EXPERIMENT 7
Spectrophotometric Iron Analysis

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 500 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.

$$-\log \frac{I}{I_0} = -\log T = A = abC$$

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 $a$ 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 a has units of $L \text{mol}^{-1} \text{cm}^{-1}$ and is termed the molar
absorbivity 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 to Analyses**

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 $C_{std}$, at an appropriate wavelength of light. The absorbance $A_{std}$ is given by $A_{std} = \varepsilon b C_{std}$. 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 $A_{unk}$ and is given by $A_{unk} = \varepsilon b C_{unk}$. Combining the two equations gives $C_{unk} = C_{std} A_{unk} / A_{std}$. Thus, we need only to measure the absorbance of a standard and the absorbance of the unknown in order to find $C_{unk}$. 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%.

A variation is 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 $A_{unk} = \varepsilon b C_{unk}$. The second solution has absorbance $A$ and the analyte concentration is $C_{unk} + C_{std}$, so that $A = \varepsilon b (C_{unk} + C_{std})$. Combining these equations gives $C_{unk} = C_{std} / [(A/A_{unk}) - 1]$. 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>analyte is $A$</th>
<th>500.0 $\mu$M A std (mL)</th>
<th>unk (mL)</th>
<th>solvent (mL)</th>
<th>$\mu$M [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 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 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 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 $10^{-5} \text{M Mn}^{2+}$ in H$_2$O is essentially colorless at all visible wavelengths of light. The absorbance of a 1.00 cm cuvet ($b = 1.00 \text{ cm}$) 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.

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 $1.00 \times 10^{-3}$ F X is $-\log(0.500) = 0.301$. The absorbance of $2.00 \times 10^{-3}$ 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
\( A(\text{standard}) = A(\text{standard, measured}) - A(\text{blank}) \).

BEFORE THE LAB

In this experiment you will make up a series of ten solutions by measuring and mixing a
number of components. The final solution volumes must each be adjusted to 15.00 mL by
adding appropriate but differing amounts of water. The preparation of the mixtures is outlined in
steps 1 - 7 in the next section. BEFORE YOU COME TO THE LAB calculate the volumes of
water that will be required to make up the volumes.

Prepare a data sheet with room for duplicate absorbance readings for each of the ten
mixtures labeled as indicated in the next section. List the required water portions on the data
sheet, which will be submitted as part of the lab report.

HINT: You might find it useful to rewrite the various solution compositions in steps 1 -
7 in the form of a table.
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 2.00 mL of 0.10 F H$_2$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. The iron standards require a 1 mL pipet gun but other components should be delivered by a 5 mL gun. (Use a fresh pipet tip for each new reagent to be added.) Mix the contents of the tubes after each addition by tapping the side of the tube and use a vortex mixer to thoroughly stir the tubes at the end.

You are going to work independently, but you will share data with a partner. One of you will prepare a set of solutions necessary for a typical Beer’s law analysis. The other will prepare a set of solutions necessary for 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 vitamine was transferred to a 500 mL 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 mark. 500.00 mL mark.

Preparation of solutions for Beer’s Law calibration plot

Line up seven clean and dry 10 mL graduated cylinders 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).

1. Use a 1 mL pipet gun to add 1.00 mL of 0.10 F H$_2$SO$_4$ to each tube. Discard the tip.

2. Use a 1 mL pipet gun to add standard iron solution (2.00 x 10^-3 F) to the tubes as follows: tube "0" - 0.00 mL, tube “1” - 0.100 mL, tube “2” - 0.200 mL, tube “3” – 0.300 mL, “4” - 0.400 mL, and “5” – 0.500 mL.

3. Then add 0.300 mL of the unknown to the “unk” test tube. Swirl the contents of each tube to mix.
4. Using a 1 mL gun equipped with a fresh tip, add 1.00 mL of 3% NH₂OH.HCl solution to each of the ten tubes. Mix.

