Analysis of Vitamin C in Fruit Juice

Learning Goals

1. Prepare a volumetric solution of L-Ascorbic acid, Vitamin C. (This solution is the Primary Standard.)
2. Determine the concentration of the provided iodine solution. (This solution is the Secondary Standard.)
3. Titrate a juice sample to find the Molar \( (M) \) concentration of L-Ascorbic acid (Vitamin C).
4. Calculate the milligrams of L-Ascorbic acid (Vitamin C) present in the juice sample.
5. Determine whether the amount of L-Ascorbic acid (Vitamin C) in the juice meets the minimum Recommended Dietary Allowance, RDA.

Abstract

Vitamin C (L-Ascorbic acid) is a water-soluble vitamin important for maintaining a healthy organism. It is found naturally in citrus fruits and leafy green vegetables. Vitamin C may be used in soft drinks as an antioxidant for flavor ingredients, in meat-containing products for curing and pickling, in flour to improve baking quality, and in a wide variety of foods for Vitamin C enrichment. Vitamin C is also found in stain removers, hair dye preparations, plastics, and photography chemicals. It is an antioxidant, appears to boost immune response, and is necessary for the formation and organization of collagen, the development of the walls of blood vessels, and tissue healing. Antioxidants help rid the body of free radicals, reducing the incidence of muscle damage, cataracts, and potentially cancerous cells. Vitamin C deficiency symptoms include bleeding gums and easy bruising or slow wound healing. Severe Vitamin C deficiencies are rare today.
However, before the widespread availability of Vitamin C containing foods, scurvy (a severe deficiency) was quite common. Humans are one of the few animal species that cannot make their own Vitamin C by utilizing beneficial bacteria in the gut. The RDA, or Recommended Dietary Allowance, is the level of intake of essential nutrients considered to be adequate to meet known nutritional requirements for the majority of people. The Recommended Dietary Allowance of Vitamin C is 60mg. However, various scientists have suggested amounts as great as 300mg are needed to maintain a healthy lifestyle. Because Vitamin C is a water-soluble vitamin, the amount of the vitamin absorbed in a single day can vary based on the daily diet. In this experiment, you will determine the amount of Vitamin C (Ascorbic acid) in commercial juices, by the titration of the juice samples with a solution of iodine to a starch endpoint.

**Prelab Assignment**

In your lab notebook, prepare the following information:

1. View the video clips on **Using the Balance** and **Performing a Titration**. You will need Quick Time video player to see them.
2. A brief (2-3 sentence) introduction to the lab.
3. A table of **safety information** including the chemicals used in the lab and any safety handling precautions. This information can be obtained from the MSDS safety sheets.
4. Before lab, calculate the molarity (moles/liter) of a standard solution prepared by dissolving 0.145g of L-ascorbic acid (C₆H₈O₆) in 250mL of distilled water.

Give this information to your TA at the beginning of the lab. You will not be allowed to work in the lab without this information.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine solution</td>
<td>Balance</td>
</tr>
<tr>
<td>Starch indicator</td>
<td>250 Volumetric flask</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>Buret</td>
</tr>
<tr>
<td>L-Ascorbic acid</td>
<td>25 mL volumetric pipet</td>
</tr>
<tr>
<td>Distilled water</td>
<td>Pipet filler</td>
</tr>
</tbody>
</table>

**Procedures:**

A. Obtain a sample:

Obtain about 100mL of the assigned juice sample. From the label, record the serving size and the amount or percentage of vitamin C per serving of the juice. If solid pulp is present filter the juice.
Dissolve 1g of oxalic acid (+0.01g) in the juice sample. The oxalic acid stabilizes the ascorbic acid.

B. Set up the buret:

Remove the buret from the buret stand. Pour out the distilled water.

Never hold a beaker with solution above eye level since spilling could cause you to get solution into your eyes. Instead, remove the buret from the stand, make sure the stopcock is in the closed position and then carefully fill the buret using a beaker.

Using a small beaker, pour about 5 mL of the iodine solution into the buret. Carefully tilt the buret and roll it between your fingers in order to rinse the entire length of the buret tube. Allow some of the iodine solution to drain through the stopcock. Discard this wash solution into a waste container. Repeat this wash twice more.

Fill the buret with the iodine solution and replace it on the buret stand. Place an empty beaker under the buret and open the stopcock to allow all of the air bubbles to escape from the tip of the buret. Adjust the level of the solution so that the bottom of the meniscus is below the zero mark on the buret. Record the initial volume of the filled buret to two decimal places (+ 0.00 mL).

C. Prepare a Standardized L-ascorbic acid solution:

Weigh 0.100 - 0.110 g (+0.001 g) of L-ascorbic acid into a clean dry beaker. Dissolve the L-ascorbic acid with about 100mL of distilled water in the beaker.

Transfer all of the solution to a 250 mL volumetric flask, rinse the beaker twice and add the rinses to the flask. Fill the volumetric flask to the mark with distilled water. Calculate the molarity (moles/liter) of this solution and record the value in your notebook.

