Vitamin C Content of Foods

Experiment #11

Objective: To measure the heat and alkaline stability of vitamin C and its quantity in juices or tablets.

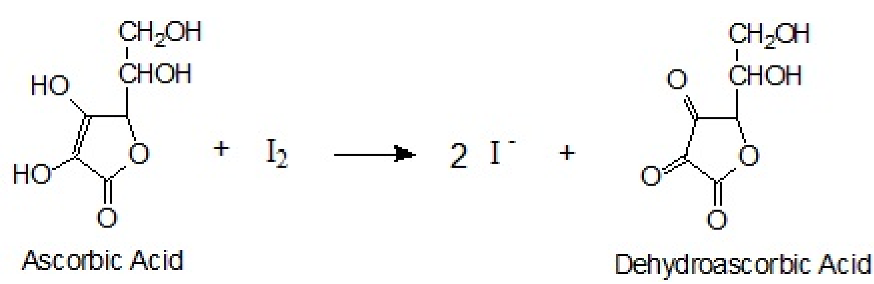
Introduction

Vitamin C is an essential component of the diet for humans and some animals because we lack the enzymes to make this substance from its natural precursor, glucose. A deficiency of vitamin C leads to a disease known as scurvy, characterized by hemorrhages throughout the body, which are especially noticeable on the gums and around the mouth and other areas of the skin with abundant vascularization (blood vessels). It is an essential cofactor in the synthesis of the protein collagen, which connects cells in the body, especially in the blood vessels.

The disease scurvy was described as early as 1500 BC. In the winter of 1535, a group of 110 French explorers with Jacques Cartier were stranded along the St. Lawrence River and became afflicted with scurvy. Several of the men died and many were totally incapacitated by the disease before the Native Americans came to their rescue and recommended a concoction from a local evergreen tree that cured the survivors. The European name for the tree is arborvitae, or tree of life. Lind investigated the causes of scurvy among British sailors in 1747 and found those given citrus fruits regularly did not develop scurvy, while sailors receiving various other dietary supplements did. He concluded there was an antiscurvy or "ascorbutic" factor in the citrus fruits that prevented the disease. The chemical structure for the ascorbutic factor was not determined until the early 1930's and was named ascorbic acid.

The recommended dietary allowance (RDA) for vitamin C is 60 mg for adults, and higher for pregnant and lactating women. This is an amount that is sufficient to prevent scurvy, although there is a lot of controversy over how much vitamin C is needed for a healthy individual. Some people claim that large doses of vitamin C will help to ward off colds, although there is not complete agreement on this aspect of its actions, primarily because individuals usually respond differently to various drugs and nutrients.

In this laboratory you will measure the amount of vitamin C in various beverages by titration with iodine solution. Some beverages will be supplied, but each student is encouraged to bring in a sample of his or her choosing to analyze for its vitamin C content. Iodine (I_2) reacts with ascorbic acid to produce iodide ion and dehydroascorbic acid.



Ascorbic acid is readily oxidized by oxygen in the air under neutral or alkaline conditions, so you will be adding acetic acid to keep the medium acidic. Starch will be added to the sample, so when the ascorbic acid has completely reacted with iodine, any excess iodine added will form a deep blue color with the starch that is present. This color formation is commonly used as an indicator for starch, but in this experiment you will use it as an indicator for excess iodine remaining at the end point of titration. Oxygen can replace iodine in this reaction, although ascorbic acid only reacts with oxygen in the presence of a transition metal catalyst (such as Fe^{3+} or Cu^{2+}) or under alkaline conditions, where it is ionized.

The following equation is used to calculate the amount of ascorbic acid in unknown samples. The denominator of the equation is the amount of iodine solution needed to titrate the standard ascorbic acid solution (Part B of procedure, 1 mg/mL concentration). The numerator represents the amount of iodine solution needed to titrate the unknown or other sample (all other parts of the procedure, except part B).

Note: You will use this equation for all calculations for the amount of vitamin C in samples.

Equation 1:

$$\text{Amount Vit C (mg/mL)} = \frac{\text{Vol } I_2 \text{ soln used for 10 mL of sample}}{\text{Vol } I_2 \text{ soln used for 10 mL of 1.0 mg/mL standard}}$$

Procedure

[See Appendix II for instructions on using the buret. Be sure to read values from top of buret down, i.e., when the liquid is at the top mark on the buret, the reading is zero mL. The value for the liquid level on the third small line below 10 and above 11 is 10.3 mL.]

A. Test Solutions for Vitamin C Stability.

Note: Be sure to label these solution to avoid getting them mixed up.

1. Add 10 mL deionized water to two beakers and add exactly 10 mL of 1 mg/mL standard ascorbic acid solution to each flask and stir. Heat the solutions to boiling and remove from the heat to cool. Do not allow the solution to boil more than a minute.

2. Add 5 mL deionized water and 5 mL of 10% sodium carbonate solution (Na_2CO_3) to two Erlenmeyer flasks and add exactly 10 mL of 1 mg/mL standard ascorbic acid solution to each of these two flasks and stir. The sodium carbonate makes the solution alkaline and will cause the ascorbic acid to ionize. Heat to boiling as you did for the first two samples and remove from heat to cool. Do not allow to boil more than a minute.

