LEARNING GOALS

1. More practice with application’s of Beer’s Law
2. Gain Experience with the Method of Standard Addition
3. Gain experience with pre-treatment using a color developing agent

INTRODUCTION

Spectrophotometric methods of analysis are fast, relatively simple and very widely applied. They rely on the fact that electromagnetic radiation may be absorbed by matter. The extent to which radiation is absorbed is related to the nature and concentration of absorbing material present in a sample as well as the wavelength of the radiation employed. In this experiment the absorption of light of 522 nm wavelength by a sample solution will lead to an analysis for a trace amount of iron in an unknown sample. We begin with a description of the spectrophotometric experiment.

Consider a sample of some solution contained in a small transparent vessel - perhaps a test tube (when employed in spectrophotometric measurements the container is called a cuvet). Imagine a beam of monochromatic light (light of a single wavelength - in practice light with a very narrow range of wavelengths) that passes through the solution. For the moment we will ignore any interaction of the beam with the cuvet itself. The intensity of the light beam as it enters the solution is called the incident intensity and is given the symbol $I_0$.

The incident intensity is essentially the number of photons per second that enters the sample solution. As the light traverses the sample some photons may be absorbed by the components of the sample depending on the nature of the components and the wavelength of the light.

NOTE: Absorption of infrared radiation relates to vibrational or rotational excitations of molecules. Absorption of visible and ultraviolet light results in electronic excitations - changes in the electron distribution in the molecules or ions of the absorbing material.

As a consequence of light absorption, the beam of light that emerges from the sample has a diminished intensity symbolized by $I$. Fewer photons leave the sample than entered it. The ratio $I/I_0$ is the fraction of light that actually passes the sample and is called the transmittance, $T$. This quantity is generally expressed as a percentage. As an example, a certain solution held in a particular cuvet may have a 10% transmittance at 450 nm wavelength. This statement means that when light of 450 nm wavelength (a shade of blue) passes the tube only 1/10 of the 450 nm photons remain in the beam; the rest are absorbed by the sample. This behavior makes no implication about the transmittance at some other wavelength of light. Indeed the same sample
might have a transmittance of 100% at 600 nm indicating that a beam of 500 nm light (a kind of green) passes through the sample tube without any detectable absorption of light.

The transmittance of a solution containing a light absorbing material, the analyte, is related to experimental conditions by Beer's Law.

\[-\log \frac{I}{I_0} = -\log T = A = \varepsilon b C\]

In this equation \(T\) is the transmittance, expressed as a decimal (10% transmittance corresponds to \(t = 0.10\)) and \(A\) is called the absorbance. \(C\) is the concentration of the analyte, \(b\) is the length of the light path through the absorbing solution and \(\varepsilon\) is the absorptivity, a number which depends both on the nature of the light absorbing substance and the wavelength of light. When \(b\) is expressed in cm and \(C\) in mol/L units \(\varepsilon\) has units of L mol\(^{-1}\)cm\(^{-1}\) and is termed the molar absorptivity or the molar extinction coefficient and may be symbolized as \(\varepsilon\). In other words, Beer's Law is sometimes written as \(A = \varepsilon b C\).

**Application of Beer's Law**

The Beer's Law equation may be applied to analyses in a variety of ways. The simplest relies on measuring the absorbance of a known sample of the absorbing material, a standard with concentration \(C_{\text{std}}\), at an appropriate wavelength of light. The absorbance \(A_{\text{std}}\) is given by \(A_{\text{std}} = abC_{\text{std}}\). The unknown is then measured at the same wavelength under the same conditions of solution composition, temperature, etc. and in the same or a "matched" cuvet. The absorbance of the unknown is \(A_{\text{unk}}\) and is given by \(A_{\text{unk}} = \varepsilon b C_{\text{unk}}\). Combining the two equations gives \(C_{\text{unk}} = C_{\text{std}}A_{\text{unk}}/A_{\text{std}}\). Thus, we need only to measure the absorbance of a standard and the absorbance of the unknown in order to find \(C_{\text{unk}}\). This simple method is called a "one-standard" or "one-point" calibration method. In favorable cases where the absorbances measured are in the range of about 0.2 to about 1.0 and where no interfering substances are present, analyses made in this way are generally reproducible to about ± 1-2%. For best results (0.1-0.5%), a series of standard solutions are prepared and a Beer's Law plot is produced (see below).

