Stability of Vitamin C

A laboratory exercise for CH121 General, Organic, and Biological Chemistry laboratories at Washburn University

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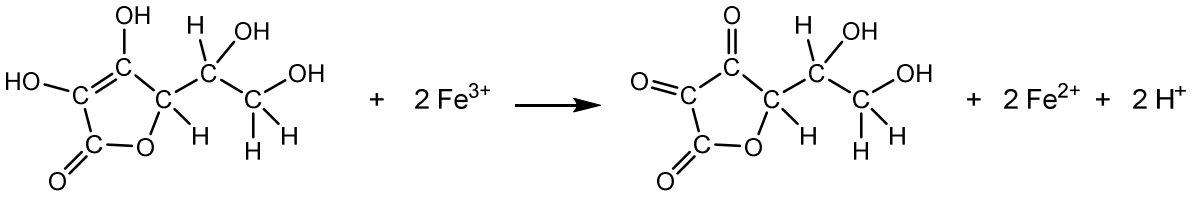
Updated: August 8, 2016

Introduction

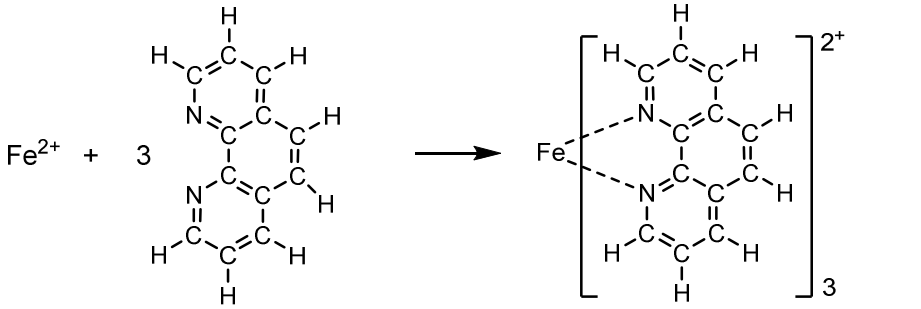
Ascorbic acid (vitamin C) is a cofactor for the enzyme involved in the synthesis of the amino acids hydroxyproline and hydroxylysine. These two amino acids are important components of collagen, which is the protein found in many connective tissues in the body including tendons, bones, and skin. A deficiency in vitamin C will result in a weakened collagen structure.

Many fruits and vegetables as well as vitamin supplements contain vitamin C. However, vitamin C is not stable when it is exposed to heat or air. In this experiment, you will investigate the effect of heat and air on vitamin C. You will determine the amount of vitamin C remaining in a solution after (1) it has been heated, (2) it has been stored open to the air for several days, and (3) it has been stored closed for several days. You will also analyze a sample of solid vitamin C that has been exposed to air for several days (4). Finally, you will have the opportunity to check a commercial vitamin C tablet to determine if it is labeled correctly.

There are many methods available to determine the amount of vitamin C in a solution. In this experiment, you will be using a spectroscopic method that involves the detection of a reddish orange-colored complex (Fe-phenanthroline) formed from the reactions shown below:



ascorbic acid (vitamin C) dehydroascorbic acid



1,10-phenanthroline

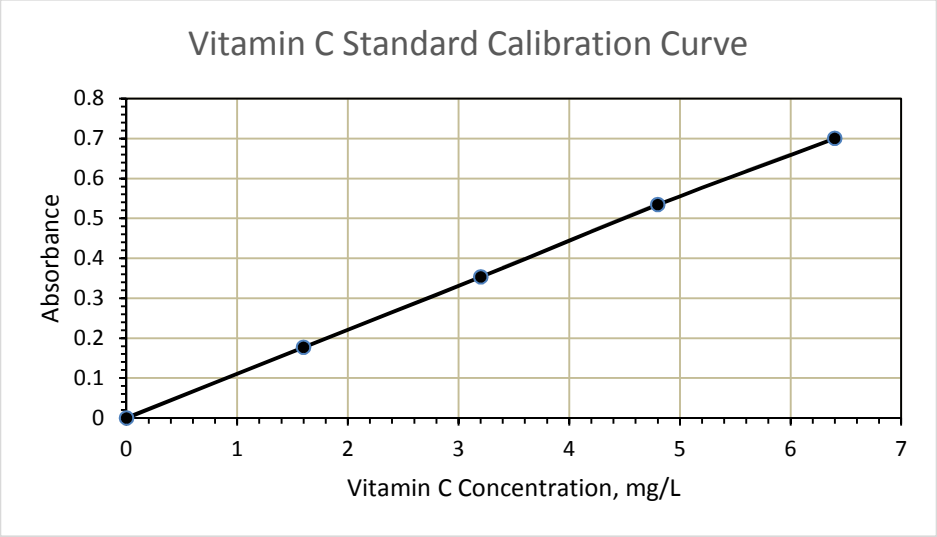
Fe-phenanthroline complex (ferroin)
(reddish-orange colored)

