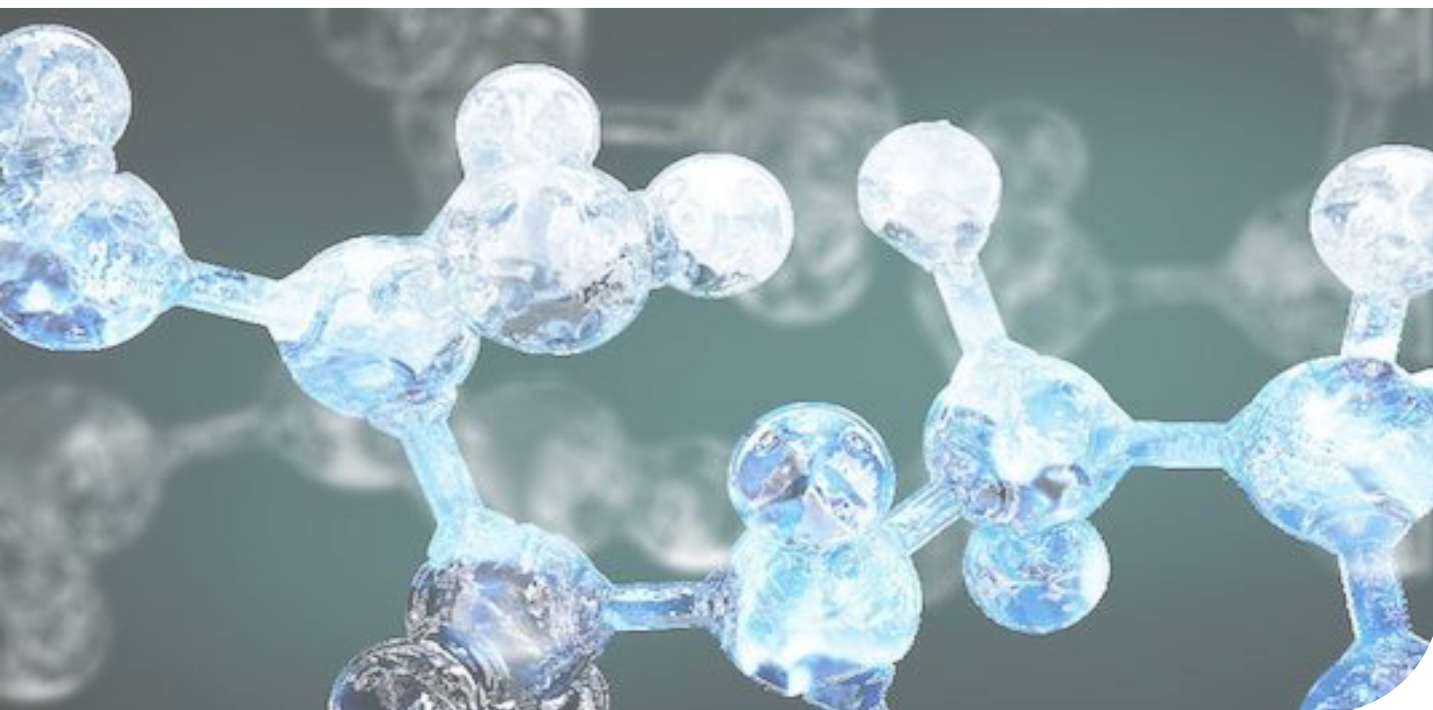


Redox Titration of Ferricyanide to Ferrocyanide with Ascorbic Acid

6



Spectroelectrochemistry: Redox Titration Using Vitamin C

While spectroscopy is often used to directly quantify compounds in solution it can also help you measure and calculate other chemical properties. In this experiment, you will use visible spectroscopy to help measure the standard reduction potential for ferricyanide/ferrocyanide. You will gain experience with oxidation and reduction (redox) reactions and use both the Nernst equation and Beer's law to calculate the standard reduction potential.

