Colorimetric analysis of aspirin content in a commercial tablet v010214

♦ Objective

In this lab, you will prepare standard solutions, and use Beer's Law to construct a calibration curve. You will determine molar absorptivity, and use your calibration curve to determine the aspirin content in a commercial preparation.

♦ Background

To get the most benefit from this lab, you should reacquaint yourself with the concepts of molarity and dilution found in section 4.4 Tro (2nd ed), and also read section 12.4 that examines other solution concentration units that will be used in this experiment.

The chemical name for aspirin is Acetyl salicylic acid (ASA). It is a drug that was first isolated in Germany by the Bayer Company in 1897. It is used as a fever reducer, pain reliever, and has antiplatelet effects. As is common with drugs made into pill form, the commercial preparations often contain other ingredients such as binders and fillers which help to form pills that do not fall apart under normal storage and transport. The amount of ASA that should be contained in a tablet differs from brand to brand and is indicated on the package. One of the advantages to the colorimetric analysis that you will perform in this procedure is that the added ingredients will have no effect on your measurement as they do not absorb visible light. So, the technique is effectively blind to all of the components in the tablet except ASA.

Absorption Spectroscopy

You learned in Chapter 7 of the Tro 2nd edition text that light has the ability to interact with matter. Absorption spectroscopy is a technique that exploits this interaction to obtain information about the matter you are observing. While there are many uses for absorption spectroscopy, such as providing information about the structure of a molecule, you can also use the amount of light absorbed at a particular wavelength to determine the concentration of a molecule in solution.

In basic terms, absorption spectroscopy works in the following manner. First a beam of white light (a light source that emits light of a broad range of wavelengths) is focused on a sample. The light interacts with the molecules in the sample, and photons of some wavelengths will be absorbed while others pass through the sample unaffected. The light then passes through a prism or a grating which separates the white light by wavelength. As you rotate the grating, different wavelengths of light strike the active area of a detector. Just as an eye can determine when light becomes more intense (brighter), a photodetector quantitatively indicates the intensity of the light that shines on it and converts this to an electronic signal that can be read from a display. By comparing the intensity of the light at each wavelength with and without the sample, the amount

1

of light absorbed by the sample is able to be calculated. The absorption of light as a function of wavelength can be plotted, and is called an absorption spectrum.
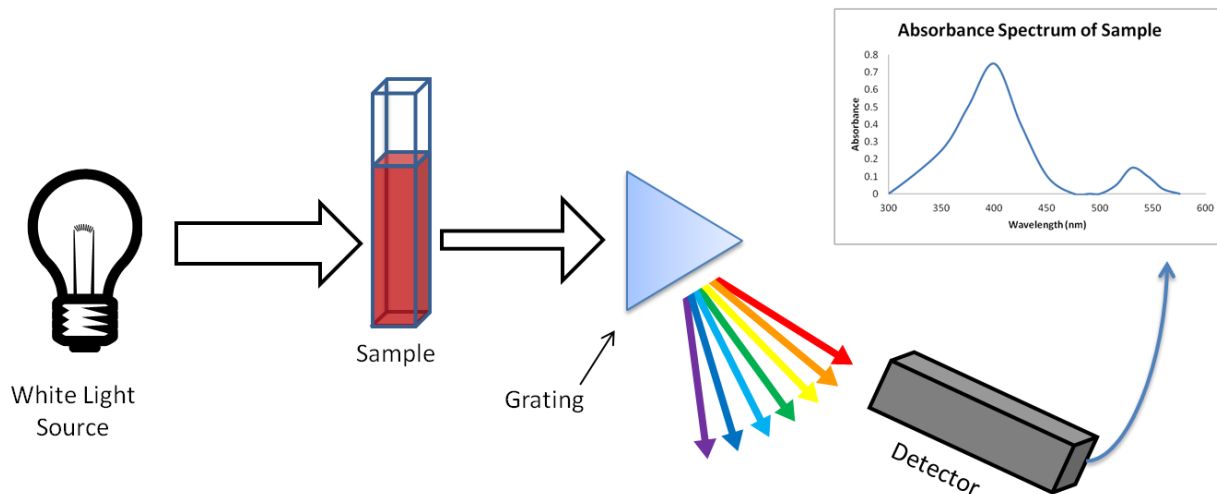

Figure 1: Schematic showing how an absorption spectrum of an aqueous sample is collected. A beam of white light is focused through the aqueous sample. Some of the light will be absorbed. The light then passes through a grating which separates the light by wavelength. As the grating is rotated, different wavelengths of light will strike the detector. An absorbance spectrum can be generated if the absorbance at each wavelength is measured and plotted.