A variation of this analysis is known as the standard addition method. A portion of the unknown is diluted with a suitable solvent to some known volume and the absorbance is measured at an appropriate wavelength. To a second, equal portion of the unknown is added an additional known amount of the analyte and the volume is adjusted as before. (The second solution contains the unknown amount of analyte plus some more that we add.) The absorbance of the unknown is given by \(A_{\text{unk}} = abC_{\text{unk}}\). The second solution has absorbance \(A\) and the analyte concentration is \(C_{\text{unk}} + C_{\text{std}}\), so that \(A = ab(C_{\text{unk}} + C_{\text{std}})\). Combining these equations gives \(C_{\text{unk}} = C_{\text{std}}/[A/(A_{\text{unk}}) - 1]\). For best results a series of standard solutions are prepared (just as in a typical Beer's law analysis), but each standard also contains the same aliquot of the unknown. This method works best when the quantity of added standard (the "spike") is comparable to the quantity of unknown present. The data is analyzed by preparing a calibration curve of “concentration added (to the unknown aliquot)” vs. absorbance (see example below). The negative of the x-intercept gives concentration of the analyte from the unknown aliquot.
Example of standard addition experiment

<table>
<thead>
<tr>
<th></th>
<th>500.0 μM A std</th>
<th>Unk</th>
<th>Solvent</th>
<th>μM [A] added</th>
<th>abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>blk</td>
<td>0.00</td>
<td>0.00</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>unk</td>
<td>0.00</td>
<td>5.00</td>
<td>5.00</td>
<td>0.00</td>
<td>0.103</td>
</tr>
<tr>
<td>standard 1</td>
<td>0.10</td>
<td>5.00</td>
<td>4.90</td>
<td>5.00</td>
<td>0.158</td>
</tr>
<tr>
<td>standard 2</td>
<td>0.20</td>
<td>5.00</td>
<td>4.80</td>
<td>10.00</td>
<td>0.219</td>
</tr>
<tr>
<td>standard 3</td>
<td>0.30</td>
<td>5.00</td>
<td>4.70</td>
<td>15.00</td>
<td>0.273</td>
</tr>
<tr>
<td>standard 4</td>
<td>0.40</td>
<td>5.00</td>
<td>4.60</td>
<td>20.00</td>
<td>0.335</td>
</tr>
<tr>
<td>standard 5</td>
<td>0.50</td>
<td>5.00</td>
<td>4.50</td>
<td>25.00</td>
<td>0.385</td>
</tr>
</tbody>
</table>

The concentration of A in each of the standards that was added from the 5 mL aliquot of unknown is approximately 8 μM (the negative of the x-intercept).

The standard addition method typically gives results reproducible to ± 1 - 3%. The method of standard addition is an important alternative to the typical Beer’s Law method when the unknown sample contains a complex matrix that influences the sensitivity of the analyte. If the sensitivity of the analyte in the unknown is markedly different than in the standards serious errors in the interpolated concentration can occur. In the method of standard addition the samples are prepared to ensure that all of the samples contain the same matrix effects. Thus, all of the solutions are on equal footing as far as matrix effects are concerned.

Both methods described above rely on an assumption that Beer's Law accurately describes the absorbance versus concentration behavior of the analyte material under the experimental conditions employed. In fact it is necessary to confirm that this is the case in each experimental circumstance. There are numerous reasons for deviations from Beer's Law from both instrumental and chemical sources.
Preparation of a calibration curve also known as a working curve and in biological sciences as a dose-response curve provides the necessary confirmation of Beer's Law and leads to yet another method of data analysis. The calibration curve is constructed by measuring the absorbances of a series of standards with accurately known analyte concentrations under the same experimental conditions of solution composition, wavelength, etc. that will be employed later with the unknowns. According to Beer's Law a plot of absorbance versus analyte concentration should be a straight line with intercept equal to zero. Once the plot is made the concentration of an unknown may simply be read from the graph after its absorbance is determined. Alternatively, the concentration of an unknown may be calculated from its measured absorbance and the regression equation of A versus C. A standard addition curve can also be produced. In the standard addition version of the calibration curve, each of the standards is spiked with the same quantity of unknown. A plot of absorbance vs. concentration added to the unknown is produced, and the concentration of the unknown is given by the negative of the x-intercept. Because 5 - 8 standards are typically employed in preparing the calibration curves random measurement errors of the standards tend to cancel, at least more so than with the single standard methods strategy described earlier. The result is that analyses made with the aid of a calibration curve are often reproducible to ± 0.5 - 1%.