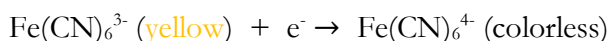
INTRODUCTION¹

Learning Goals

While spectroscopy is often used to directly quantify compounds in solution it can also help you measure/calculate other chemical properties. In this experiment, you will use visible spectroscopy to measure the standard reduction potential for ferricyanide/ferrocyanide. You will gain experience with oxidation and reduction (redox) reactions and use both the Nernst equation and Beer's law to calculate the standard reduction potential.

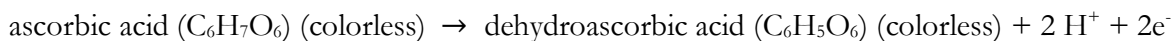
Redox Reactions

Ferricyanide, $\text{Fe}(\text{CN})_6^{3-}$, is an iron containing complex that can easily undergo redox reactions. Ferricyanide, a yellow Fe^{3+} complex, can be reduced (gain an electron) to ferrocyanide, a colorless Fe^{2+} complex:



Since this redox reaction involves a color change (from yellow to colorless) it can easily be monitored using visible spectroscopy. You will use a spectrometer to measure the amount of light absorbed by the ferricyanide/ferrocyanide solution. Changes in absorbance will let you determine the progress of the redox reaction.

Redox reactions involve both oxidation and reduction. An electron is needed to reduce ferricyanide to ferrocyanide. This electron is provided by oxidizing ascorbic acid (vitamin C) to dehydroascorbic acid:



Both the ascorbic acid and the dehydroascorbic acid are colorless. That means that ferricyanide is the only colored ion in solution making it easy to spectroscopically measure the amount of ferricyanide in the solution.

¹ **This experiment was adapted from:** Huang, T., Salter, G., Kahn, S. L., & Gindt, Y. M. (2007). Redox Titration of Ferricyanide to Ferrocyanide with Ascorbic Acid: Illustrating the Nernst Equation and Beer-Lambert Law, 1–3. <http://doi.org/10.1021/ed084p1461>

Nernst Equation

The overall Nernst equation for this redox reaction is:

$$E_{\text{overall}} = E^0 - \frac{RT}{nF} \ln Q \quad \text{where } Q = \frac{[\text{Fe}(\text{CN})_6^{4-}]^2 [\text{H}^+]^2 [\text{C}_6\text{H}_5\text{O}_6]}{[\text{Fe}(\text{CN})_6^{3-}]^2 [\text{C}_6\text{H}_7\text{O}_6]}$$

E^0 is the standard reduction potential for the overall reaction, R is the gas constant (8.31451 J/K•mol), T is the temperature in Kelvin, n is the number of electrons transferred (2 electrons for this redox reaction), and F is Faraday constant (96485 C/mol).

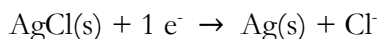
This equation can be simplified by only looking at the reduction of the ferricyanide ion. The reduction half reaction is:

$$E_{\text{Ferricyanide/Ferrocyanide}} = E^0 - \frac{RT}{nF} \ln \frac{[\text{Fe}(\text{CN})_6^{4-}]}{[\text{Fe}(\text{CN})_6^{3-}]}$$

Thus, this simplified equation shows that if you know the potential of the solution and the ratio of ferricyanide to ferrocyanide you can calculate the standard reduction potential, E^0 .

Measuring the potential of the solution

The potential of the solution can be measured using a voltmeter and a standard reference electrode (Ag/AgCl) and platinum electrode. Standard potentials are measured against a given reaction under very specific conditions; generally, a standard hydrogen electrode is the measure of zero potential. For this redox reaction you will use the silver-silver chloride (Ag/AgCl) standard:



The potential for the solution is measured relative to the reference electrode. The difference between the standard reference electrode and platinum electrode can be measured by a simple voltmeter. This reading gives you the potential for the solution.