5. Use a 1 mL gun and a fresh tip to add 2.00 mL of 0.5 F sodium acetate (NaOAc) to each tube. Mix.

6. Add 2.00 mL of 0.2% 2,2'-dipyridine solution to each tube and mix.

7. You have now added various quantities of liquid to each of the seven tubes. Dilute with DI water to total volume of 15.0 mL. Cap the tube and stir with a vortex mixer. Make up the other solutions in the same way.

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

<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>
</tr>
</thead>
<tbody>
<tr>
<td>Blk</td>
<td>1.00 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.00 mL</td>
<td>0.100 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>2</td>
<td>1.00 mL</td>
<td>0.200 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>3</td>
<td>1.00 mL</td>
<td>0.300 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>4</td>
<td>1.00 mL</td>
<td>0.400 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>5</td>
<td>1.00 mL</td>
<td>0.500 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>unk</td>
<td>1.00 mL</td>
<td>0.000 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
</tbody>
</table>

### Preparation of solutions for Standard Additions Analysis

(Also see table below for guide on preparing the solutions)

Line up seven clean and dry 10 mL graduated cylinders and label them 0, x, 1x, 2x, 3x, 4x, and 5x. The "0" tube will be the blank with no added iron. Tubes 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.

1. Use a 1 mL pipet gun to add 1.00 mL of 0.10 F H₂SO₄ to each tube. Discard the tip.

2. Use a 1 mL pipet gun to add standard iron solution (2.00 x 10⁻³ F) to the tubes as follows: tube "0" and “x” - 0.000 mL, tube “1x” - 0.100 mL, tube “2x” - 0.200 mL, tube “3x” – 0.300 mL, “4x” - 0.400 mL, and “5x” – 0.500 mL.

3. Then add 0.300 mL of the unknown to each of the tubes, “x”, “2x”, “4x”, “6x”, “8x”, and “10x” (But not to the blank). Swirl the contents of each tube to mix.

4. Using a 1 mL gun equipped with a fresh tip, add 1.00 mL of 3% NH₂OH.HCl solution to each of the seven tubes. Mix.
5. Use a 1 mL gun and a fresh tip to add 2.00 mL of 0.5 F sodium acetate (NaOAc) to each tube. Mix.

6. Add 2.00 mL of 0.2% 2,2'-dipyridine solution to each tube and mix.

7. You have now added various quantities of liquid to the graduated cylinder. Dilute to the 10.0 mL mark with distilled water. Pure the solution into a clean and dry small beaker, and swirl the solution to mix thoroughly. Make up the other solutions in the same way.

### Preparation of solutions for Standard Additions Analysis

<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·HC N</th>
<th>NaOAc</th>
<th>Dipy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blk</td>
<td>1.00 mL</td>
<td>0.000 mL</td>
<td>0.000 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>x</td>
<td>1.00 mL</td>
<td>0.000 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>1x</td>
<td>1.00 mL</td>
<td>0.100 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>2x</td>
<td>1.00 mL</td>
<td>0.200 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>3x</td>
<td>1.00 mL</td>
<td>0.300 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>4x</td>
<td>1.00 mL</td>
<td>0.400 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
<tr>
<td>5x</td>
<td>1.00 mL</td>
<td>0.500 mL</td>
<td>0.300 mL</td>
<td>1.00 mL</td>
<td>2.00 mL</td>
<td>2.00 mL</td>
</tr>
</tbody>
</table>

The instructor will demonstrate the use of the spectrophotometer. Adjust the spectrophotometer wavelength selector to 522.0 nm. Rinse and fill two cuvets with blank (solution"0") using a 5 mL pipet gun set to about 2.5 mL. Carefully wipe the cuvets and place them in the "reference" and "sample" receptacles in the spectrophotometer. Adjust the electrical and optical zeros of the instrument. 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 2.5 mL of solution x; 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, 1, 2, 3, 4, 5, unk, and 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 tubes. Clean up.
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 sample for each standard, 0, 1, 2, 3, 4, and 5. You must calculate the total mg of Fe in each 10 µL standard.
   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.)
   c. Perform a regression analysis on the data. Are the regression statistics typical of Beer's Law analysis? In order to answer this you must comment on several things: The standard error of regression should be less than 0.01 (see note at the end).
   d. Determine the concentration of the unknown from the calibration curve in units of "micrograms Fe per sample".
   e. Determine the error in the concentration of the unknown derived from the calibration plot.