The Scope and Limitations of the Method
Spectrophotometric analyses made by any of the methods above rely on several important assumptions:

1. The analyte substance must strongly absorb light of the wavelength employed for measurement.

Many substances simply do not absorb light (or absorb only weakly) at any convenient measurement wavelength. For example, a solution containing $10^{-5} \text{Fe}^{2+}$ in H$_2$O is essentially colorless at all visible wavelengths of light. The absorbance of a 1.00 cm cuvet (b = 1.00 cm) containing this solution is almost exactly 0.000 over the entire visible range from 400 nm to 700 nm. Nevertheless, it is a simple matter to make a spectrophotometric analysis of the solution for manganese. This is accomplished by a pre-treatment that involves acidifying the solution and heating with excess S$_2$O$_8^{2-}$ (peroxydisulfate). The peroxydisulfate ion is colorless to visible light. It is a very strong oxidizer that quantitatively converts nearly colorless Mn$^{2+}$ to the strongly colored MnO$_4^-$ (permanganate ion). Permanganate absorbs light most strongly near 525 nm and measurement of the absorbance at this wavelength leads to a value of the original Mn$^{2+}$ concentration by any of the methods we have described. In this example, peroxydisulfate has served as a color developing reagent. In the experiment that follows you will analyze a solution for iron (Fe$^{3+}$) at a very low concentration. Fe$^{3+}$ is only weakly colored to visible light and in dilute solution appears colorless. However, 2, 2'-dipyridine (which we will symbolize as dipy) forms an intensely colored complex with Fe$^{2+}$. We will take advantage of this by adding an excess of dipy to the Fe$^{3+}$ sample along with NH$_2$OH.HCl (hydroxylamine hydrochloride). This substance reduces Fe$^{3+}$ to Fe$^{2+}$ which is subsequently complexed by dipy. The result is complete conversion of Fe$^{3+}$ to Fe(dipy)$_3^{2+}$ which strongly absorbs light at 522 nm.

2. The analyte must be the only substance that absorbs light at the wavelength of measurement.

Recall that the absorbance, which is proportional to the concentration of analyte, is simply a measure of how much a light beam is attenuated by the cuvet. If several substances in
the cuvet absorb light each will attenuate the beam. In that case the measured absorbance will depend on the nature and concentration of all the absorbing substances present and will no longer be proportional to the concentration of analyte. In fact the absorbance of the mixture is the sum of the absorbances of the individual components (as we have seen in the previous experiment),

AN EXAMPLE: A solution contains two absorbing substances: X and Y. A \(1.00 \times 10^{-3}\) F solution of X has a transmittance of 50.0%. A \(2.00 \times 10^{-3}\) F solution of Y has a transmittance of 25.0%. What is the transmittance of a solution that contains both \(1.00 \times 10^{-3}\) F X and \(2.00 \times 10^{-3}\) F Y?

The absorbance of a \(1.00 \times 10^{-3}\) F X is \(-\log(0.500) = 0.301\). The absorbance of a \(2.00 \times 10^{-3}\) F Y is \(-\log(0.250) = 0.602\). The absorbance of the mixture is the sum, 0.903. This corresponds to a transmittance of 0.125 or 12.5%.

Elimination of the interfering substances (ones that absorb at the same wavelength as the analyte) is a difficult problem that must be dealt with one analysis at a time. There exists a large body of reference literature that describes specific experimental procedures designed to deal with elimination of many interferences in many thousands of spectrophotometric analyses. Some methods rely on chemical reactions or on separation and others involve numerical analysis procedures based on absorbance measurements at several different wavelengths of light. In any case, it is essential that any attenuation of the light beam from sources other than the analyte be either eliminated or accounted for in some other way. In this connection it is important to recognize that the cuvet itself as well as any small impurities present in the color developing reagents may contribute to the absorbance. For this reason, analyses based on measurements of absorbance almost always involve a blank. A blank is a cuvet, as closely matched as possible to the cuvet containing the sample, but containing none of the analyte substance. In the experiment that follows you will use a blank cuvet made of the same material and of the same dimensions as the sample cuvet. The blank contains a solution of all of the same substances and at the same concentrations as the sample cuvet. The single difference between the blank and sample cuvets is that no iron is added to the blank. In this way we (hope to) assure that the measured absorbance is related only to the quantity of iron added to the sample cuvet. If this is indeed the case we may employ the methods described above to make a reasonably accurate analysis for a very small quantity of iron in an unknown sample.