Measuring the ratio of ferricyanide to ferrocyanide

The ratio of ferricyanide to ferrocyanide can be measured using visible spectroscopy. Conveniently, the only compound in that solution that absorbs visible light is the ferricyanide. Absorbance is an incredibly useful measurement because it is directly proportional to the concentration, c , of the light absorbing species in a sample. This relationship is known as Beer's law:

$$A = \epsilon l c \quad (\text{Beer's Law})$$

Absorbance is dimensionless but is sometimes given ‘dummy’ units of absorbance units or AU. The concentration, c , of the sample is usually given in moles per liter (M). The pathlength, l , is the distance the beam of light travels through the sample is commonly given in centimeters. The pathlength is usually 1.000 cm. Epsilon, ϵ , is the molar absorptivity (or extinction coefficient) and has units of $M^{-1}cm^{-1}$. The molar absorptivity depends on the wavelength of the light and is determined by measuring the absorbance of a series of standard solutions of known concentration. Once ϵ and l are known, you can determine the concentration of an unknown sample from its absorbance.

Ferricyanide has a molar absorptivity of $1.02 \times 10^3 M^{-1}cm^{-1}$ at 420 nm. In this experiment, you will place your samples in cuvettes that have a pathlength of 1.000 cm. Using Beer’s Law, you can calculate the concentration of ferricyanide by monitoring the absorbance at 420 nm. You can then get the concentration of ferrocyanide at any point by using the following relationship:

$$[\text{Ferricyanide}]_{\text{initial}} = [\text{Ferricyanide}]_x + [\text{Ferrocyanide}]_x$$

You will add ascorbic acid to the ferricyanide/ferrocyanide solution to change the ratio of the two ions.

EXPERIMENT PREPARATION

Before you come to lab

In preparation for this experiment, you should complete the prelab and read through the procedure. Make sure you understand how to use the spectrometer, voltmeter, and micropipette. Prepare your lab notebook with any tables and examples calculations that would be useful for you. Please ask your instructor if you have questions.

Collaboration

You will work with a partner to complete this laboratory experiment. You and your partner should work together during lab and make sure that both of you have all the necessary data to complete the lab report. Both of you should understand each step of the procedure (both the *how* and *why*) and how to perform each calculation.

Safety

In preparation for this experiment, you should look up hazard information on the chemicals and equipment used in this laboratory. This might include looking at the Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) for the chemicals used in this experiment. Additionally, the Pharos Project's Chemical and Materials database (<https://www.pharosproject.net>) provides a wealth of hazard and safety information on thousands of chemicals. You should identify the hazards associated with each chemical used in this experiment, the necessary personal protective equipment (e.g. lab coats, goggles) you should use, and any toxicology and ecological information. After completing this search, you should understand both how to handle chemicals safely in lab and how these chemicals effect the environment outside of your laboratory. Remember, the chemicals you use and the waste you generate don't stay in lab – they have to be disposed of after you complete your experiment.

PRELAB QUESTIONS

1. In your own words, define oxidation and reduction.
2. The ferricyanide anion is a yellow colored complex. What do you expect the wavelength of maximum absorbance (λ_{max}) to be? Why?
3. For the overall Nernst equation for this redox reaction $n = 2$ electrons. What does n equal for the reduction half reaction of ferricyanide to ferrocyanide?
4. Familiarize yourself with the spectrometer system you will use for this experiment. Using Figure 1 as a guide, explain how the spectrometer, light source, and computer (with spectroscopic software) will allow you to measure absorbance.
5. What is the purpose of using blank? What is the purpose of zeroing the spectrometer (or taking a dark reference spectrum)?
6. Describe the hazards associated with the chemicals for this lab. What precautions should you take when working with these chemicals? What would you tell someone who wanted to pour these chemicals down the drain (remember, many sinks eventually connect into your local rivers, lakes, and coastal waters)?