In order to calculate the absorbance (A) of light at each wavelength, the intensity of the light that reaches the detector when no sample is present (I_0) must be compared to the intensity of the light transmitted when the sample is present (I_t). The ratio of these intensities corresponds to the **transmittance (T)** at that wavelength, and is calculated using Equation 1a. The **percent transmittance (%T)** is commonly reported by instrumentation. The functional form for %T is given in Equation 1b.

$$T = \left(\frac{I_t}{I_0}\right) \qquad\qquad\qquad \textit{Equation 1a}$$

$$\%T = \left(\frac{I_t}{I_0}\right) * 100\% \qquad\qquad\qquad \textit{Equation 1b}$$

The **Beer-Lambert Law** (or simply Beer's Law) relates the amount of light absorbed to properties of the material in the sample. It states that there is a logarithmic dependence between the transmitted light (T) and the product of the molar absorptivity (ε), the molarity of the sample (c), and the distance the light travels through the sample or pathlength (l). The molar absorptivity is a constant is basically a sensitivity factor. If it is large, it means that the molecule

is very likely to absorb photons of that particular wavelength. If small, it means that most photons of that wavelength will pass through the sample unabsorbed. The molarity (c) and pathlength (l) are factors in this equation because the more sample molecules the beam of light passes through, the more photons absorbed. In algebraic terms, the law is written as Equation 2:

$$log(T) = -\varepsilon * l * c \hspace{3cm} \textit{Equation 2}$$

where: molar absorptivity, ε, is in units of $\dfrac{L}{mol*cm}$
(which is the same as $\dfrac{1}{M*cm}$)
pathlength, l, is in units of cm
concentration, c, is in units of mol/L or M

The **absorbance (A)** at a particular wavelength is defined as the negative logarithm of the transmittance and is related to transmittance (T) and percent transmittance (%T) through Equation 3:

$$A = -log(T) = 2.00 - log(\%T) \hspace{2cm} \textit{Equation 3}$$

Absorbance (A) is a unit-less number with values ranging from 0.00 if all of the photons pass through the sample unabsorbed to a maximum of nearly 2.00 if almost every photon is absorbed by the sample molecules.

The introduction of absorbance (A) makes Beer's Law a very simple equation where absorbance (A) has a linear relationship with the concentration (c) as shown in Equation 4. Below Equation 4 is the algebraic equation for a line.

$$A = \varepsilon * l * c \hspace{3cm} \textit{Equation 4}$$
$$y = m * x + b$$

Note that absorbance (A) and concentration (c) are the variables
*y and x, respectively. The slope is the product ε * l (which is a*
constant). And, the y-intercept (b) is zero.

Constructing a Beer's Law Plot with Trendline

When the absorbances of solutions of known concentrations are measured and plotted on a rectangular coordinate system, a best fit line can be generated using a graphing calculator or a computer graphing program such as Excel. *Note that with either method, you must ensure that the y-intercept is zero!* An example of a Beer's Law Plot is shown in Figure 2.

x	y
Concentration (M)	Absorbance
0.0005	0.09
0.001	0.222
0.002	0.41
0.003	0.531
0.004	0.76
0.005	1.05

Beer's Law Plot of Absorbance vs. Sample Concentration at 530 nm
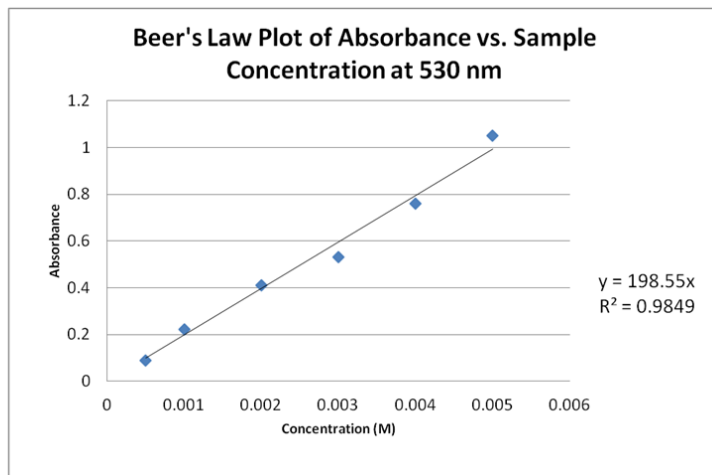
y = 198.55x
R² = 0.9849

Figure 2: A Beer's Law Plot of a sample obtained at 530 nm. Note that the titles on the graph are descriptive and include units when needed. The best fit line equation is shown on the graph as well as the R² value. The data in this figure are only meant as an example of how to generate a Beer's Law Plot and should not be the same as the numbers you measure in this procedure!

The data points shown in Figure 2 correspond to the blue sample points on the plot. It is imperative that concentration is plotted on the x-axis and absorbance is plotted along the y-axis. The best fit line generated from the data is useful in two ways. First, the molar absorptivity (ε) can be calculated using the slope of the best fit line. Remember that the slope of the line is the product of the molar absorptivity (ε) and the pathlength (l). The pathlength is the distance the light travels through the sample in centimeters. In today's lab, you will be using cylindrical cuvettes (shaped like test tubes) where the spectrophotometer's light passes through the curved side of the cuvette as shown in Figure 3. The distance measured as the pathlength is also indicated in Figure 3. In this procedure, the pathlength of your cuvette is 1 cm which makes the molar absorptivity calculation very simple.
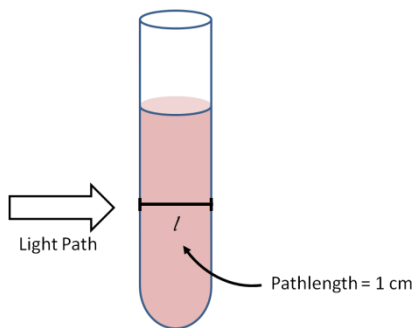
$$m = \varepsilon * l$$

$$198.55\frac{1}{M} = \varepsilon * 1cm$$

$$\frac{198.55\frac{1}{M}}{1cm} = \varepsilon$$

$$198.55\frac{1}{M * cm} = \varepsilon$$

Light Path

Pathlength = 1 cm

Figure 3a

Figure 3b

Figure 3a: Diagram showing a how the pathlength is measured. The distance measured as the pathlength is indicated by the black bar drawn across the cuvette. Figure 3b: Sample calculation showing how the molar absorptivity is calculated from the slope of the Beers Law best fit line.