NOTE: In many analyses the blank is handled as a separate sample. Consider an analysis by the "one-standard method".

We have an unknown sample, a standard and a blank (that contains none of what we are analyzing for). Each of these is carried through a complex but identical series of operations. The absorbance of each final product is measured and the absorbances of the unknown and standard are each "corrected" by subtracting the blank absorbance. That is \(A(\text{unknown}) = A(\text{unknown, measured}) - A(\text{blank})\) and \(A(\text{standard}) = A(\text{standard, measured}) - A(\text{blank})\).

**IN THE LABORATORY**

You will work with a partner in this experiment. Arrange the work so that one person pipets all of the portions of a given component. For example, one person adds 0.500 mL of 0.10 F \(\text{H}_2\text{SO}_4\) to each mixing tube and after the entire series is done, the second person adds the iron solutions and mixes each tube, etc.
The various solution components should be added in the order: H$_2$SO$_4$, iron solution, NH$_2$OH.HCl, NaOAc, dipyridine and water. Mix the contents of the bottles after each addition.

You are going to work in pairs, but you will share data with another pair of students. One pair will perform a typical Beer’s law analysis. The other pair will perform a standard additions analysis. The both sets of data will be used to measure the amount of iron in a nutritional supplement. A comparison of the data will reveal if there are matrix effects from the other components of the supplement that interfere with the analysis using a typical Beer’s Law calibration curve.

**Preparing the unknown**  
*Your instructor will do this prior to lab.*
Obtain a vitamin supplement and place it in a clean, dry 100 mL beaker. Add about 5-10 mL of concentrated HCl. Let it sit in the hood for 5 minutes, occasionally stirring. Slowly add about 50 mL of water from a wash bottle. Using a funnel and a piece of filter paper the dissolved vitamin was transferred to a 1 L volumetric flask. The filter paper was repeatedly washed with water to ensure all of the iron was transferred to the flask. Finally, the flask was diluted to the 1.000 L mark.

**Preparation of solutions for Beer’s Law calibration plot**

Line up seven clean and dry 20 mL screw cap bottles and label them 0, 1, 2, 3, 4, 5, and unk. The ”0” tube will be the blank with no added iron. Tubes 1, 2, 3, 4, 5 are prepared by adding a known amount of standard iron solution (see table below).

<table>
<thead>
<tr>
<th>Std</th>
<th>H$_2$SO$_4$</th>
<th>Fe$^{3+}$ Std</th>
<th>Fe$^{3+}$ unk</th>
<th>NH$_2$OH.HCl</th>
<th>NaOAc</th>
<th>Dipy</th>
<th>DI water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blk</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>1.000 mL</td>
</tr>
<tr>
<td>1</td>
<td>0.500 mL</td>
<td>0.200 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.800 mL</td>
</tr>
<tr>
<td>2</td>
<td>0.500 mL</td>
<td>0.400 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.600 mL</td>
</tr>
<tr>
<td>3</td>
<td>0.500 mL</td>
<td>0.600 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.400 mL</td>
</tr>
<tr>
<td>4</td>
<td>0.500 mL</td>
<td>0.800 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.200 mL</td>
</tr>
<tr>
<td>5</td>
<td>0.500 mL</td>
<td>1.000 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
</tr>
<tr>
<td>Unk</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
</tr>
</tbody>
</table>

1. Use a 500 µL pipet gun to add 0.500 mL of 0.060 F H$_2$SO$_4$ to each screw cap bottle. Discard the tip.

2. Use a 1 mL pipet gun to add standard iron solution (3.00 x 10$^{-4}$ F) to the screw cap bottles as follows: tube ”0” - 0.00 mL, bottle “1” - 0.200 mL, bottle “2” - 0.400 mL, bottle “3” – 0.600 mL, bottle “4” - 0.800 mL, and bottle “5” – 1.000 mL.