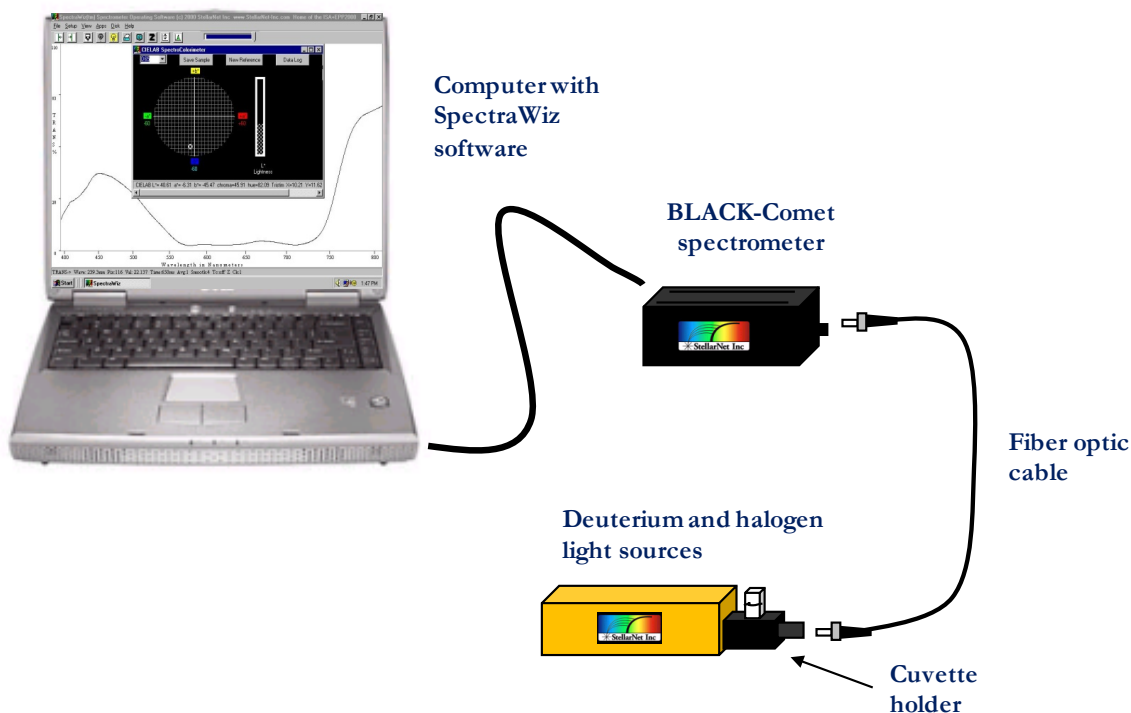


Figure 1: StellarNet UV-visible spectroscopy system

PROCEDURE

Material and Equipment

Chemicals

- ✓ 1 mL of 0.060 M ascorbic acid
- ✓ 30 mL of 0.500 mM $K_3Fe(CN)_6$ in 0.1 M phosphate buffer pH 7.0

Equipment

- ✓ Micropipette
- ✓ Saturated Ag/AgCl electrodes
- ✓ Platinum electrodes
- ✓ Voltmeters
- ✓ Magnetic stirrer
- ✓ Cuvettes with caps
- ✓ Lint free wipes
- ✓ Spectrometer (Black Comet)
- ✓ Light source (deuterium and halogen) and cuvette holder
- ✓ Computer or tablet
- ✓ Fiber optic cable
- ✓ USB cable

Solution Setup

1. Add approximately 30 mL of 0.500 mM $K_3Fe(CN)_6$ to a 50 mL beaker with a stir bar. Place the beaker with solution on a stir plate and stir gently.
2. Place the reference (Ag/AgCl) electrode and a platinum electrode into the of 0.500 mM $K_3Fe(CN)_6$ solution in your beaker.

Make sure that the stir bar is not hitting either electrode and that the electrodes are not touching.

3. In your lab notebook create a table similar to the one below to record (and calculate) data:

Table 1: Data collection.

Sample	Absorbance of ferricyanide (at λ_{max})	[Ferricyanide]	[Ferrocyanide]	[Ferro]/[Ferri]	$\ln([Ferro]/[Ferri])$	Potential
#1						
#2						
#x						

- Transfer several milliliters of ferricyanide solution to a cuvette from your beaker. Fill each cuvette approximately $\frac{3}{4}$ full and cap. Do not shake or agitate the cuvettes as that can introduce air bubbles which will alter your absorbance readings.

Make sure to check each cuvette is free of scratches or discoloration on the transparent sides. Gently clean the transparent sides using a lint-free wipe to remove any residual particulates. Make sure to not touch the transparent sides with your (ungloved) fingers. Your fingers can transfer small amounts of oil onto the cuvettes which will obstruct light passing through the cuvette and alter your absorbance readings.