When a solution containing vitamin C is mixed with a **Color Reagent Solution** containing Fe^{3+} ions and 1,10-phenanthroline, the Fe^{3+} ions are reduced to Fe^{2+} ions by vitamin C, and the resulting Fe^{2+} ions then react with 1,10-phenanthroline to form a reddish orange-colored Fe-phenanthroline complex that absorbs light at a wavelength of 510 nm.

An ultraviolet-visible (UV-Vis) spectrometer is used to measure the **absorbance** of light at 510 nm by the reddish orange-colored Fe-phenanthroline complex. Because the Fe-phenanthroline complex only forms in the

presence of vitamin C, the absorbance of light at 510 nm will be directly proportional to the amount (concentration) of vitamin C in the solution being analyzed in accordance with **Beer's Law**, which states that the absorbance of a chemical solution is directly proportional to the product of its concentration and the distance light travels through it.

In order to use this spectroscopic method to determine the amount of vitamin C in an unknown solution, you must first create a **Standard Calibration Curve** from a series of solutions with known vitamin C concentrations. Then you will measure the absorbance values of each known vitamin C solution (after they have been treated with Fe^{3+} and 1,10-phenanthroline Color Reagent Solution). These measured absorbance values will be plotted versus the corresponding vitamin C concentrations to generate a standard curve (see example below). You will then use this curve to determine the concentration of vitamin C in different unknown solutions by measuring the absorbance of each solution (after they have been treated with Fe^{3+} and 1,10-phenanthroline Color Reagent) and matching it up on the standard calibration curve. (See "how to read standard calibration curves" below.)



Procedure

Waste Disposal: All wastes from this experiment should be flushed down the sink with plenty of water.

UV-Vis Spectrophotometers: Use and calibrate the instrument in accordance with the manufacturer's instructions. Make sure the instrument is warmed up about 15 minutes prior to use, set to the proper wavelength, set to the proper filter setting, the cuvettes are wiped clean with a tissue to remove any foreign matter, the cuvettes are filled about ¾ full with liquid, and the doors are closed on the instrument.

Part I. Preparation of an experimental Standard Calibration Curve

A) Preparation of known vitamin C concentration solutions

- Label five clean 35- or 50-mL beakers using a wax pencil as: **Blank, 1, 2, 3, 4**.

Using a disposable plastic pipet add the ***Standard Vitamin C Solution*** (40 mg/L) as follows:

- Add 1.0 mL of the Standard to the beaker labeled **1**.
- Add 2.0 mL of the Standard to the beaker labeled **2**.
- Add 3.0 mL of the Standard to the beaker labeled **3**.
- Add 4.0 mL of the Standard to the beaker labeled **4**.

Using a disposable plastic pipet add 4.0 mL of the ***Color Reagent Solution*** to all five of the beakers, including the "Blank".

Using a 25 mL graduated cylinder add ***DI water*** to make the volume of the solution in each beaker equal to 25 mL as follows:

- Add 21.0 mL of DI water to the beaker labeled "**Blank**".
- Add 20.0 mL of DI water to the beaker labeled **1**.

The final concentration of vitamin C becomes**:** $\frac{1\ mL\ x\ 40\ mg/L}{25\ mL} = \mathbf{1.6\ mg/L}$

- Add 19.0 mL of DI water to the beaker labeled **2**.