3. Then use a 500 µL pipet gun add 0.500 mL of the unknown to the “unk” bottle. Cap and mix the contents of each.
4. Use a 500 µL pipet gun with a fresh tip to add 0.500 mL of 1.3% NH₂OH.HCl solution to each of the bottles. Cap and mix.

5. Use a 500 mL gun and a fresh tip to add 0.400 mL of 0.6 F sodium acetate (NaOAc) to each bottle. Cap and mix.

6. Use a 500 µL pipet gun and a fresh tip to add 0.500 mL of 0.25 % 2,2'-dipyridine solution to each bottle and cap and mix.

7. You have now added various quantities of liquid to the bottles. Use the pipet guns to add the appropriate amount of DI water so that the final volume in each bottle is exactly 3.000 mL. Put the caps on and mix thoroughly.

Preparation of solutions for Standard Additions Analysis
(also see table below for guide on preparing the solutions)

Line up seven clean and dry 20 mL screw cap bottles and label them 0, x, 1x, 2x, 3x, 4x, and 5x. The “0” bottle will be the blank with no added iron. Bottles x, 1x, 2x, 3x, 4x, and 5x are prepared by adding a portion of the unknown iron solution plus a known added amount of standard iron solution.

<table>
<thead>
<tr>
<th>Std</th>
<th>H₂SO₄</th>
<th>Fe³⁺ Std</th>
<th>Fe³⁺ unk</th>
<th>NH₂OH.HCl</th>
<th>NaOAc</th>
<th>Dipy</th>
<th>DI water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blk</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
<td>0.000 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>1.200 mL</td>
</tr>
<tr>
<td>X</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
<td>0.200 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>1.000 mL</td>
</tr>
<tr>
<td>1x</td>
<td>0.500 mL</td>
<td>0.200 mL</td>
<td>0.200 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.800 mL</td>
</tr>
<tr>
<td>2x</td>
<td>0.500 mL</td>
<td>0.400 mL</td>
<td>0.200 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.600 mL</td>
</tr>
<tr>
<td>3x</td>
<td>0.500 mL</td>
<td>0.600 mL</td>
<td>0.200 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.400 mL</td>
</tr>
<tr>
<td>4x</td>
<td>0.500 mL</td>
<td>0.800 mL</td>
<td>0.200 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.200 mL</td>
</tr>
<tr>
<td>5x</td>
<td>0.500 mL</td>
<td>1.000 mL</td>
<td>0.200 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
</tr>
</tbody>
</table>

1. Use a 500 µL pipet gun to add 0.500 mL of 0.060 F H₂SO₄ to each of the screw cap bottles. Discard the tip.

2. Use a 1 mL pipet gun to add the standard iron solution (3.00 x 10⁻² F) to the screw cap bottles as follows: bottle "0" and “x” - 0.000 mL, bottle “1x” - 0.200 mL, bottle “2x” - 0.400 mL, bottle “3x” – 0.600 mL, bottle “4x” - 0.800 mL, and bottle “5x” – 1.000 mL.

3. Then use a 200 µL pipet gun to add 0.200 mL of the unknown to each of the bottles, “x”, “2x”, “4x”, “6x”, “8x”, and “10x” (But not to the blank). Cap and mix the contents of each bottle.

4. Using a 500 µL pipet gun equipped with a fresh tip, add 0.500 mL of 1.3% NH₂OH.HCl
solution to each of the six bottles. Cap and mix.

5. Use a 500 μL pipet gun equipped with a fresh tip to add 0.500 mL of 0.6 F sodium acetate (NaOAc) to each bottle. Cap and mix.

6. Use a 500 μL pipet gun equipped with a fresh tip to add 0.500 mL of 0.25 % 2,2'-dipyridine solution to each bottle and cap and mix.

7. You have now added various quantities of liquid to the bottles. Use the pipet guns to add the appropriate amount of DI water so that the final volume in each bottle is exactly 3.200 mL. Put the caps on and mix thoroughly.