- Fill a cuvette approximately $\frac{3}{4}$ full with water and cap the cuvette. This sample will serve as your **blank solution**.

Blank solutions are solutions that contain all the reagents and solvents used in analysis but not the analyte. They measure the response of the instrument to impurities or interfering species in the reagents.

Spectrometer Setup

- Transfer your ferricyanide sample and blank cuvettes to the spectrometer. Make sure that your cuvettes are capped so you don't accidentally spill solution on or near the spectrometer.
- Review the spectrometer system setup. Make sure that the spectrometer system resembles Figure 2. Make sure that you can identify all the components of the spectrometer system.
- Remove the cuvette holder cap. Place your **blank** cuvette into the cuvette holder and replace the cuvette holder cap.
- Turn on the computer. Open the StellarNet software by double clicking on the desktop icon *SpectraWiz* to open the application.

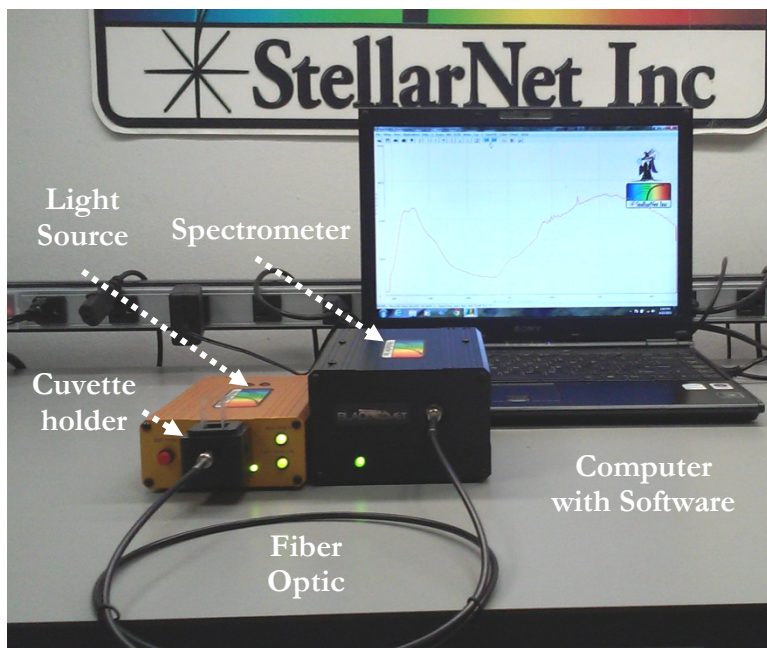
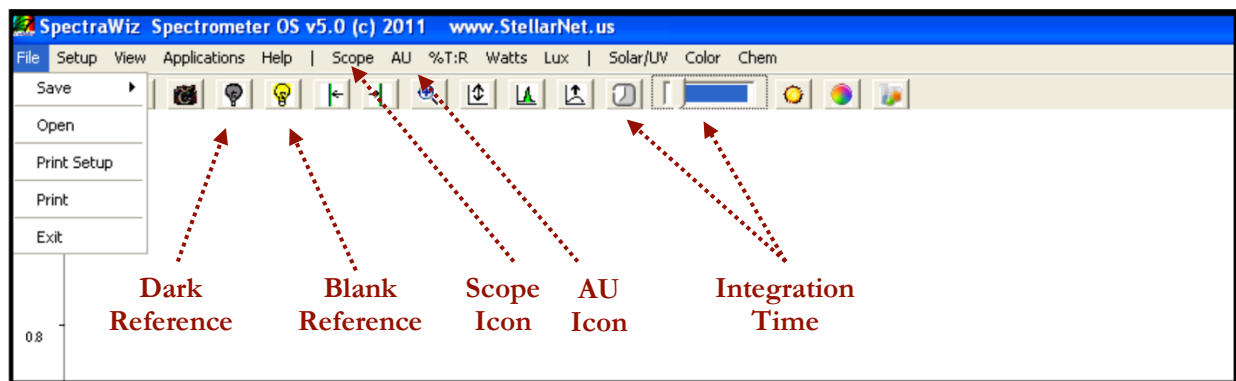
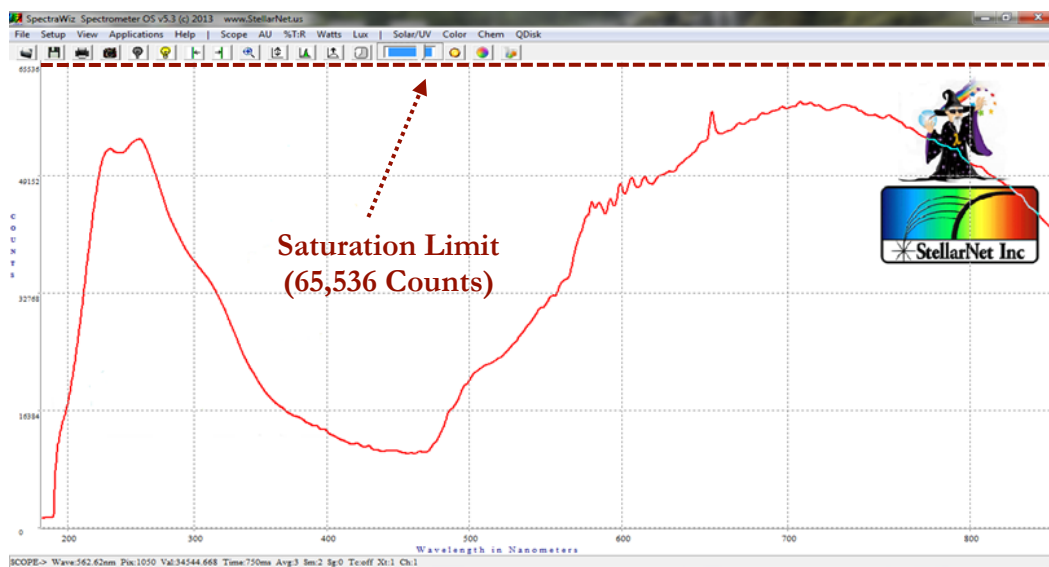