3. These 4 solutions will be titrated in Part C to determine the amount of ascorbic acid remaining under these conditions.

Part B. Titration of Ascorbic Acid Solutions with Iodine Solution.

1. Add 10 mL deionized water and 2 mL of 6 M acetic acid to each of two Erlenmeyer flasks. Add exactly 10.0 mL of 1 mg/mL ascorbic acid standard solution to each flask and about 1 mL of 1% starch solution to each.

2. Obtain about 100 mL of iodine solution in a beaker to use for all parts of this experiment. Set up a 50 mL buret using the buret clamp, make sure the stopcock is close at the bottom

and fill it with iodine solution. Turn the stopcock to fill the tip of the buret before you start the titration, making sure you have no air bubbles in the tip. [See appendix II].

3. Record on the Report Sheet the initial level of the iodine solution in the buret. Remember zero is at the top and 50 mL is at the bottom, so the scale is increasing down the buret.

4. Slowly add the iodine solution to the ascorbic acid solution in one of the Erlenmeyer flasks containing acetic acid and starch (prepared in step B-1). You will see a deep blue color appear that disappears as you swirl the solution. If you don't see a blue color appear you forgot to add the starch solution. Continue adding the iodine solution a little at a time and swirl the flask after each addition until the blue color persists. As you get closer to the endpoint, it will take longer for the blue color to disappear. That means you should try to add smaller and smaller amounts of the iodine solution between swirling. When you have added just enough, so the blue color remains for more than 30 seconds, record on the Report Sheet the final level of iodine solution in the buret.

5. Subtract the initial volume from the final volume to determine the volume of iodine solution added to completely oxidize the ascorbic acid standard solution.

   Make sure there is enough iodine solution in the buret before starting each titration.

6. You can use the final reading for your first titration (step B-4) as the initial reading for the second titration. Repeat the procedure with the other flask containing the ascorbic acid standard with acetic acid and starch, recording on the Report Sheet the initial volume and final volume of iodine in the buret and determine the amount used for each titration.

7. If the difference in volumes used for these two titrations is greater than 1.0 mL repeat this entire part, being more careful in adding small amounts of iodine solution near the end.

8. The average volume used for these titrations, from first page of report sheet Part B will be used in the denominator of Eq. 1 for all subsequent calculations.

9. You can discard these solutions in the sink.

Part C. Titration of Ascorbic Acid Stability Test Solutions.

1. Transfer one of the test solutions in a beaker from Part A to a clean Erlenmeyer flask, add 2 mL of 6 M acetic acid and 1 mL of 1% starch solution. Mix well by swirling.

2. Make sure there is sufficient iodine solution in the buret and record on the Report Sheet the initial volume. If you used about 10 mL of iodine solution in part B, make sure the buret reads above 40 mL. You can always fill it to be safe. Titrate as you did in Part B, adding just enough iodine solution from the buret to the test solution so the blue color remains.

3. Repeat steps 1 and 2 for each test solution from part A, making sure to add 2 mL acetic

acid and 1 mL of 1% starch solution before titrating.

Make sure there is sufficient iodine solution in the buret each time and the initial volume is recorded before titrating.

4. Calculate the amount of ascorbic acid remaining in each of the test solutions using Equation 1 [see introduction]. The volumes used for titrations in this section will go in the numerator of Eq. 1.

Part D. Determination of Ascorbic Acid in Beverages.

Note: You may wish to compare freshly squeezed orange juice from an orange with commercial orange juice in a container. Squeeze juice from the orange and use 10.0 mL of the fresh juice. You may also wish to measure vitamin C in a supplement tablet from home or one provided for this lab as described below.

1. Add 10 mL deionized water and 2 mL of 6 M acetic acid to each of two clean Erlenmeyer flasks. Add exactly 10.0 mL of juice or other beverage sample to each flask and 1 mL of 1% starch solution.

   Make sure there is sufficient iodine solution in the buret and the initial volume is recorded before titrating.

2. Titrate these samples in the same way that you did in Part B. Record the initial and final volume in the buret for each sample. You can discard solutions in the sink.

3. Calculate the amount of vitamin C in the food sample using Equation 1.

4. If you measure the amount of vitamin C in a tablet, crush the tablet using your mortar and pestle and transfer all the powder to a large Erlenmeyer flask or beaker. Add exactly 200 mL of deionized water to dissolve the vitamin C. Some of the binding agents for the tablet may not dissolve, but the vitamin C should dissolve relatively fast (in a minute with stirring).

5. Add 10 mL deionized water to a clean Erlenmeyer flask with 2 mL of 6 M acetic acid and 1 mL of 1% starch solution. Add exactly 10.0 mL of the vitamin C solution you obtained after dissolving the tablet in 200 mL of water.

   Fill the buret with iodine solution before starting to titrate 10.0 mL of solution prepared from the tablet. You will <u>NOT</u> titrate all 200 mL of that solution.