The data shown in Figure 2 is also useful in determining the concentration of an unknown sample using the absorbance of that sample. For example, if the absorbance at 530 nm of a sample of unknown concentration is 0.645, the concentration can be calculated as shown using the constants found in the Beer's Law plot:

From the best fit line in Figure 2 we have:
$$\varepsilon = 198.55\,\frac{1}{M*cm} \quad \text{and} \quad l = 1cm$$

Including these in Beer's Law $(A = \varepsilon * l * c)$ gives:
$$A = 198.55\,\frac{1}{M*cm} * 1cm * c$$

Substituting 0.645 for A, the value of c is able to be calculated:

$$\frac{0.645}{198.55\,\frac{1}{M*cm} * 1cm} = c = 0.00325M$$

One practical aspect that must be considered so that your Beer's Law plot produces quantitative, repeatable results is that we must ensure that I_0 and I_t are measured accurately so that the loss of light measured by the detector is only due to sample absorption. To do this, we must minimize and account for any loss of light due to scattering and absorption by the sample container and other components of the solution. The cuvettes you use in the spectrometer are made of optical grade glass or quartz in order to minimize the effect of reflection and scattering caused by imperfections in the walls of the cuvette. You should use the same cuvette for all of your measurements and note the position of the marking on the front of the cuvette to ensure that the position of the cuvette is the same each time you make a measurement. This ensures that if there is a flaw in the cuvette you are using, your measurements will account and compensate for it. In order to have an accurate I_0 reference point, you will also need to use an appropriate blank for your system. A blank is a cuvette filled with a solution that is identical to your sample solutions in every way with the exception of the molecule you are trying to measure. This way any absorbance or scatter due to the solvent or other dissolved molecules will not affect your measurement.

 Colorimetric Analysis of ASA

Acetylsalicylic acid (ASA) does not absorb light in the visible range. This is easily seen when you look at a dissolved sample of ASA as it is colorless. Colorimetry is a technique that uses a chemical reaction between reagents to produce a measurable color change. In order to use absorption spectroscopy and Beer's law to measure the concentration of ASA in an unknown sample, a reaction with Fe^{3+} converts ASA into a violet complex that strongly absorbs light around 530 nm. This occurs via a two step process shown in the following reactions.

First, a strong base is added to the ASA forming the salicylate dianion and an acetate ion.

Acetyl salicylic acid   Salicylate dianion   acetate anion
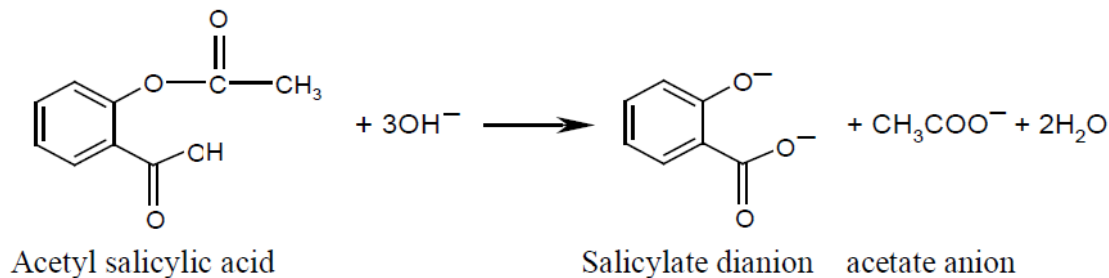
After this, the solution is acidified which causes the addition of an H^+ to the salicylate dianion. Then, $FeCl_3$ is added. When the $FeCl_3$ dissolves, the Fe^{3+} will form a complex with 6 water molecules surrounding it. As shown in the reaction below, the hydrated iron(III) ion forms a complex ion by binding to 2 oxygen atoms in the salicylate.
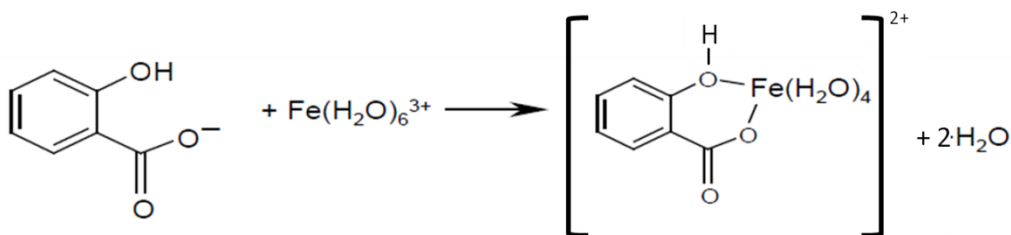
Because this reaction proceeds to completion and is very fast, the concentration of the colored complex will be the same as the concentration of ASA in the original sample. To ensure that every molecule of ASA is detected, an excess of iron (III) cations will be used.