Figure 2: StellarNet spectrometer system (Black Comet spectrometer and SL5-CUV deuterium and halogen light source)

5. When you first open the software, you should see a screen that looks like:



6. Click on the **Scope** icon (highlighted above). This will show you the number of counts (photons) the detector receives. You should see a spectrum of counts versus wavelength.




7. You now need to make sure that there are not too many photons making it to the detector. Click on the **integration time** icon  (and type in the integration time you want – a value between 1 and 1000 ms) or use **sliding scale**  (both highlighted above). Set the integration time such that your spectrum is not saturated but as close to 65,000 counts as possible.


If too many photons reach the detector it will become saturated and you will not be able to get accurate absorbance readings for your samples. *Why is this?*

This means you will want to be able to see a complete spectrum of your blank on the computer screen. In the picture above, the spectrum is correctly optimized to be right below the saturation limit (65,536 counts). If a portion of the spectrum is off the screen you need to reduce the integration time.

Blanking and Zeroing the Spectrometer

1. Make sure that your **blank cuvette** is in the cuvette holder and the cap is on the cuvette holder. **Blank** the spectrometer by clicking on the **yellow light bulb** icon  in the toolbar at the top of the screen.

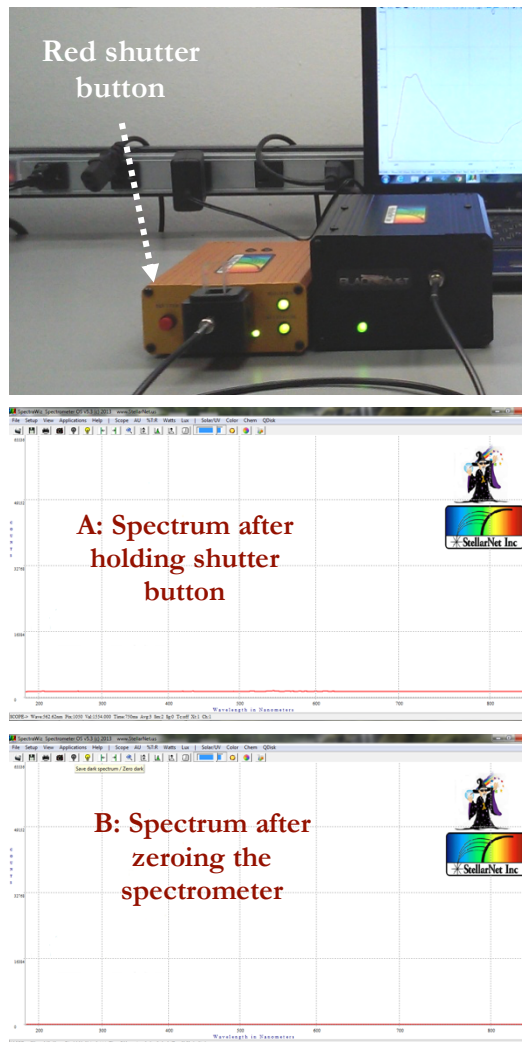
2. Zero the spectrometer. First, hold the red shutter button for at least three seconds. You should see the spectrum fall flat everywhere on your graph (A).

Second, while still holding the shutter button, click on the **dark light bulb** icon  in the toolbar at the top of the screen. The baseline will drop to zero (B).

Finally, you can release the shutter button. You should see the spectrum return to its original profile.

3. Now that you have blanked and zeroed your spectrometer, click on the **AU (absorbance)** icon in the toolbar at the top of the screen to display a spectrum in absorbance units.
4. You should see a nearly flat absorbance spectrum once you switch to absorbance if you have blanked and zeroed the spectrometer correctly.

Depending on the cuvettes you use, you may see a lot of fluctuations below 300 nm. This is simply due to the cuvette absorbing UV radiation at these wavelengths. *Why is this not an issue for your analysis?*

