Introduction:

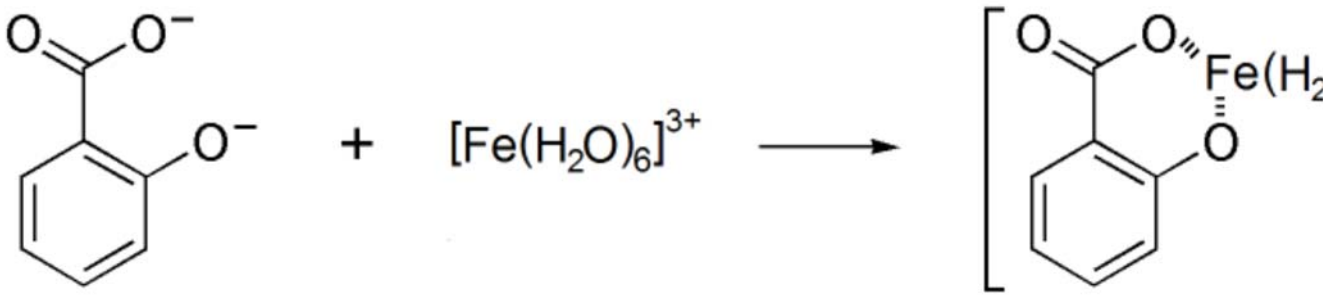
Earlier this semester you synthesized aspirin from salicylic acid. Unlike previous reactions where you assumed a 100% yield, this was an equilibrium reaction which resulted in less than 100% yield. In addition, you started with one white compound and produced another white compound. So how do you figure out the purity of your aspirin?

There are actually several ways to determine your product's purity: melting point, chromatography, mass spectrometry, spectrophotometry, and others. Of course that last one, spectrophotometry, should ring a bell since we used it previously to determine the thickness of the copper clad on newer pennies. It turns out that salicylic acid will react with iron (III) nitrate to produce a complex that absorbs green light, but aspirin does not. So you can use this to determine the amount of unreacted salicylic acid that remains in your aspirin, and ultimately determine its purity.

Theory:

Note: I have added two videos from the Kahn Academy that which should give you a better understanding of the theory and practice of spectrophotometry. The first video covers the theory of how spectrophotometry works using both an intuitive and algebraic approach. The second video works through a standard spectrophotometry problem. Warning: both videos are approximately 13 minutes long. The first video is 16MB, and the second is 20MB.

When salicylic acid is dissolved in water, it produces a salicylate *dianion*, which reacts with an acidic solution of iron (III) nitrate, $Fe(NO_3)_{3(aq)}$, to produce a highly colored (violet) tetraaquosalicylatroiron (III) complex:



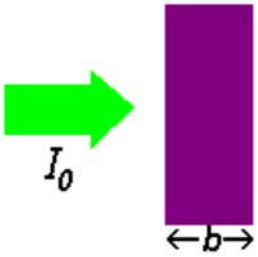
Salicylate dianion
$C_7H_4O_3$
MW = 136.09

Iron (III) ion

Tetraaquosalicylatroiron

The violet color of the complex results from the fact that the complex strongly absorbs green light. When this green is removed from normal white light, we observe violet (therefore, green is the compliment of violet). This absorption of green light can be used to quantitatively determine the amount of aspirin present in the solution. The more green light that is absorbed, the more violet the solution, and hence, the more salicylate is present.

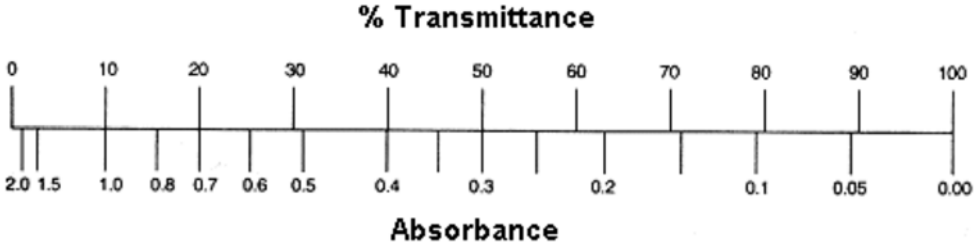
If green-yellow light with a wavelength of 530 nanometer is directed into a solution that contains this aspirin complex, some of the green light will be absorbed:



As you can see, the intensity of the green light leaving the sample, ***I***, is less than the original intensity of the green light, I_0. There are two ways of expressing this difference. We can talk about the fraction of light that was transmitted through the sample, **transmittance (T);** or we can talk about the amount of light that was absorbed by the sample, **absorbance** (**A**). As you can see, one is opposite of the other:

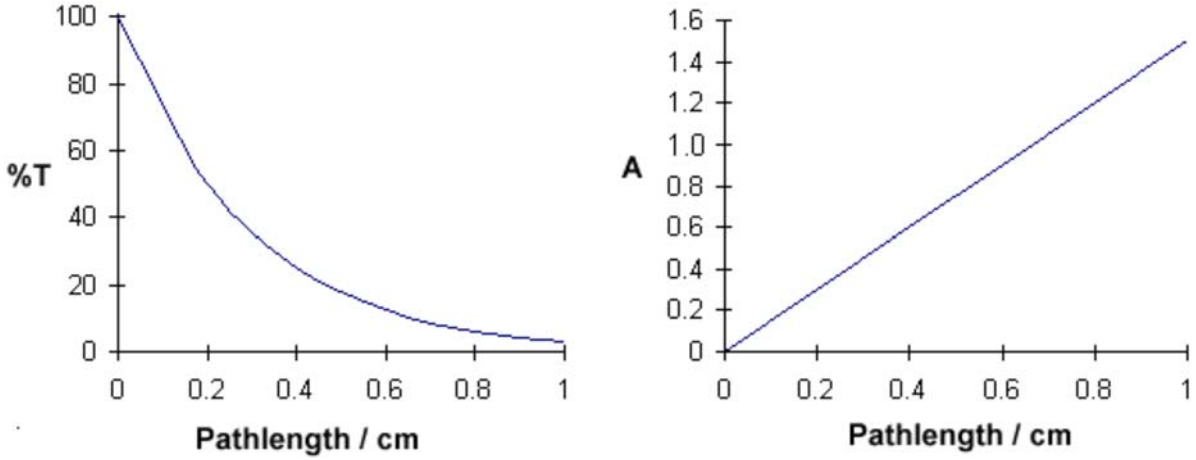
transmittance (T)	absorbance (A)
$T = I / I_0$	$A = \log (I_0 / I) = \log (1 / T)$

The inverse relationship between transmittance and absorbance can best be seen in the following figure:



Notice that the %T can vary from 0 to 100% whereas the absorbance varies from 2.00 to 0.00 absorbance units. The more light that passes through the sample, the higher the transmittance and the lower the absorbance. Conversely, the less light that passes through the sample, the lower the transmittance and the higher the absorbance.

Unfortunately, a plot of transmittance versus concentration does not result in a straight line. However, a plot of absorbance, versus concentration does provide a straight line:



In a typical experiment, several solutions of known concentration of the salicylate complex are prepared. Since the concentration of these solutions is known, they are called standard solutions. The absorbance of each standard solution is measured at the wavelength of maximum absorption (530 nanometer from the spectrum above) using a spectrophotometer. A graph of these absorbance values versus the concentration of each of the standards should yield a straight line. This relationship is known as Beers' Law:

$$A = a\,b\,c$$

In this equation, ***A*** is the absorbance of the solution, ***a*** is the molar absorptivity (a constant for this complex), ***b*** is the path length of cuvette (in cm), and ***c*** is the molar concentration of the solution being measured. If the same cuvette is used to measure all of the solutions, then ***a*** and ***b*** are constant. This means that the absorbance of a solution is directly proportional to the concentration of that solution. Therefore, the molar concentration, ***c***, of a solution can be determined by simply measuring the absorbance, ***A,*** of that solution. Although we are actually measuring the absorbance of the complex, the stoichiometry of the reaction producing the complex is 1:1. So, if we know the concentration of the complex, we know the concentration of the aspirin is the same.

