

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.

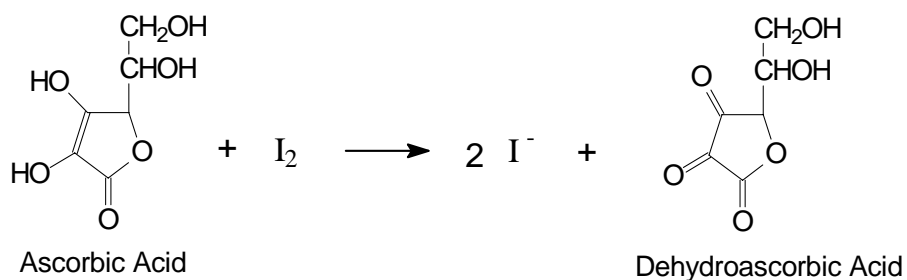
Introduction

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 antiscorbutic or "ascorbic" factor in the citrus fruits that prevented the disease. The chemical structure for the ascorbic 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 (1 mg/mL concentration). The numerator represents the amount of iodine solution needed to titrate the unknown or other sample.

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]

A. Test Solutions for Vitamin C Stability.

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

1. Add 25 ml deionized water to two Erlenmeyer flasks or beakers and add exactly 10 mL of 1 mg/mL standard ascorbic acid solution to each flask. 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 15 ml deionized water and 10 ml of 10% Na_2CO_3 (sodium carbonate solution) to each of two other Erlenmeyer flasks or beakers. Add exactly 10 ml of 1 mg/ml standard ascorbic acid solution to each of these two flasks. Heat to boiling and remove from heat to cool. Do not allow to boil more than a minute.
3. These 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 25 ml deionized water and 2 ml of 6 M acetic acid to each of two 125 ml Erlenmeyer flasks. Add exactly 10.0 ml of 1 mg/ml ascorbic acid standard solution to each flask and about 2 ml of 1% starch solution to each.
2. Set up a 50 ml buret and fill it with the iodine solution. Turn the stopcock of the buret to

fill the tip 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.
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. 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. 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.

Part C. Titration of Ascorbic Acid Stability Test Solutions.

1. Transfer one of the test solutions from Part A to an Erlenmeyer flask, if you used a beaker for this solution, and 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.
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 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].

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 25 ml deionized water and 2 ml of 6 M acetic acid to each of two clean 125 ml 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 the ascorbic acid standard, making sure there is enough iodine solution in the buret for each titration and record the initial and final volume in the buret.
3. Calculate the amount of vitamin C in the food sample by comparing the amount of iodine used to titrate the sample with the amount used to titrate the standard [Equation 1].
4. If you measure the amount of vitamin C in a tablet, crush the tablet using your mortar and pestle and place it in an Erlenmeyer flask or beaker. Add exactly 100 mL of deionized water to dissolve the vitamin C tablet. Some of the binding agents for the tablet may not dissolve, but the vitamin C should dissolve relatively fast (in a minute or two with stirring).
5. Add 25 mL deionized water to an Erlenmeyer flask with 2 mL of 6 M acetic acid and 1 mL of 1% starch solution. Add 5.0 mL of the vitamin C solution after dissolving the tablet.

Make sure there is sufficient iodine solution in the buret and the initial volume is recorded before titrating. This titration may require as much as 50 mL of iodine solution.

6. Titrate this solution in the same way that you did the ascorbic acid standard, recording the initial and final volume in the buret.

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Data & Report Sheet

Part B. Titration of Ascorbic Acid Standard Solution (1 mg/ml):

	Flask 1	Flask 2
Final level of I ₂ soln. in buret: (Read from top of buret down)	_____	_____
Initial level of I ₂ soln. in buret: (Zero is at top of the buret)	- _____	- _____
Total vol. of I ₂ soln. used:	_____	_____
Average volume used:	_____ ml	

B-1. When you mix 10.0 ml of ascorbic acid standard solution in the flask with 25 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 ₂ O		alkaline solution	
	1	2	1	2
Fin vol, ml				
Init vol, ml				
Tot used, ml				
Vit C, mg/ml				
% Original Vit C Remaining in Sample				

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 conditions?

C-2. What effect does heating have on any disappearance of vitamin C?

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Part D. Vitamin C in Beverages.

Beverage Samples: A) _____ mL of _____
Type of Beverage

B) _____ mL of _____
Type of Beverage

C) 5 mL of vitamin C tablet dissolved in 100 mL water.

Titration Results

A

B

C

Fin Vol, ml			
Init vol, ml			
Total vol, ml			
Conc Vit C, mg/ml			
Vit C/serving (mg/ml x ml/serv)			
% RDA for Vit C (RDA = 60 mg)			

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?

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 question on back of this 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. What may account for any significant difference between what you measured and what is claimed?

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.