    Adjust the spectrophotometer wavelength selector to 522.0 nm. Rinse and fill two cuvets with blank (solution"0") using a Pasteur pipet. Carefully wipe the cuvets and place them in the spectrophotometer. Set the 100 % T or the zero absorbance. Discard the solution in the sample cuvet and add a second portion of the blank. Measure the absorbance. The value should be within 0.001 or 0.002 of zero. If not, consult the instructor.

    Discard the blank in the sample cuvet; rinse the cuvet twice with std 1; fill the cuvet and measure the absorbance; record the value.

    Discard the sample and repeat the measurement with a fresh portion of the same sample. The measurements should agree to within about 0.002 absorbance units. Repeat as necessary. Discard the sample solution and use the same procedure to measure the remaining solutions, 2, 3, 4, 5, unk or the x, 1x, 2x, 3x, 4x, and 5x standards.

    When you have completed the measurements rinse the cuvets with several portions of distilled water and return them to the instructor. Discard the solutions and thoroughly rinse the mixing bottles. Clean up. Trade data with another pair of students so that you have a Beer’s law data set and a standard addition data set.
THE LAB REPORT

1. Analysis using a typical Beer’s law plot

   a. Using Excel prepare a plot of the absorbance versus the iron concentration in units of "micrograms Fe per bottle" for each standard, 0, 1, 2, 3, 4, and 5. You must calculate the total mg of Fe added from the master Fe standard in each standard. [HINT: The first step in this calculation is to convert the Master Fe standard from units of mol/L to mg/mL] (5 pts)

   b. Does the plot appear to conform to Beer's Law? [In order to answer this question you must comment on whether the plot appears to be linear or show curvature and whether or not the intercept is reasonably close to zero.] (5 pts)

   c. Perform a regression analysis on the data. Are the regression statistics typical of a Beer's Law analysis? The standard error of regression should be less than 0.01. (5 pts)

   d. Determine the concentration of the unknown from the calibration curve in units of "micrograms Fe per sample". Also determine its uncertainty (see the “Introduction to Measurement” documented posted on the course website). (5 pts)

2. Analysis by the Standard Addition

   a. Using Excel prepare a plot of the absorbance versus the iron concentration added in units of "micrograms Fe of Master standard added" for bottles x, 2x, 4x, 6x, 8x, and 10x. You must calculate the total mg of Fe added from the master Fe standard in each 3.2 mL standard. (5 pts)

   b. Perform a regression analysis on the data. Are the regression statistics typical of a Beer's Law analysis? The standard error of regression should be less than 0.01. (5 pts)

   c. Determine the concentration of the unknown from the calibration curve units of "micrograms Fe per tube" (the negative of the x-intercept). (5 pts)

   d. Determine the uncertainty in the concentration of the unknown derived from the standard additions calibration plot (the error in the x-intercept; see the “Introduction to Measurement” documented posted on the course website). (5 pts)
3. **Iron in the supplement and Propagation of error**  
   (do this analysis for both sets of data)

   a. The mg of Fe in the unknown sample corresponds to the Fe in the 500 μL taken from the 1000 mL sample of the dissolved vitamin (for the Beer’s Law analysis) and to the Fe in the 200 μL taken from the 1000 mL sample of the dissolved vitamin (for the Standard Addition’s analysis). Determine the mg of Fe in the vitamin tablet as determined by both methods. (65 pts)

   b. Propagate errors to determine the uncertainty in the mass of iron in the tablet. (5 pts)

4. **Comparison of the results (include this in the discussion)**

   a. Do the results from the Beer’s Law plot and the Standard Additions Experiment Agree to the 95 % CL? Perform the t-test using \((n_1 - 2)\) and \((n_2 - 2)\) for degrees of freedom where \(n_1\) is the number of standards in the Beer’s Law plot and \(n_2\) is the number of points in the Standard Addition curve. Use the results from Part 3 (the mg Fe in the tablet) for this analysis, which is a reasonable approach to comparing the two methods, since the relative uncertainty of the result from the standard curves dominates over the relative uncertainties from the micropipette and the 1 L volumetric flask. (5 pts)

   b. Does the t-test suggest the presence of a likely matrix effect? (5 pts)

   c. The bottle claims that each tablet provides 18 mg of Fe. Do your results agree? Which method gave a result that is closer to the listing on the vitamin bottle? Does this finding impact whether or not you suspect there is a matrix effect. (5 pts)

In your report …

Show both standard curves.