Summary of Procedure

In the following procedure, you will dilute a solution of known ASA concentration with a $FeCl_3$-KCl-HCl solution to make a series of standard ASA solutions of known concentration. You will then use a Spec-20 spectrophotometer to measure the absorbance of each of these solutions at 530 nm. Using the absorbance data you collect, you will construct a Beer's law plot and calculate a line of best fit. Using this line, you will calculate the molar absorptivity (ε) of the ASA complex at 530 nm.

Finally, you will use the Beer's law plot to determine the concentration of ASA solutions made from commercial preparations of aspirin. You will then use the molarities of these solutions to calculate the mass of the ASA that was in the original tablet and the percent mass of ASA in the tablet.

♦ *Procedure*

Part 1: Preparing the ASA stock solution and Commercial Aspirin Tablet Solutions

* In the interest of time, your instructor may omit Part 1 of the procedure opting instead to use solutions previously prepared. If this is the case, you should take care to record the data from these solutions in Part 1 of your Data Sheet as they will be needed to complete your calculations.*

A. Preparation of ASA Stock Solution

Before you start, collect the following:

- 10 mL graduated cylinder
- 250 mL volumetric flask
- 125 mL Erlenmeyer flask
- glass funnel
- Hot Plate

1. Add 0.100 g reagent grade ASA to a 125 mL Erlenmeyer flask. Record this mass.
2. Measure 10 mL of 1M NaOH solution using a clean, dry graduated cylinder.   Add this to the Erlenmeyer flask from Step 1.
3. Heat the mixture to boiling on a hot plate to hydrolyze the ASA. *This step must be done carefully to avoid splatters!* Periodically rinse the inside walls of the flask with small amounts of deionized water to ensure all of the ASA has been reacted. The addition of deionized water will also ensure that the water in the boiling flask does not evaporate away. Note that this step will form the salicylate dianion shown as the product of Reaction 1.
4. Transfer the resulting solution to a 250 mL volumetric flask using a glass funnel. Use deionized water to thouroughly rinse the flask and funnel so that the salicylate is quantitatively added to the volumetric flask.
5. Add deionized water to the flask to the bottom of the neck of the volumetric. Stopper the flask and, while holding the stopper with your forefinger to prevent spilling the solution, invert the flask 10 times to ensure complete mixing. After all air bubbles rise to the surface, add water to the mark on the neck of the flask. Again stopper the flask and invert 10 times to mix the solution.

B. Preparation of Commercial Aspirin Tablet Stock Solutions

Before you start, collect the following:

- 10 mL graduated cylinder
- 250 mL volumetric flask
- 125 mL Erlenmeyer flask
- glass funnel
- Hot Plate

1. Record the mass of one commercial aspirin tablet.
2. Add the tablet to a 125 mL Erlenmeyer flask. So that the tablet dissolves and the reaction of ASA occurs in a timely manner, it is advised that you crush the tablet before adding it to the flask.
3. Measure 10 mL of 1M NaOH solution using a clean, dry graduated cylinder. Add this to the Erlenmeyer flask from Step 2.
4. Heat the mixture to boiling on a hot plate to hydrolyze the ASA. *This step must be done carefully to avoid splatters!* Periodically rinse the inside walls of the flask with small amounts of deionized water to ensure all of the ASA has been reacted. The addition of deionized water will also ensure that the water in the boiling flask does not evaporate away. Note that this step will form the salicylate dianion shown as the product of Reaction 1.
   It should be noted that cloudiness caused by starch fillers should disappear when the solution is acidified. Some buffering agents may also remain undissolved and should be allowed to settle before using the solution.
5. Transfer the resulting solution to a 250 mL volumetric flask using a glass funnel. Use deionized water to thouroughly rinse the flask and funnel so that the salicylate is quantitatively added to the volumetric flask.
6. Add deionized water to the flask to the bottom of the neck of the volumetric. Stopper the flask and, while holding the stopper with your forefinger to prevent spilling the solution, invert the flask 10 times to ensure complete mixing. After all air bubbles rise to the surface, add water to the mark on the neck of the flask. Again stopper the flask and invert 10 times to mix the solution.
7. Repeat steps 1-6 of this section for each commercial tablet you are to analyze.

> - *Before Beginning Part 2, you should begin warming up the Spec-20 so that it is ready for use when you need it in Part 4.*
>
> ***Warming up the Spec-20***
> 1. Your Spec-20 must be warmed up for at least 15-20 minutes before use. This allows the light source to become stable so that the intensity of light striking the sample is constant. It also warms up the detector so that it is able to make consistent, reproducible readings. To warm up the Spec-20, simply plug it in, and turn the On/Off Knob located on the front left of the instrument. You should hear the fan come on and the Readout light up.

- ***Part 2: Preparation of ASA standard solutions***

In this section of the procedure, you will use ASA Stock Solution from Part 1, and $2x10^{-2}M$ $FeCl_3$-KCl-HCl solution to prepare 5 standard ASA solutions of known concentration. When you complete this section, you should have 5 small beakers of violet Fe-ASA complex solutions.