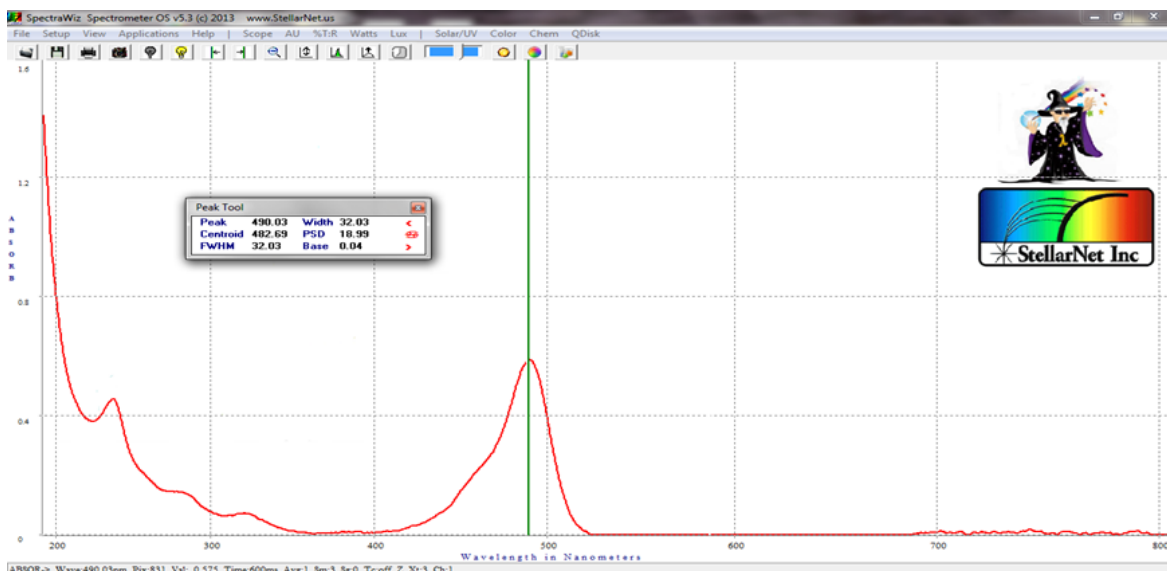


Taking Absorbance Measurements

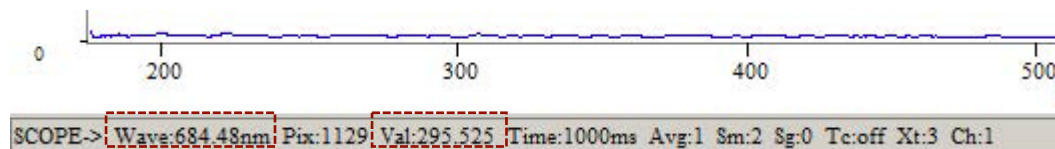
1. Remove your blank sample from the cuvette holder. Place the cuvette containing your sample in the holder and replace the cuvette cap. The instrument will now display absorbance in real time.
2. To find a peak, use the mouse to **right click** on either of the following icons:



The left arrow will find a peak to the left of the vertical line in the spectra. The right arrow will find a peak to the right of the vertical line in the spectra.



3. You can also **right click** directly onto the spectrum to find the absorbance of a specific point. To find the specific wavelength your cursor is at, look to the bottom of the spectrum. **Wave** gives the specific wavelength and **Val** gives the absorbance for that particular point in the spectrum.



4. Look at the spectrum and select the wavelength of maximum absorbance. Sketch this spectrum in your lab notebook and label the wavelength of maximum absorbance. Make sure to label your axes. Use this wavelength for subsequent absorbance measurements. Record the absorbance for your ferricyanide solution at λ_{max} .

Re-blank and zero between separate trials or if you have let the instrument sit for more than five minutes between sample. *Why is this necessary?*

5. Calculate the initial concentration of ferricyanide on the report and record in the table in your lab notebook.

Redox Titration

1. After you have measured the initial absorbance of ferricyanide return the ferricyanide solution in the cuvette back to the beaker containing your original ferricyanide solution.
2. Obtain about 1 mL of 0.060 M ascorbic acid.
3. Using a micropipette, add 5 μL of the 0.060 M ascorbic acid to the ferricyanide solution. Make sure the stir bar is not touching the electrodes and the electrodes are not touching each other.