O.K., lets work through an example to see how all of this theory works (**DO NOT** include this section in your pre-lab). Lets assume that 0.273 g of pure salicylic acid is treated as outlined in the experimental procedure below. The concentration of the complex in the "Stock Solution" can be calculated as follows (remember, the molar mass of salicylic acid $C_7H_6O_3$ is 138.09 g/mol):

0.273 g salicylic acid x (1 mol salicylic acid / 138.09 g) = 1.98×10^{-3} mol of salicylic in the Erlenmeyer flask

Upon hydrolysis, and dilution to 1000 mL (1.0 L), the molarity of the solution is:

$$M = 1.98 \times 10^{-3} \text{ mol} / 1.0 \text{ L} = 1.98 \times 10^{-3} \text{ M}$$

The "Stock Solution" is then diluted in varying proportions (aliquots) to yield the standard solutions "A", "B", "C", "D", "E", and "F". Solution "A" is produced by diluting 10.0 mL of the "Stock Solution" with 50 mL of $Fe(NO_3)_3$. The concentration of aspirin in solution "A" can be found using the relationship:

$$M_1V_1 = M_2V_2$$

where M_1 is the molarity of the "Stock Solution", M_2 is the molarity of the solution "A", V_1 is the volume of the "Stock Solution", and V_2 is the volume of the solution "A":

$$(10.0 \text{ mL})(1.98 \times 10^{-3} \text{ M}) = (50.0 \text{ mL})(M_2)$$

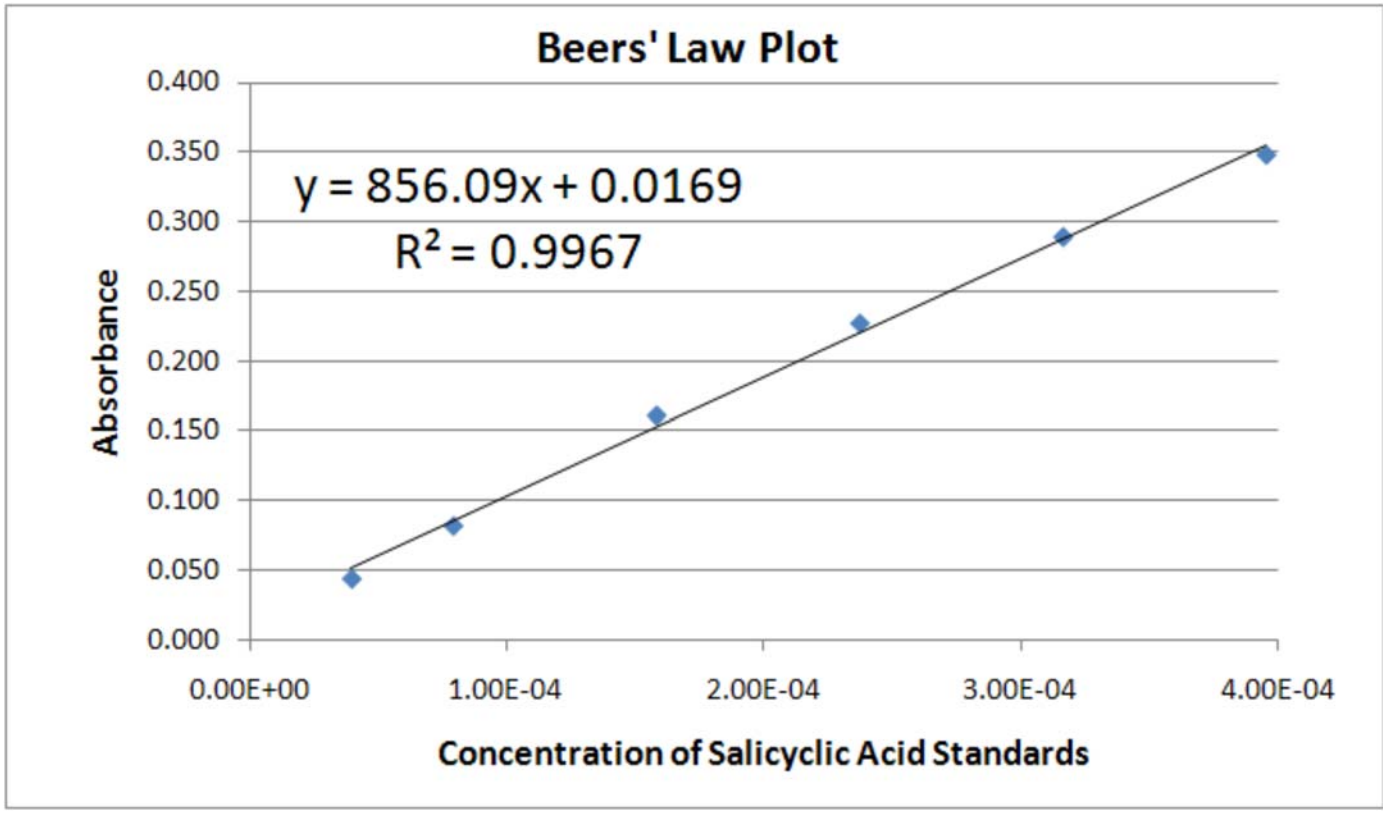
Therefore, the concentration of standard "A" is 3.95×10^{-4} M. Now that you know the concentration of standard "A", you can use the spectrophotometer to measure it's absorbance. In this example, it had an absorbance of 0.348. Likewise, you can determine the concentration and absorbance for each of the other standard solutions:

Solution	mL of Stock	Concentration	Absorbance
"A"	10.0	3.95×10^{-4} M	0.348
"B"	8.0	3.16×10^{-4} M	0.289
"C"	6.0	2.37×10^{-4} M	0.227
"D"	4.0	1.58×10^{-4} M	0.161
"E"	2.0	7.91×10^{-5} M	0.082
"F"	1.0	3.95×10^{-5} M	0.044

Now you have the data you need to create your Beers' Law plot. However, it would be a good idea to check your data to make sure it is consistent before you throw away your "Stock Solution". Remember, the whole idea behind this experiment is that the absorbance of a given solution will be directly proportional to the concentration of the aspirin in that solution. If that is the case, then the Absorbance of a solution divided by the mL of Stock used to create it should be very nearly constant. For example, if I divide the measured Absorbance of Solution "A" (0.348) by the mLs of Stock solution (10.0 mL), I obtain

a value of approximately 0..035 Absorbance/mL. Likewise, I obtain values of 0.036, 0.038, 0.040, 0.041, and 0.044 for solutions "B", "C", "D", "E", and "F" respectively. Since values are all within about 10% of each other, I am confident in the data I have collected and am ready to create my Beers' Law plot. Remember that this is sample data that I have create to make the Beers' Law plot look good. You may notice that the higher concentration solutions don't show as much Absorbance/mL as the lower concentration solutions. This can happen if you use a large sample of aspirin. If this happen, you will have to throw out the higher concentration result and only used the lower concentration results.

Once you have determined the concentration and absorbance for all five standards, you will plot these points using an 'X-Y Scatter' plot (Excel). Your Beers' Law plot should look like the one below:



Note that most of the points do not fall directly on the line. So, we have asked the software to draw the 'best' straight line through the data. This is the 'Least Squares Fit' or 'Trend line'. The plot is fairly straight and has a 'goodness' of fit (R^2) of 0.9967, where 1.000 is a perfect fit. It also gives us an equation for the line which we will use to calculate the concentration of the salicylic acid remaining in your aspirin sample.

Next you will need to process a sample of the aspirin you synthesized previously. Lets assume that you used 0.327 g of your aspirin and processed it in exactly the same manner as you did the pure salicyclic acid above. Since we are looking for the amount of salicylic acid, use the molecular weight of salicylic acid (138.09 g/mol) to calculate the molarity of "My Aspirin" solution. You will end up with 100.00 mL of a 2.37×10^{-2} M "My Aspirin" solution (assuming it is pure salicylic acid). You then take 5.0 mL of this "My Aspirin" solution and diluted it to 50.0 mL with $Fe(NO_3)_3$. The resulting solution has a concentration of 2.37×10^{-3} M (again, assuming it is pure). You then measure its absorbance and obtain a value of 0.079.