The final concentration of vitamin C becomes: $\frac{2\ mL\ x\ 40\ mg/L}{25\ mL} = \mathbf{3.2\ mg/L}$

- Add 18.0 mL of DI water to the beaker labeled **3**.

The final concentration of vitamin C becomes: $\frac{3\ mL\ x\ 40\ mg/L}{25\ mL} = \mathbf{4.8\ mg/L}$

- Add 17.0 mL of DI water to the beaker labeled **4**.

The final concentration of vitamin C becomes: $\frac{4\ mL\ x\ 40\ mg/L}{25\ mL} = \mathbf{6.4\ mg/L}$

Carefully mix the contents of each beaker.

B) Absorbance Measurement of Calibration Standards

The Spec 20 is the spectrophotometer to be used in this experiment. Follow the instructions on the instrument or use the following steps to set up the spectrophotometer.

- Turn on and warm up the instrument for at least 15 minutes.

- Using the wavelength knob, set the wavelength to 510 nm.
- Select the correct filter position.
- With the Mode button Set to Transmittance, set %T to zero.
- Using the Mode button, set mode to Absorbance. (This is the mode to be used in this experiment.)
- Transfer the blank solution from beaker labeled blank into a cuvette to nearly 3/4 full. Don't label or mark the cuvette.
- Hold the cuvette at the top and wipe it with a tissue to clean off fingerprints and water drops.
- Insert the cuvette into the sample holder of the Spec 20. Make sure the line mark on the cuvette matches with the line mark on the sample holder of the instrument.
- Close the lid and set absorbance to zero with the right knob on the instrument. Don't change the instrument settings from now on.
- The Blank absorbance reading of 0.000 is already recorded on the Report Sheet.
- **Save the "Blank" solution for Part II. Sample Analysis below.**
- Similarly, transfer the contents of the other beakers to a cuvette (3/4 full) one by one. Record the absorbance of each solution in the table of the Report Sheet. Record your absorbance readings to three decimal places, or as indicated on the Spec 20 display.
- Plot the calibration absorbance vs. concentration data to the blank vitamin C Standard Calibration Curve chart provided for you on the report sheet. (Your experimental data should follow a relative straight line passing through zero similar to the example chart given above.)
- Draw the best-fit straight line for the data on the chart (NOT A CONNECT-THE-DOTS PLOT). Use a ruler or straight edge to draw your line.

Part II: Unknown Vitamin C Concentration Analysis

A) Effect of Temperature on the Stability of Vitamin C in Solution

- Label two clean 35- or 50-mL beakers using a wax pencil as: **1 min and 15 min**.
- To two clean 150-mm test tubes, transfer about 10 mL (use graduated cylinder) of the Standard Vitamin C Solution (40 mg/L). ***(The same Standard Solution you used to prepare your Standard Calibration Curve.)*** Clamp them in the 50 °C water bath provided.
- Record the actual temperature of the water bath on the bottom box of the Report Sheet. (It should be about 50 °C.)
- Remove one of the test tubes after **1 min** and immediately place it in an ice bath to cool it off (4-5 minutes).
- Take 4.0 mL (use a disposable plastic pipet) and transfer it to the beaker labeled **1 min**. Add 4.0 mL of the ***Color Reagent Solution*** and then 17.0 mL of ***DI water*** (use a 25-mL graduated cylinder).
- Measure and record the absorbance of the solution as in **Part IB**.
- Remove the other test tube from the 50 °C water after **15 min** and place it in an ice bath to cool it off (4-5 minutes).
- Take 4.0 mL (use a disposable plastic pipet) and transfer it to the beaker labeled **15 min**. Add 4.0 mL of the ***Color Reagent Solution*** and then 17.0 mL of ***DI water*** (use a 25-mL graduated cylinder).
- Measure and record the absorbance of the solution as in **Part IB**.

B) Effect of Storage on the Stability of Vitamin C

In this part, you will be provided with three samples of vitamin C solutions to test their stability. These three vitamin C solutions were prepared with the same concentration of Vitamin C as the Standard Solution used in **Part I** (40 mg/L), but were treated differently than the Standard Solution, as noted below. Our objective here is to determine if vitamin C concentration decreases over time under different storage conditions; **Open solution –** a standard vitamin C solution (40 mg/L) left exposed to air for one week, **Closed solution –** a standard vitamin C solution (40 mg/L) left closed to air for one week, **Open solid** – a standard vitamin C solution (40 mg/L) prepared fresh from a solid that had been exposed to air for one week.