6. Titrate this solution in the same way that you did all the others, recording the initial and final volume in the buret. You can discard the solution in the sink.

7. Return any unused iodine solution to one of the iodine solution bottles from which it came and rinse the buret with water before returning it.

Vitamin C Content of Foods

Experiment #11 Pre-lab Exercise

1. Vitamin C is considered to be unstable during cooking. What kind of chemical reaction do you expect vitamin C to undergo in solution (hydrolysis, oxidation, reduction)? [Hint: look at the reaction with iodine in the introduction].

2. Show the chemical reaction you would expect for loss of ascorbic acid when heated in air (O_2). Again, look at the reaction with iodine, oxygen should react similarly with regard to this reaction?

3. The formula shown in the introduction (equation 1) can be used to calculate the amount of ascorbic acid (mg) in test solutions. If 10.5 mL of iodine solution is needed to titrate 10.0 mL of 1.0 mg/mL ascorbic acid standard solution and 7.0 mL of iodine solution is needed to titrate 10.0 mL of an ascorbic acid test solution. What is the concentration (mg/mL) of ascorbic acid in the test solution?

4. In Experiment #5, The Chemistry of Carbohydrates, you tested for starch with iodine solution. What color change is observed when iodine solution is added to starch solution?

5. What is the recommended dietary allowance (RDA) for vitamin C for adults? See text book or find this information on the internet.

6. What disease results if a person becomes deficient in vitamin C? What are the symptoms of this disease?

7. Given the symptoms of vitamin C deficiency, what is one biochemical function for vitamin C in the body? You should describe the biochemical or metabolic function in which vitamin C is involved (see textbook).

Name ____ Section ______________

Vitamin C Content of Foods

Experiment #11 Data & Report Sheet

<u>Part B. Titration of Ascorbic Acid Standard Solution (1 mg/mL):</u>

	Flask 1	Flask 2
<u>Final</u> level of iodine soln. in buret: (Read from top of buret down)	________	________
<u>Initial</u> level of iodine soln. in buret: (Zero is at top of the buret)	- ________	- ________
Total vol. of iodine soln. used:	________	________

Average volume used: ________ mL
Use this number in the denominator of Equation 1
for all calculations using Equation 1.

B-1. When you mix 10.0 mL of ascorbic acid standard solution in the flask with 10 mL water, 2 mL of 6M acetic acid solution and 1 mL of starch solution, is it necessary to know the exact final volume of this solution to get an accurate determination of ascorbic acid in the titrations of test solutions? Explain. Hint: Do the volumes of water, acetic acid and starch solutions enter into the calculations to determine the amount of ascorbic acid? Does the volume of ascorbic acid solution added before titration influence how much iodine solution will be needed?

Part C. Determination of Ascorbic Acid in Test Solutions:

Titration Results	deionized H_2O		sodium carbonate solution	
	1	2	1	2
Final vol, mL				
Initial vol, mL				
Total used, mL (numerator in Eq. 1)				
Vit C, mg/mL				
% Original Vit C Remaining in Sample (100 x vit C, mg/mL above)				

C-1. Give a brief explanation for any differences in the amount of ascorbic acid remaining in the heated test solutions, *i.e.*, what may cause differences between deionized water and alkaline sodium carbonate conditions?

C-2. Does heating cause any disappearance of vitamin C in the water solutions? Why would heating affect vitamin C?

Name ____ Section _____________

Part D. Vitamin C in Beverages.

Beverage Samples: A) ________ mL of ___________________________
Type of Beverage

B) ________ mL of ___________________________
Type of Beverage

C) 10 mL of vitamin C tablet dissolved in 200 mL water.

Titration Results

	A	B	C
Final vol, mL			
Initial vol, mL			
Total vol used, mL (numerator in Eq. 1)			
Concentration of Vit C (mg/mL)			
Vit C per serving (mg/mL x mL/serving)*			
% RDA for Vit C (RDA = 60 mg)			

* If you use 8 ounces as a typical serving size, that is equivalent to about 235 mL/serving.

D-1. Check the labels for the juice or beverage containers and record whether vitamin C is listed for that beverage and indicate how much is supposed to be in the beverage if that information is given. If it's not given, do you think it should be? If the label indicates 100% (of RDA), how does your result compare with the manufacturer's claim?

D-2.Would you consider either of the beverage samples a "good" source of vitamin C in the diet? Give your own clarification of what a "good" source would be.

[Answer questions on next page].

D-3. If you measured vitamin C in the solution prepared from a vitamin C tablet, calculate how much vitamin C was in the tablet (show your work) and compare that with the amount claimed to be in the tablet given on the label. [Note: You dissolved the tablet in 200 mL of water, so the serving size for the calculation would be 200 mL]. What may account for any significant difference between what you measured and what is claimed? (Check the expiration date on the vitamin C bottle).

Give a brief summary of your own conclusions about the chemical stability of vitamin C and how this might affect the amount of vitamin C that may be found in foods, such as cooked vegetables.