tablets

	Sample 1	Sample 2
Mass of sample	_____	_____
Absorbance from graph	_____	_____
Concentration in diluted solution	_____	_____
Concentration in Sample Stock solution	_____	_____
Moles of acetylsalicylic acid in Stock solution	_____	_____
Mass of acetylsalicylic acid in Stock solution (g)	_____	_____
Percent acetylsalicylic acid in sample	_____	_____
Average percent acetylsalicylic acid		_____

Show sample calculations here:

Operating instructions for the Spectronic 200

Your TA should have already turned on the instruments so they warm up during the short lecture before the experiment begins. In addition, the instrument has been set to measure absorbance at 530 nm.

1. Open the cover and place the cuvette containing the iron(III) chloride solution (the blank) in the holder.
2. Close cover and press zero 

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3. After zero is complete, open cover to remove the blank. Next measure the absorbance of each standard and sample. Be sure to wait for the reading and record all digits displayed before moving to the next sample.