- Label 3 more clean 35- or 50-mL beakers using a wax pencil as: **Open solution**, **Closed solution**, and **Open solid**.

Be sure to re-zero the spectrophotometer with the "Blank" from the calibration set above (**Part I)** if time has elapsed since you used the spectrophotometer to read the Standard Solutions.

- Take 4.0 mL (use a disposable plastic pipet) of each of the stability solutions and transfer to the appropriately labeled beakers – *be careful*. Add 4.0 mL of the ***Color Reagent Solution*** and then 17.0 mL of ***DI water*** (use a 25-mL graduated cylinder).
- Measure and record the absorbance of the solutions as in **Part IB**.

Part III. Vitamin C Tablet or Nutritional Supplement Analysis

Obtain from your instructor a vitamin C tablet (or nutritional supplement) for analysis. The objective here is to determine the accuracy of the manufacturer's label with regard to the experimentally measured vitamin C content. Using the report sheet on *page 8* of this procedure as a guide, you will weigh one tablet, (or one unit of the product) then calculate how much of the product to weigh out to make a 0.040 gram/Liter vitamin C solution. This is a conversion factor calculation. For example, if the nutrition label shows that a tablet contains 500 mg of vitamin C per tablet and we wish to weigh out 40 mg of vitamin C, then dividing 40 mg by 500 mg yields the conversion factor of 0.080. One then needs to weigh the 500 mg vitamin C tablet and multiply the mass times the conversion factor 0.080 to obtain the mass of the tablet we need to weigh in order to obtain 40 mg.

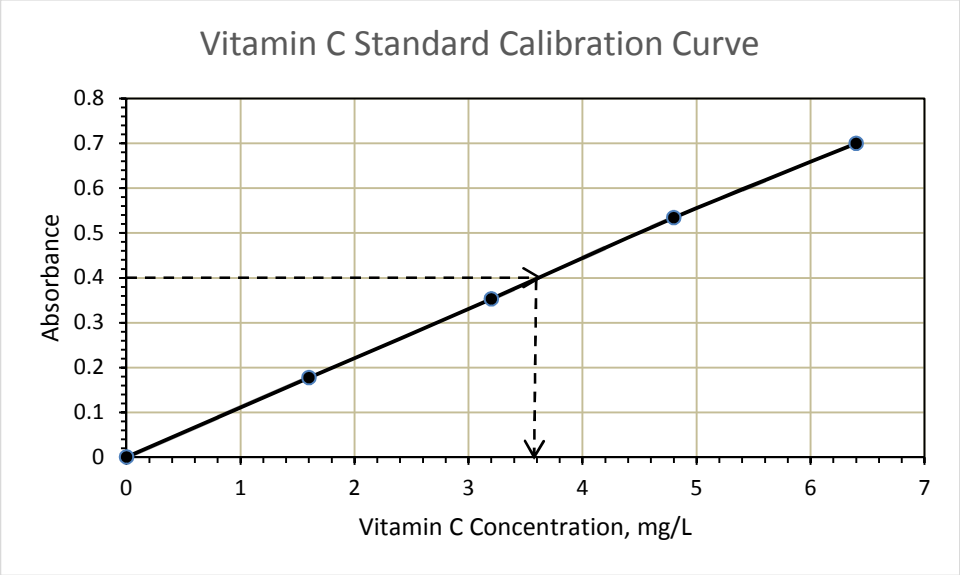
$\frac{\text{40 mg vitamin C}}{\text{500 mg vitamin C}} = 0.080$ For a tablet weighing 0.697 g, we need to weigh 0.697 x 0.080 = 0.0558 g

After weighing the whole tablet, grind the tablet in a mortar and pestle and then weigh the calculated grams of ground tablet, transfer it to a one liter volumetric flask, then dilute to volume with deionized water. Mix well. The mass you weigh should be slightly more than 0.040 grams because most vitamin tablets contain other ingredients such as binders.