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 per tube" using tubes x, 2x, 4x, 6x, 8x, and 10x. You must calculate the total mg of Fe added from the master Fe standard in each 10 mL standard.
   b. Perform a regression analysis on the data. Are the regression statistics typical of Beer's Law analysis? In order to answer this you must comment on several things: The standard error of regression should be less than 0.01.
   c. Determine the concentration of the unknown from the calibration curve units of "micrograms Fe per tube" (the negative of the x-intercept).
   e. Determine the error in the concentration of the unknown derived from the standard additions calibration plot (the error in the x-intercept).

3. Comparison of the results
   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 (n1-2) and (n2-2) for degrees of freedom where n1 is the number of standards in the Beer’s Law plot and n2 is the number of points in the Standard Addition curve.

b. Does the t-test suggest the presence of a likely matrix effect?

c. Use the standard addition results in the subsequent calculations.

4. Iron in the supplement and Propagation of error

   a. Calculate the mass of iron in the tablet.
   b. Propagate errors to determine the uncertainty in the mass of iron in the tablet.
   c. The bottle claims that each tablet provides 18 mg of Fe. Do your results agree?

NOTE: What is acceptable? We may estimate what the scatter should be as follows.

The absorbance depends on the concentration of iron in the mixture. This in turn depends on the quantity of iron and on the volume.

   a. The quantity of iron depends on the pipetting of the 0.2 mL, 0.4 mL, etc. portions of the iron standard. This was done with a 1 mL pipet gun supposedly reproducible to ±0.006 mL. We need to express this uncertainty in terms of absorbance. To do this we note that the most concentrated mixture, made with 1.00 mL of the iron standard, has an absorbance near 1. In that case, an uncertainty of 0.006 mL in the quantity of iron corresponds to a scatter of about 0.006 absorbance units.

   b. The 15 mL volume of the mixtures depends on adding together 5 portions of liquid from the 5 mL pipet gun, supposedly reproducible to ±0.015 mL. The uncertainty of the 15.00 mL volume is then about 0.015√5 = 0.03 mL. This is quite small compared to the 15.00 mL volume and we will ignore it.

As a consequence, we expect a random scatter of about 0.006 absorbance units. A value of the standard error of regression, s_y, should be less than 0.01.

   c. Calculate the x intercept and it standard error using the equations give in the introduction section of your lab manual. Calculate the Fe^{3+} concentration and error in the original unknown, according to the standard addition data.
(EXTRA CREDIT - 10 points) Ammonia can be determined spectrophotometrically by its reaction with phenol and hypochlorite (ClO⁻). 

A 30.14 mg sample of protein was digested to convert all of the nitrogen present to ammonia. After treatment the sample mixture was diluted to 100 mL. A 10.0 mL portion of this mixture was placed in a 50 mL flask and treated with excess phenol and hypochlorite and diluted to the mark. The absorbance at 625 nm of this solution in a 1.00 cm cuvet was 0.605.

A 10 mL portion of standard NH₄Cl containing 0.200 mg NH₄Cl per mL was added to a 50 mL flask, treated with excess phenol and hypochlorite and diluted to the mark as above. The absorbance at 625 nm was 0.502 in a 1.00 cm cuvet.

A blank was prepared by adding phenol and hypochlorite reagents to a 50 mL flask and diluting to the mark. The absorbance was 0.114 in a 1.00 cm cuvet at 625 nm.

Calculate the percentage by weight of nitrogen in the protein sample.