4. Wait until voltmeter reading stabilizes (approximately 2 minutes) and then record the potential of the solution in the table in your lab notebook.
5. Transfer several milliliters of this solution to a cuvette from your beaker. Record the absorbance of this solution in the table in your lab notebook. Return the solution in the cuvette back to the beaker.

Remember, re-blank and zero between separate trials or if you have let the instrument sit for more than five minutes between sample.

6. Continue adding 5 μL of the 0.060 M ascorbic acid (and recording the potential and absorbance of the resulting solution) until $\ln [\text{Ferro}]/[\text{Ferri}]$ is greater than 1.

DATA ANALYSIS

1. Use Beer's Law to calculate the molarity of ferricyanide before adding ascorbic acid (initial [ferricyanide]).
2. After adding each 5 μL of the 0.060 M ascorbic acid calculate:
 - a. the concentrations of ferricyanide,
 - b. the concentration of ferrocyanide,
 - c. the $[\text{Ferro}]/[\text{Ferri}]$ ratio, and
 - d. the $\ln [\text{Ferro}]/[\text{Ferri}]$ ratio.

DISCUSSION QUESTIONS

1. From your experimental data, what is the relationship between the yellow color of the ferricyanide/ferrocyanide solution and the potential?
2. From the Nernst equation, what is the relationship between the potential observed and the [Ferricyanide]?
3. Plot $\ln([\text{Ferri}]/[\text{Ferro}])$ versus E (your measured potential) for values between 1 and -1. Add a linear trend line.
4. Use your trendline to calculate the number of electrons transferred and the reduction potential of ferricyanide relative to the reference electrode (Ag/AgCl) potential.
5. The standard reduction potential of the Ag/AgCl electrode measured against the standard hydrogen electrode (SHE) as zero potential is 0.197 V. Calculate the standard reduction potential for the reduction of ferricyanide to ferrocyanide measured relative to the SHE as zero potential.