Transfer 4.0 mL of this solution to a 30 or 50 mL beaker using a disposable plastic pipet. Add 4.0 mL of Color Reagent and 17.0 mL of deionized water to the beaker and mix. Read the absorbance. Determine the vitamin C concentration in the one-liter flask, using the absorbance reading and your vitamin C Standard Calibration Curve. From the vitamin C concentration in the flask, calculate the vitamin C content of the original tablet or formulation. This is accomplished by multiplying by the respective dilution factors.

$$Concentration\ of\ vitamin\ C\ from\ Std\ Cruve \times 6.25 \times mass\ of\ tablet, g \div mass\ \text{"x" above, g} = \text{vitamin C content of tablet or formulation}$$

How to read Standard Calibration Curves:
Below is an example of how to read a Standard Calibration Curve. *(You will use your own Standard Calibration Curve to read the results of this experiment, NOT this one.)*



- Assume you have completed Part I of this experiment where you have obtained your data for the Standard Calibration Curve, plotted the points, and drawn the best-fit straight line to fit the data. Assume the curve above represents our results.
- You will now use this Curve to determine the vitamin C Concentration of your unknown solutions involved in the experiment.
- For example, if your unknown sample had an Absorbance reading of 0.400, find 0.400 on the Absorbance scale (vertical y-axis). Draw a horizontal dashed line from 0.400 to the right until it intersects the solid Calibration Curve. Then draw a vertical line down to the "Vitamin C Concentration, mg/mL" axis (horizontal x-axis). The point this vertical line intersects the Concentration axis (x-axis) is the concentration of your solution. (In our example, that is ~3.60 mg/mL.)
- If using a computer to plot your data, another method would be to use the equation for your line of best-fit and solve for x using your measured absorbance as the y value.

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Pre-lab Questions (Answer before you come to lab and turn in on a separate piece of paper)
1. What happens when Fe^{3+} ions are mixed with vitamin C in a solution?

2. When an unknown solution was mixed with a solution of Fe^{3+} ions and 1,10-phenanthroline, no orange color was observed. What does it mean? How do you know?

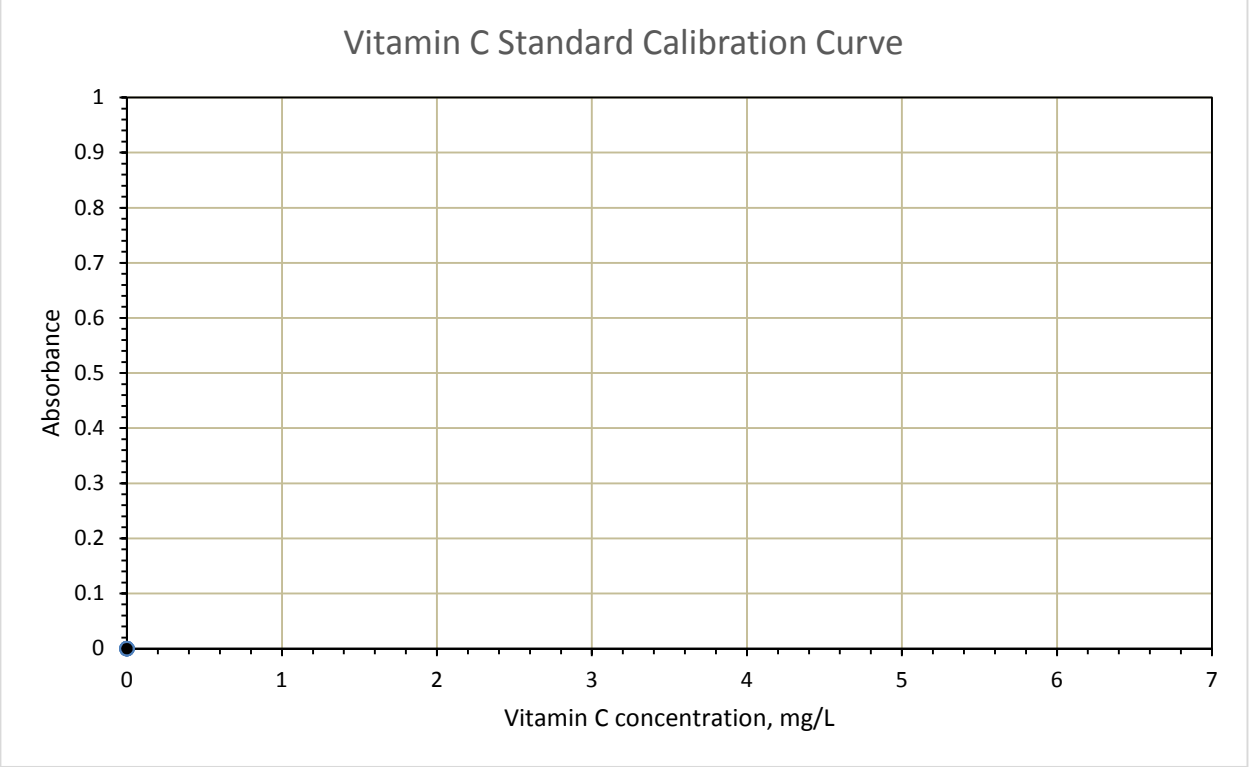
3. What is the purpose of a standard calibration curve?

