**Spectrophotometric Determination of Iron in an Unknown**

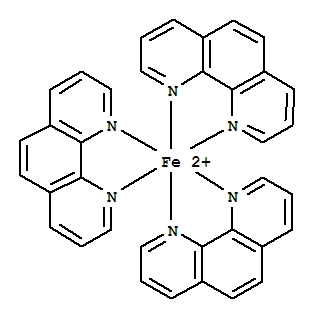
**Background**

*Spectrophotometry* is simply any technique that uses light to measure chemical concentrations. The utilization of spectrophotometry as a quantitative technique relies heavily on the Beer-Lambert (Beer’s) Law: , where “A” is absorbance, “ε” (epsilon) is molar absorptivity (also called the extinction coefficient) with units of per Molarity per centimeter (M-1 cm-1), “b” is the path length in centimeters (cm), and c is the sample concentration in molarity (M). Absorbance is dimensionless. What should be realized in Beer’s Law is that absorbance is proportional to the concentration of light-absorbing molecules within the solution being analyzed. As the concentration of light absorbing molecules increases, absorbance increases. This is an extremely useful relationship. There are limitations where Beer’s Law deviates from this proportionality, but the conditions in this experiment do follow Beer’s Law. Therefore, when a standard [calibration] curve is generated it should be linear. Using standard reference points (standard solutions), the concentration of an unknown solution can be determined simply from its absorbance value & the linear regression equation of the standard [calibration] curve.

A *spectrophotometer* is an instrument that measures the transmission of light through a substance. Say a light source of radiant power, P0, is passed through a substance and some of the light is absorbed. The radiant power after the light passes through the sample (P) is different than radiant power (P0) if energy is absorbed by the molecule. The difference in the light’s radiant power is measured/detected by the spectrophotometer.

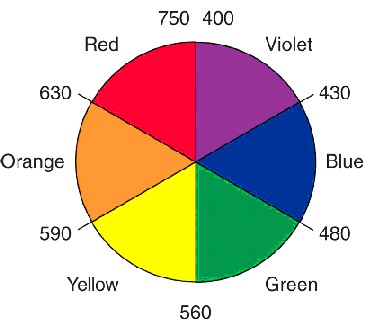
Measuring transmittance of light may be used instead of absorbance, depending on the type of experiment being completed. Absorbance is defined as  where T is transmittance and  (the fraction of the original light that passes through the sample).

Molecules absorb different wavelengths (energies) of light as a result of present structural features. This means that when performing a spectrophotometric analysis, it is important to select a wavelength of light that will result in the most intense signal. This particular wavelength is commonly referred to as lambda max (λmax). Selecting λmax ensures that the molecule is absorbing the greatest amount of light possible, resulting in a more sensitive [and presumably accurate] analysis.

The light absorbing molecule in this experiment is a complex ion composed of iron (II) and orthophenanothroline. The uniqueness of this complex ion is that it is orange/red in color. Therefore, it absorbs light in the visible range and can be detected using the spectrophotometer.

The wavelength chosen for this experiment is based on the notion of complementary colors. In other words, the color we see is opposite the color (wavelength energy) being absorbed by the molecule or ion. The color of the *prepared* samples is a varying shade of orange/red so it is likely that λmax is in the region of blue wavelengths.

For the iron(II)-orthophenanthroline complex, λmax is found to be around 510 nanometers (nm).



* **Violet:**   400 - 420 nm
* **Indigo:**   420 - 440 nm
* **Blue:**   440 - 490 nm
* **Green:**   490 - 570 nm
* **Yellow:**   570 - 585 nm
* **Orange:**   585 - 620 nm
* **Red:**   620 - 780 nm

Wavelength range by color Color wheel

**Prepare Iron Standard Solutions**

Iron (Fe) is most common in the +2 or +3 oxidation state. The aqueous stock solution of iron used in this experiment is Fe3+. In order to produce the colored iron(II)-orthophenanthroline complex for the iron standard solutions and the unknown iron solution, the iron species must be Fe2+. A reducing agent, hydroxylamine hydrochloride, is utilized to reduce Fe3+ to Fe2+. Following this, a sodium acetate buffer solution is added which maintains stability of the overall reaction mixture’s pH. At too high of a pH, the iron will oxidize back to Fe3+; at too low of a pH, the excess H+ ions compete with iron in the complex formation. The orthophenanthroline solution is then added to complex with all Fe2+, forming the orange/red colored complex. The order in which the chemicals are added is extremely important!

Obtain about ~40-45 mL of the stock standard iron solution. Record the concentration! Rinse, then fill a buret with the stock iron solution. Use a buret to add 0 (blank), 2.50, 5.00, 6.00, 7.50, and 10.00 mL of standard iron solution to six respective [clean] 25.00 mL volumetric flasks. Record the EXACT buret readings to two places past the decimal (i.e. if the buret volumes are NOT exactly those listed above, record the actual *buret reading*.) This is important for calibration curve accuracy! Do not force volumes!

Then add the following reagents (using a 10.0 mL graduated cylinder) **IN THE ORDER SHOWN**:

2.6 mL hydroxylamine hydrochloride (reducing agent – MUST add first)

2**.**6 mL sodium acetate solution (buffer – slightly basic)

2**.**6 mL orthophenanthroline (complexing agent)

Dilute each flask to volume with Nanopure H2O. Parafilm, invert to mix, and let stand for 5 minutes.