This solution of L-ascorbic acid (the Primary Standard) is used to "standardize" or determine the exact concentration of the iodine solution. Once the exact concentration of the iodine solution (the Secondary Standard) is known, it can be used to find the concentration of vitamin C in the fruit juice.

Pipet 25mL of the Standardized L-ascorbic acid solution into a clean 250mL Erlenmeyer flask and add 10 drops (about ½ mL) of the starch indicator and about 100ml of distilled water. (Why do you not need to know exactly how much water or starch is added to the solution to be titrated?)

Record the starting volume of the solution in the buret. Titrate your sample by adding the iodine solution to the ascorbic acid standard with stirring until the solution just turns blue-black and remains that color for 20 seconds while
stirring. To reach the end point very accurately, you can use a technique called **splitting drops**.

**Splitting Drops.** Open the stopcock very slowly until a drop is suspended from the tip of the buret. Touch the side of the flask to the tip and wash the drop down into the solution with a stream of distilled water from your squeeze bottle. When splitting drops gives you a permanent color change (one that does not fade before 30 seconds), you have reached the endpoint. If you are not sure whether or not you have reached the endpoint, add another split drop.

Record the volume needed to reach the endpoint.

Repeat the procedure twice more for a total of three titration values for the Standard Iodine solution.

These titrations are used to determine the exact concentration of the iodine solution. At the end point, when the solution turns color, the molar amounts of ascorbic acid and iodine are equal. The molar amount of ascorbic acid is determined by the redox titration with a standardized solution of iodine. The iodine is reduced by the ascorbic acid to form iodide according to the following equation.

\[
\text{L-Ascorbic acid} + I_3^- \rightarrow \text{Dehydroascorbic acid} + 2H^+ + I^- 
\]

Once all of the ascorbic acid has been oxidized, there is nothing left to react with any additional iodide-iodine \((I_3^-)\). The next small amount of iodide-iodine \((I_3^-)\) solution will react with the starch indicator and the solution will change color. Since the ascorbic acid and the iodine react in a 1:1 ration, use \(M_1V_1 = M_2V_2\), where \(M_1\) = unknown molarity of iodine solution, \(V_1\) = volume of iodine solution from the buret, \(M_2\) = molarity of the ascorbic acid solution and \(V_2\) = volume of ascorbic acid sample to determine the Molar concentration of the iodine solution in the buret.

From your three values for the concentration of the Iodine solution, calculate the **average concentration**, the **deviation from the average concentration**, the **average deviation** and the **percent deviation** for each of the concentration values. **See example.** If any
value is off by more than 5% of the average, it should be discarded and another titration performed. Repeat until you have three values all within 5% of the average concentration.

D. Titration of the assigned commercial Fruit Juice sample:

Use a pipet to transfer 25mL of your juice sample into a clean 250mL Erlenmeyer flask. Add 10 drops of starch indicator and 100 mL of distilled water. Record the exact volume of iodine solution in the buret.

Add the iodine solution to the flask slowly. Use the splitting drops technique used above to find the point of a definite, permanent color change (to blue-black) in the solution.

Record the volume of iodine needed to complete the titration.

Repeat the procedure twice more for your juice sample for a total of three titration values.

Record the initial and final volumes of iodine solution for each titration.

Use \( M_1V_1 = M_2V_2 \), where \( M_1 \) = known molarity of iodine solution, \( V_1 \) = volume of iodine solution from the buret, \( M_2 \) = unknown molarity of the ascorbic acid solution and \( V_2 \) = volume of ascorbic acid determine the Molar concentration of the vitamin C (ascorbic acid) in the juice.

Dispose of your chemicals in the appropriate containers.

Post Lab Assignment

Include the following information in a lab report to give to your TA:

*(Be sure to show clear examples of all calculations)*

1. Data and calculations from all six titrations.
2. The concentration of the standardized Iodine solution.
3. The ascorbic acid concentration \((M)\) in your assigned juice samples.
4. The average the three values for the juice sample.
5. Calculate the number of milligrams of Vitamin C per milliliter of juice for your juice sample.
6. Based on your results, calculate the amount of Vitamin C in a serving. The serving size for each juice is on the container label. How do these values compare to the recommended daily allowance of vitamin C for an individual? Does one serving of your juice sample meet the RDA value?
7. The number of class results and the class average of milligrams of Vitamin C per milliliter of juice for your juice sample.
8. Determine the deviation and the percentage deviation of your results from the class average result.

Answer the following questions in your report

1. After calculating the ascorbic acid concentration (M) of each sample of your assigned juice and finding the average, determine if all of your values fall within 3% of each other and if not how far from the average they are. Present at least one reasonable explanation for an error greater than 3%.
2. If an air bubble is originally trapped in the buret tip but disappears during the titration, how does this affect the calculation of the ascorbic acid in the sample of juice? Explain.