Before you start, collect the following:

- 1,2,3,4,and 5 mL Pipettes
- One 25 mL volumetric flask
- 5 small (50 mL is plenty) Erlenmeyer flasks or beakers for storing your solutions
- Small glass funnel
- A beaker of about 200 mL $FeCl_3$Solution.
- A beaker of about 200mL of deionized water for rinsing glassware
- A small beaker holding about 30 mL ASA Stock Solution prepared in Part 1 or provided by the instructor.
- One large beaker for collecting waste

1. Label 5 small flasks or beakers "Standard ASA Solution 1" through "Standard ASA Solution 5" for storage of your standard solutions. Also label the beakers for waste, deionized water, $FeCl_3$ stock solution, and ASA stock solutions before using them to ensure that solutions are not confused. At the completion of this section, you should not discard any solutions as you will use these stock solutions in Part 3.
2. Clean, using 2-3 deionized water rinses only, all pipettes, the 25 mL volumetric flask, and your storage beakers.
3. Rinse the 1 mL pipette with a small aliquot of ASA stock solution. Discard rinse into waste beaker. Repeat once more.
4. Measure 1 mL of ASA stock solution using the clean pipette and add to the 25 mL volumetric flask. Be sure to allow the pipette to drain on its own, not forcing the last bit of solution from the pipette tip.

5. Dilute the ASA stock solution to the marking on the volumetric flask using the $FeCl_3$-KCl-HCl solution. The solution should immediately turn violet indicating that you have made the complex ion that is the product of Reaction 2.
6. Pour this solution into the storage beaker marked "Standard ASA Solution 1."
7. Repeat this process using the 2 mL, 3 mL, 4 mL and 5 mL pipettes making the other 4 standard solutions

- *Part 3: Preparation of commercial aspirin unknown solutions*

In this section of the procedure, you will use the commercial aspirin stock solutions made in Part 1 (or provided by the instructor), and $2x10^{-2}M$ $FeCl_3$-KCl-HCl solution to prepare unknown concentration solutions. When you complete this section, you should have 1 small beaker of violet Fe-ASA complex solution for every commercial tablet you are planning to analyze.

Before you start, collect the following:

- Small (50 mL is plenty) Erlenmeyer flasks or beakers for storing solutions
- Small glass funnel
- 1 mL Pipette
- One 25 mL volumetric flask

1. Label small Erlenmeyer flasks or beakers for storing the solutions you prepare in this step. You will need one beaker or flask for each commercial preparation that you will analyze.
2. Rinse your 1 mL volumetric pipette from Part 2 first with deionized water, and then with one or two small aliquots of the commercial aspirin stock solution. Discard rinses into the waste beaker.
3. Measure 1 mL of the commercial aspirin solution using the clean pipette and add to the 25 mL volumetric flask.
4. Dilute the solution to the marking on the volumetric flask using the $FeCl_3$-KCl-HCl solution. The solution should immediately turn violet indicating that you have made the complex ion that is the product of Reaction 2.
5. Pour this solution into a labeled storage beaker.
6. Repeat this process (steps 2-5) for each commercial preparation you are analyzing.

- *Part 4: Absorbance Measurements of Standard ASA solutions and Commercial aspirin preparations*

Before you start, collect the following:

- 2 plastic transfer pipettes
- 1 cuvette
- Test tube rack
- Spec-20

A. Setting up the Spec-20

The following instructions walk you through preparing the Spec-20 spectrophotometer for absorbance measurements. Recall from the Background Section the importance of using a blank to ensure that the I_0 measured by the detector is accurate. Note that you should use the same cuvette for all of the measurements as well as the blank so that any imperfections in the cuvette are accounted for. You should also note that the cuvettes have a marking on the top that should be lined up with the arrow in the cuvette holder each time a reading is made.
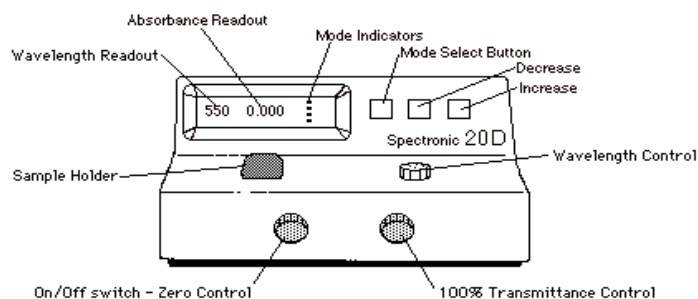

Figure 4: Labeled Diagram of the Digital Spec-20 Spectrophotometer. This figure will be referenced in the text.

Warming up the Spec-20 (If this was completed prior to beginning Part 2, skip this step.
1. Your Spec-20 must be warmed up for at least 15-20 minutes before use. This allows the light source to become stable so that the intensity of light striking the sample is constant. It also warms up the detector so that it is able to make consistent, reproducible readings. To warm up the Spec-20, simply plug it in, and turn the On/Off Knob located on the front left of the instrument. You should hear the fan come on and the Readout light up.