Obtain an aqueous unknown iron sample in a 25.00 mL volumetric flask from the TA. Add hydroxylamine hydrochloride, sodium acetate, and orthophenanthroline solutions as done previously for the iron standard solutions. Then, dilute this flask to volume with Nanopure H2O. Parafilm, invert to mix, and let stand for 5 minutes. ALL of the volumetric flasks’ solutions, including the unknown, should be a varying hue of orange/red (except the blank; it should be *clear/colorless*).

At this point of the experiment, the concentration of each of the iron standard solutions MUST be calculated! Notably, these solutions are of *known* concentration [the stock iron solution concentration, volume of the stock solution used, and final volume of standards are all known values].

**Analyze the Standard Solutions and Unknown**

Follow the procedure below to use the Genesys 20 Spectrophotometer. Determine the absorbance of each standard iron solution and then the unknown iron solution.

1. Turn the power switch on. This switch is located on the back (lower left) of the instrument. It is a black toggle button. Allow 5-10 minutes for instrument warm-up.
2. Push the up or down arrow button labeled “nm” (nanometers) to select the proper wavelength for maximum absorbance. The wavelength maximum (λmax) for this experiment is **510 nm**.

\*\*Remember, the color (wavelength) absorbed is complementary to the color we see. Because we see an orange/red solution, the energy absorbed by the solution is in the blue/green region in the visible range of the electromagnetic spectrum, 440-570 nm. That is why we set the spectrophotometer to 510 nm.\*\*

1. Rinse a plastic cuvette with the blank solution, discard rinse in a waste beaker. Then, fill the cuvette ¾ full with the blank solution and cap it. Wipe cuvette dry with a Kimwipe making sure there are no fingerprints or marks on the clear sides. Check for bubbles; there should be none in the cuvette’s solution. Place the cuvette into the cell compartment\* of the Spec 20. Close the cell compartment cover.

\*Position cuvette so the light beam passes through the flat (not ribbed) sides of the cuvette!

1. Press the “0 ABS / 100 % T” button. The readout should say 0.000 A. Remove the cuvette and discard the blank solution. You will personally not have to Blank the instrument again during analysis (BUT each individual student has to blank the instrument).
2. Using the same cuvette, fill with the LEAST concentrated standard solution (solution lightest in orange/red color). Then, discard this “rinse” solution into a waste beaker. Refill cuvette ¾ full with a fresh aliquot of the same standard solution. Cap the cuvette (dry with Kimwipe, check for bubbles) and place it into the cell compartment, closing the cover. Record the absorbance.
3. Continue this procedure (step 5) with the remaining standard solutions, always analyzing from the least to most concentrated standard. Always, first fill the cuvette with standard and then dispose of the “rinse” solution into a waste beaker before re-filling with the same standard that will be analyzed. Record all absorbance values in a lab notebook as they are not stored by the instrument. Each standard solution should have a *different* absorbance value. None of the standards’ absorbance values should be > 1.00. If this does occur, or if two standards have too similar absorbance, you must remake the standard solutions in question and re-analyze them.
4. Lastly, fill the cuvette with the unknown iron solution to thoroughly rinse it! Discard the rinse into a waste beaker. Re-fill the cuvette ¾full with a fresh aliquot of unknown solution. Cap the cuvette (dry with Kimwipe, check for bubbles) and place it into the cell compartment, closing the cover. Record the absorbance.

Note: The unknown absorbance *should* fall within the range of the standard solutions and should not be greater than or equal to 1.00.

**Tips for Obtaining Good Calibration Standard Curves and Unknown Absorbance:**

* Set the instrument to the proper wavelength for the experiment.
* Use a clean cuvette & handle it only by the ribbed sides.
* Wipe the clear sides of the cuvette with a Kimwipe to remove oils or droplets of solution.
* Always cap the cuvette and close the cell compartment to prevent stray light from entering the solution, which will skew the results.
* Use the SAME cuvette for all absorbance readings.
* Always place the cuvette in the holder with the arrow on the cuvette facing toward you.
* Always rinse out the cuvette with the solution being tested before measuring its absorbance.
* Always test a blank solution first before standards or unknowns.
* Always test the solutions from LEAST to MOST concentrated.
* NEVER dry a cuvette with a paper towel, as towel fibers can stick onto the sides. Think about how paper towel fibers may affect results.

**Added Notes:**

* If desired, periodically check the “zero” absorbance of the instrument with the blank solution.
* Plot the Iron (Fe) concentration (x axis) vs. corresponding absorbance (y axis) for all of the standard solutions, **using Excel or other graphing software.** Use a least squares fit or linear regression to “determine the line of best fit” (y = m*x* + b) through the points. Display the equation and R2value on the graph. Label the graph with appropriate title and axes labels.
* After obtaining the linear regression equation with the standard solutions, substitute the unknown absorbance (y value) into the linear equation to mathematically determine the concentration of the Fe unknown in Molarity (x value). Fun fact: the slope of the line (m) is the molar absorptivity (ε) of the Iron (II) phenanthroline complex.

**The figure below is an example of a calibration curve [also known as a standard curve], generated via the analysis of standard iron solutions of *known* concentration (molarity):**