When you plotted your standards (five or six depending on whether you decided use the 10 mL aliquot), you obtained an equation for the linear regression equation. In our example, that equation was:

$$Y = 856.09 \cdot X + 0.0169$$

In this equation, 'Y' is the absorbance, 'X' is the concentration of the solution, '856.09' is the slope of the line, and '0.0169' is the y-intercept. Since we know the absorbance ('Y'), we can solve for the

concentration ('X'):

$$X = (Y - 0.0169) / 856.09$$
$$X = (0.079 - 0.0169) / 856.09$$
$$X = 7.25 \times 10^{-5} \text{ M}$$

This is the actual concentration of unreacted salicylic acid remaining in your aspirin sample. However, we calculated that if your sample was pure salicylic acid, it should have a concentration of 2.37×10^{-3} M. This means your aspirin sample actually contains:

$$(7.25 \times 10^{-5} \text{ M} / 2.37 \times 10^{-3} \text{ M}) \times 100 = 3.06\% \text{ salicylic acid}$$

Therefore, the remainder, 96.94%, must be pure aspirin!

Procedure:

Hints for using the cuvette and colorimeter:

1. A cuvette have two clear sides (the light passes through these), and two ribbed sides, perpendicular to each other.
2. Always handle the cuvette using the ribbed sides. You must avoid fingerprints on the clear sides.
3. The cuvette must be clean and dry on the outside. Use a ChemWipe for this. **DO NOT** use a regular paper towel. This will scratch the clear sides.
4. After filling with solution, make sure there are no bubbles. You may have to tap it vigorously to remove them.
5. Make sure you check that the colorimeter is working properly by putting in a cuvette of distilled water. It should have an absorbance of zero. It is is larger than 0.002, let your instructor know so it can be recalibrated.
6. Make sure you put the cuvette in the colorimeter with the ribbed side facing you. The light beam travels from right to left in the colorimeter.

Preparation of Standards for the Beers' Law Plot:

In this section you will produce five acetylsalicylic acid standards of known concentrations. Spectrophotometric determination of each standard's absorbance will be recorded and this data will be graphically plotted against concentrations to give a standard curve (Beers' Law Plot).

1. This lab is very time intensive and you must 'multi task' if you are going to finish. It is important to study the procedure before coming to lab and not just 'cookbook' it.
2. Obtain two 125 mL Erlenmeyer flask and clean them thoroughly with soap and water. Rinse them with distilled water and use paper towels to remove as much of the excess water as possible. Make sure you label each of them before using.
3. Tare one of the flasks and add approximately 0.2 g (**Note:** do not use more than 0.25 g) of pure salicylic acid and record the mass to the nearest 0.001 g.