Stability of Vitamin C **Name:** _____

Report Sheet

Sample	**Concentration of Vitamin C, mg/L**	**Absorbance @510 nm**
Part IB: **Blank**	0.00	0.000
Part IB: **1**	1.6	
Part IB: **2**	3.2	
Part IB: **3**	4.8	
Part IB: **4**	6.4	
Part IIA: heated **1 min**	Unknown	
Part IIA: heated **15 min**	Unknown	
Part IIB: **Open Solution** (Solution left open for 1 week)	Unknown	
Part IIB: **Closed Solution** (Solution kept closed for 1 week)	Unknown	
Part IIB: **Open Solid** (Solution prepared fresh from solid left open for 1 week)	Unknown	
Actual Temperature of Water Bath	_____ °C	

Use the concentration of the standard solutions (Blank, 1, 2, 3, and 4) and the corresponding absorbance to generate a Standard Calibration Curve. Use the blank chart below. Use the Standard Calibration Curve to determine the concentration of the other five solutions and record them on the next page of this report. Alternatively, plot these on excel.



Calculations (Parts I & II)
Obtain your Concentration results for the first column from the Standard Calibration Curve on the previous page. Because you used 4.0 mL of solution and diluted it to a final volume of 25 mL, it is now necessary to correct your concentration readings for that dilution factor which is 25/4.0 = 6.25. Finally, calculate the percent decrease in vitamin C concentration in the respective samples using the formula in the header.

Experiment or Source of the sample	**Concentration of vitamin C in the diluted sample from the calibration curve, mg/L**	**Concentration of vitamin C in the original sample (previous column x 6.25 dilution factor), mg/L**	**Percent decrease in vitamin C concentration in the original sample** $= \frac{(40\,\frac{mg}{L} - previous\ column)}{40\ mg/L}$ x 100%
≈ 1 min. in 50°C bath			
≈ 15 min. in 50°C bath			
Open Solution			
Closed Solution			
Open Solid			

Part III. Vitamin C Tablet or Formulation Analysis

Type of Tablet or Formulation	
Mass of One Tablet or One Unit, grams	
Mass of Ground Tablet or Formulation to be Diluted (calculated mass "x"), grams	
Absorbance of Solution	
Concentration of Vitamin C from Std. Calib. Curve, mg/L	
Experimental Vitamin C in Whole Tablet or Formulation, mg	
Product Label Vitamin C for Tablet or Formulation, mg	

Final Lab Questions

1. Based on your experimental data, what can you conclude about the stability of vitamin C when exposed to heat and long-term storage?

2. Which form of vitamin C is less stable: solution form or solid form? Can you suggest a reason for your choice?

3. Can we trust food nutrition labels on fruit juices to contain the amount of vitamin C listed? Why or why not?

4. What can we do to stabilize the vitamin C in fruits and vegetables for longer shelf-life?

5. Comment on the accuracy of the nutrition information on your vitamin C tablet or formulation.

6. Is the supplement or formulation tested a good source of vitamin C for your diet? Why or why not?