Set the wavelength of the Spec-20
1. Set the wavelength to 530 nm using the wavelength control knob shown in Figure 4.
2. Ensure that the filter setting is moved all the way to the 340-599nm setting. This is a lever located under the On/Off knob, but not pictured in Figure 4.

Setting 0% Transmittance
When there is no sample in the sample holder, there is a shutter that closes, blocking any light from hitting the detector. The reading should be 0 %T when this happens. This step adjusts the electronic detector amplification so that the detector reads 0 %T as it should.

1. Make sure that no sample cuvettes are in the sample holder of the Spec-20.
2. The "Mode indicator" light should indicate that the Spec-20 is reading in %T Mode. If it is not, change the mode by pushing the "Mode Selection Button" (See Fig. 4) to toggle the mode to %T.

3. Using the "Zero Control" knob (The same one used to turn on the instrument) adjust until the readout shows 0.000 %T. The reading may fluctuate some in the last digit, but should be stable otherwise. If the readout is not stable, your Spec-20 may not be warmed properly. If you believe it is warm, and still experience fluctuations, see your instructor.

Setting 100% Transmittance
*This is the step of the process where you will use a blank to cancel out effects from the cuvette and other substances in the sample solution. The purpose of this process is to tell the detector that the light striking it when there is only a cuvette and reference solution as a sample is the I_0, or the maximum amount of light that the detector will see when there is no absorbing sample present. *

1. Clean your cuvette using the steps in the box. You will be preparing your blank using $FeCl_3$-KCl-HCl solution as your reference solution for this experiment.   (Think about why you would use this solution rather than water…)

> **Steps for Cleaning the Cuvette**
> **NEVER use a brush inside the cuvette! It will scratch the surface and make the cuvette useless.**
> a. Rinse the cuvette 2-3 times with deionized water.
> b. Rinse the cuvette 2 times with about 1 mL of the sample to be measured. Make sure the rinse contacts all of the inside surface of the cuvette. Discard the rinse in your waste beaker.
> c. Fill the cuvette 2/3 full with the sample solution.
> d. Clean the outside of the cuvette with a dry Kimwipe. (These come from a small green box. It is NOT a paper towel!) Make sure to remove smudges or fingerprints before putting it in the Spec-20 for measurement.
> e. Measure the absorbance for all 5 standards and two unknowns.
> f. **You will need to repeat this process each time you change the sample solution in the cuvette.**

2. Insert the cuvette with the reference solution into the Spec-20.
3. Using the "100% Transmittance Control" knob, adjust until the readout shows 100.0 %T.

B. Measure the Absorbances of Standard Solutions and Unknown Solutions
You are ready to measure absorbances (A) for your standard solutions and your commercial aspirin tablet solutions (which are referred to as unknowns because you do not know the concentrations).

1. Clean your cuvette using the steps in the box. Filling the cuvette with Standard ASA Solution 1.
2. Using the "Mode Select Button" toggle the mode indicator to Absorbance. If no cuvette is in the sample holder, this will cause the readout to flash which is expected and correct.
3. Place the cuvette in the sample holder and record the absorbance reading on your Data Sheet.
4. Discard the used solution in your waste beaker, and repeat these steps for all standard solutions and unknown solutions .

NAME_____

♦ Data Sheets

Don't Forget Sig figs and Units!

- **Part 1: Preparing Stock Solutions**

Even if you do not complete Part 1 of the Procedure, you should complete this portion of the Data Sheets as you will need these values for your calculations!

A. Preparation of ASA Stock Solution

Mass of ASA Used: _____

Volume of Solution: _____

B. Preparation of Commercial Aspirin Tablet Stock Solutions

Brand of Sample Tablet 1:_____
Mass of Sample Tablet Used: _____
Advertized mass of ASA in 1 Tablet: _____
Volume of Solution: _____
Brand of Sample Tablet 2:_____
Mass of Sample Tablet Used: _____
Advertized mass of ASA in 1 Tablet: _____
Volume of Solution: _____
Brand of Sample Tablet 3:_____
Mass of Sample Tablet Used: _____
Advertized mass of ASA in 1 Tablet: _____
Volume of Solution: _____

- **Part 2: Preparation of Standard ASA Solutions**

Standard Solution	Volume of ASA Stock Solution Used	Final Volume of solution after dilution with FeCl$_3$ solution (These are all 25 mL, but you should check your volumetric to ensure correct sig figs!)
1		
2		
3		
4		
5		

- **Part 3: Preparation of Commercial Aspirin Unknown Solutions**

Unknown Solution	Volume of Commercial Aspirin Tablet Stock Solution Used	Final Volume of solution after dilution with FeCl$_3$ solution (These are all 25 mL, but you should check your volumetric to ensure correct sig figs!)
1		
2		
3		

- **Part 4: Absorbance Measurements**

Absorbance Measurement (no units)

Stock Solutions		
	1	
	2	
	3	
	4	
	5	

Unknown Solutions		
	1	
	2	
	3	

Results and Calculations

Use the following Instructions as help for your Calculations.