4. Tare the other flask and add approximately 0.3 g (**Note:** do not use more than 0.35 g) of your aspirin and record the mass to the nearest 0.001 g.
5. Wash down the inside of each flask with 20-30 mLs of distilled water. Use your hot plate to heat these solutions until they have completely dissolved. If the water level becomes low, add small portions (3-5 mL) of distilled water to ensure that the solutions do not dry up.
6. Add approximately 25 mL of distilled water to each flask and allow the solutions to cool to room temperature. While the solutions are cooling, obtain a 100mL volumetric flask and a 1000 mL volumetric flask and clean them by rinsing several times with distilled water (**Note: DO NOT use soap to clean it.**)
7. Quantitatively transfer the solution of pure salicylic acid to the clean 1000 mL volumetric flask and then dilute with distilled water to the 1000 mL mark. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "STOCK SOLUTION."
8. Make sure you clean your graduated pipette by filling it with the "STOCK SOLUTION" and then dumping it down the drain.
9. Using a 10-mL graduated pipette, transfer a 10.0 mL aliquot into a 50 mL volumetric flask and dilute to the 50 mL mark with 0.02 M $Fe(NO_3)_3$ solution. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "Solution A".
10. Rinse your cuvette with "Solution A" and then discard. Refill the cuvette with "Solution A" and measure its absorbance.
11. Using a 10-mL graduated pipette, transfer a 8.0 mL aliquot into a 50 mL volumetric flask and dilute to the 50 mL mark with 0.02 M $Fe(NO_3)_3$ solution. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "Solution B".
12. Rinse your cuvette with "Solution B" and then discard. Refill the cuvette with "Solution B" and measure its absorbance.
13. Using a 10-mL graduated pipette, transfer a 6.0 mL aliquot into a 50 mL volumetric flask and dilute to the 50 mL mark with 0.02 M $Fe(NO_3)_3$ solution. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "Solution C".
14. Rinse your cuvette with "Solution C" and then discard. Refill the cuvette with "Solution C" and measure its absorbance.
15. Using a 10-mL graduated pipette, transfer a 4.0 mL aliquot into a 50 mL volumetric flask and dilute to the 50 mL mark with 0.02 M $Fe(NO_3)_3$ solution. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "Solution D".
16. Rinse your cuvette with "Solution D" and then discard. Refill the cuvette with "Solution D" and measure its absorbance.
17. Using a 10-mL graduated pipette, transfer a 2.0 mL aliquot into a 50 mL volumetric flask and dilute to the 50 mL mark with 0.02 M $Fe(NO_3)_3$ solution. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "Solution E".
18. Rinse your cuvette with "Solution E" and then discard. Refill the cuvette with "Solution E" and measure its absorbance.
19. Check your data to make sure your absorbance data is decreasing relative to the decreasing concentration of each solution. For example, the absorbance for the 4 mL solution should be half of that for the 8 mL solution and the absorbance for the 2 mL solution should be half of that for the 4 mL solution, etc. If you find that the 10 mL solution shows significantly less absorbance that it should, it is possible that it is too concentrated and has fallen off the linear portion of the Beer's Law plot.
20. Once you are confident with your data from the pure salicylic acid, you can dump the rest of the 1000 mL solution down the drain.
21. Clean a 100 mL volumetric by rinsing several times with distilled water (**Note: DO NOT use soap to clean it.**)
22. Quantitatively transfer the solution of your aspirin to the clean 100 mL volumetric flask and then dilute with distilled water to the 100.00 mL mark. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times. Label the flask as "MY ASPIRIN."
23. Since this is a new solution, make sure you clean your graduated pipette by filling it with "MY ASPIRIN" solution and dumping it down the drain.

24. Using the cleaned 10-mL graduated pipette, transfer a 5.0 mL aliquot into a 50 mL volumetric flask and dilute to the 50 mL mark with 0.02 M $Fe(NO_3)_3$ solution. Be sure to thoroughly mix this solution by inverting the volumetric flask at least ten times.
25. Rinse your cuvette with "MY ASPIRIN" and then discard. Refill the cuvette with "MY ASPIRIN" and measure its absorbance.
26. Rinse the cuvette and all of the glassware you used with distilled water and return them to where you found them.
27. Dispose of your left over aspirin in the trash. Remove the label from the test tube, clean and dry it and return it to the instructor's desk. Use a dry paper towel to remove any aspirin from the cork and return it to the instructor's desk as well.

Waste Disposal. All materials can be washed down the sink with plenty of water to neutralize the acids and bases.

Calculations:

1. Calculate the number of mols of pure salicylic acid used in your "Stock Solution".
2. Calculate the molarity of your "Stock Solution".
3. Calculate the molarity of each of your standard solutions, "A", "B", "C", "D", and "E".
4. Use Excel to produce your Beers' Law plot. Enter your concentration and absorbances in two columns and insert a 'Scatter Plot'. Then make sure to add a 'Trend Line'. This 'Trend Line' is the least squares line through your data. You will also want to set the plot options to show the equation of the line and the 'R^2'on the graph. You can use this equation to calculate the concentration of your aspirin sample by using your absorbance value for 'y' and solving for 'x'.

(Updated 10/31/12 by C.R. Snelling)