A. Calculate the Molarities of each of your 5 standard solutions
    This takes two steps!
    1. Calc Molarity of ASA Stock Solution.
        i. The Molar Mass of ASA is 180.2 g/mol
        ii. Convert g $\rightarrow$ mol, then divide by Liters of solution.
    2. Now, you are ready to use $M_1V_1=M_2V_2$ to calculate the concentrations of your standards.
        i. For each calculation, you should use the molarity of the ASA Stock solution you just calculated as M_1, the amount of this solution you used to make the standard (1-5mL) as V_1, and the final volume you diluted the stock solution (always 25mL) as V_2. You will calculate for M_2.
B. Beer's Law Plot
    1. Use the molarities of the standard solutions as your x-values, with the absorbance being the y-value.
    2. Force the intercept to be 0.
    3. Use the slope and the measured absorbance of the unknowns to calculate the molar absorptivity (ε) as shown in the Background section
C. Calculate the mass of ASA in the commercial tablet. (Repeat this process for each commercial Preparation you are analyzing.)
    1. Use the Beer's Law Best fit line to calculate the Molarity of each of the unknown solutions.
    2. Use $M_1V_1=M_2V_2$ to calculate the molarity in the commercial aspirin tablet stock solution.
        i. This time, you know the concentration of the dilute solution from your Beer's Law plot, the volume of the dilute solution (25 mL) and the volume of the stock solution used to make this dilute solution (1mL). You are calculating the Molarity of the solution made in Part 1 or provided by the instructor.
    3. Now, you know that one tablet was dissolved in 250 mL to make the stock solution. Use this knowledge and the molarity of the stock to calculate the total number of moles in the stock and then use the molar mass of ASA to convert that to grams.
    4. The mass of the tablet that was dissolved to make the unknown stock should have been recorded. Use this to calculate the mass % of ASA in the tablet.

Results and Calculations

A. Molarities of Standard ASA Solutions

> **Molarity of ASA Stock Solution:** ————————————————
>
> **Molarity of ASA Standard Solutions**
>
> > **1:** ——————————————
> >
> > **2:** ——————————————
> >
> > **3:** ——————————————
> >
> > **4:** ——————————————
> >
> > **5:** ——————————————

B. Beer's Law Plot: You should attach a graph showing your Beer's Law plot.

1. Make sure to include axis labels, a chart title, a Best Fit Line, and the equation for the best fit line on your chart if you use a computer program.
2. If your instructor allows it, you may use the graph page provided to hand-draw your graph. The graph should still include all parts listed above, but the equation of best fit will be found using a graphing calculator.

> **Equation of Best Fit:** _____
>
> **Molar Absorptivity (ε):** _____

B. Mass of ASA in Commercial Tablet

	Tablet 1	Tablet 2	Tablet 3
Molarity of Unknown Solutions			
Molarity of Commercial Aspirin Stock Solution			
Moles of ASA Contained in Tablet			
Mass of ASA contained in Tablet			
Mass% of ASA in Tablet			
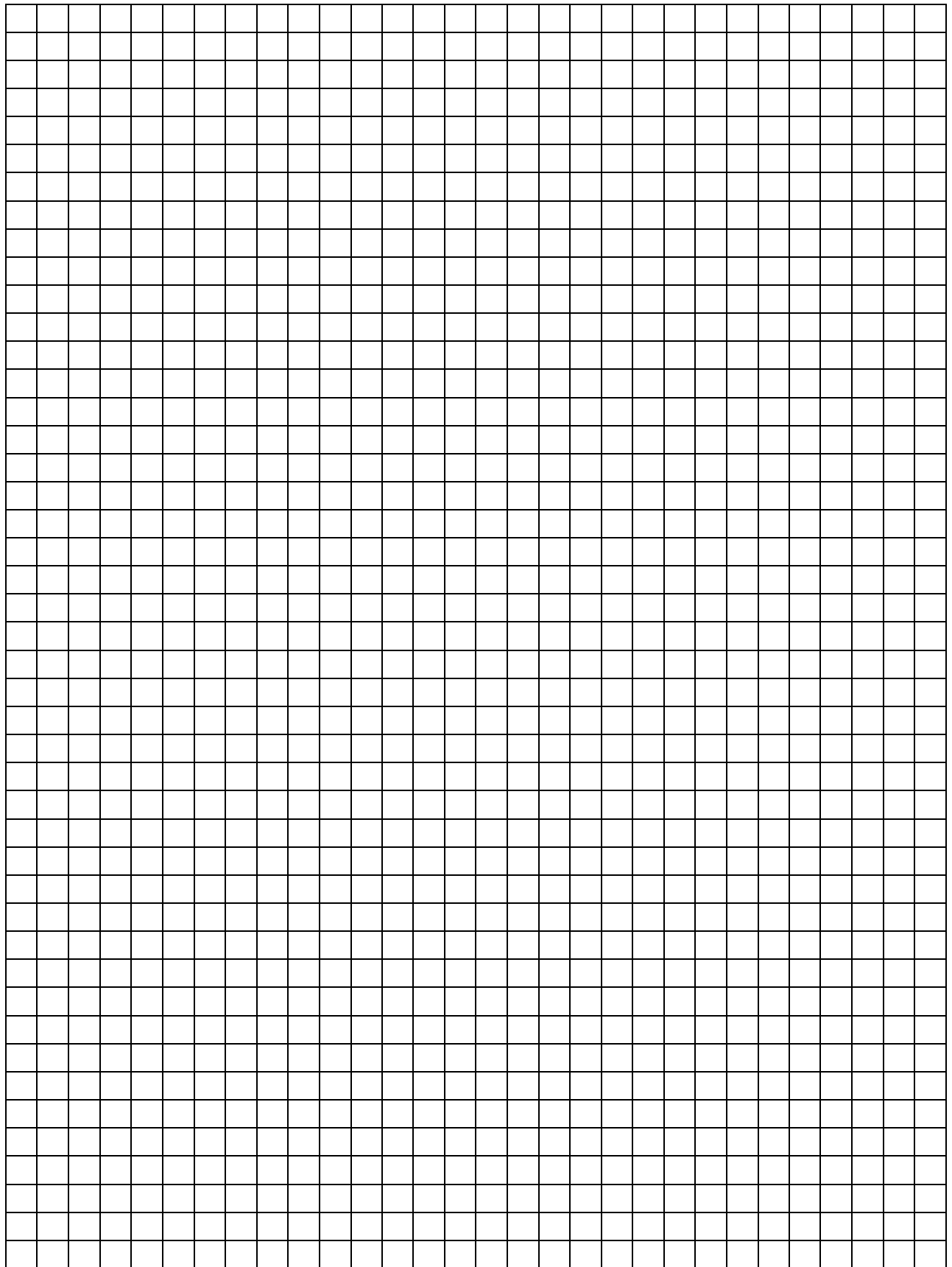
Post Laboratory Questions

1. **Why did you use the $FeCl_3$-KCl-HCl solution as a blank? Would water have worked? Explain your reasoning.**

2. **Fill in the table below with the ASA mass per commercial tablet you calculated to and the advertized mass of ASA in each tablet? (You have these numbers in your data and results tables.)**

	Experimentally Determined Mass of ASA contained in Tablet	Advertized mass of ASA per tablet (found on pill bottle)
Tablet 1		
Tablet 2		
Tablet 3		

    Use the table above to critique your analysis in 2 or 3 sentences. Are your calculated values consistently high or low? Are your values accurate, precise, both, or neither?

3. **What is the advantage of using a 5 mL pipette once to make your 5th ASA standard solution rather than using the 1 mL pipette 5 times?**

4. **Look at the ASA mass % you calculated for each tablet. Is the majority of the mass of the tablet from ASA? Is this what you expected given the brand and list of ingredients for the tablets you analyzed.**

5. **A student who uses a pipette incorrectly forces all of the ASA stock solution out of the end of her pipette.**
   a. **How will this mistake affect the calculated molarities of the ASA standard solutions? Explain your reasoning.**

   b. **If the commercial tablet unknown solutions are made correctly, will her error lead to a high or low calculated ASA mass per tablet? Explain your reasoning.**

NAME_____

Pre-Laboratory Questions

1. A stock solution of NaCl is made using 25.738g NaCl and diluting to a final volume of 250mL. What is the molarity of this stock solution?

2. You use the stock solution above to make 3 dilute solutions. Each of the new solutions are diluted to a total of 150mL. What is the molarity of each new solution?

Dilute Solution	Amount of Stock NaCl solution used	Molarity of Dilute Solution
1	25 mL	
2	15 mL	
3	1 mL	

3. Colorimetry is a technique used to determine the concentration of many molecules in solution that do not absorb visible light. One example is the detection of glucose in a sample. Glucose itself will not absorb visible light, but forms a blue-green complex when reacted with toluidine in acetic acid. The analytical wavelength for this complex is 635 nm. A student prepares the following Beer's Law plot. Use the plot to answer the following questions.

Beer's Law Plot of Absorbance vs. Glucose concentration at 635 nm

$y = 23.75x$
$R^2 = 0.9922$
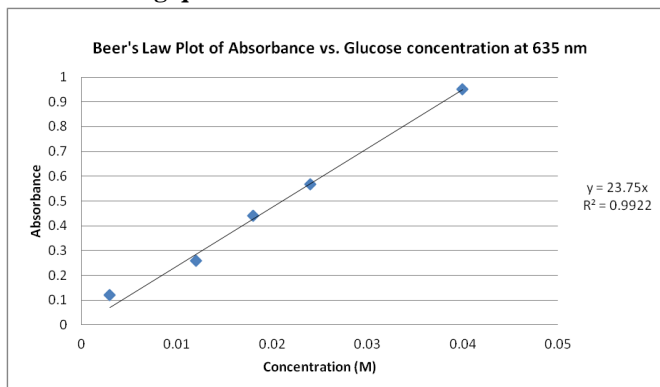
The student wants to use his data shown above to analyze a tablet containing glucose as well as other ingredients to determine the amount of glucose in the tablet. The tablet has a mass of 2.500g and is dissolved to a total solution volume of 300mL using a solution containing toluidine and acetic acid. This student measures an absorbance of .732 for this solution at 630 nm.

a) Calculate the molar absorptivity (ε) of this complex given that the pathlength is 1 cm. Include units!

b) Calculate the glucose concentration of the unknown solution the student prepared.

c) Calculate the number of moles of glucose in the tablet.

d) Calculate the mass of glucose in the tablet using the molar mass of glucose (180.16 g/mol)

e) Calculate the mass % of glucose in the tablet.