EXPERIMENT 7  
CHEM 209 Lab, Fall 2002

Determination of Vitamin C Content in Fruit Juice and in a Vitamin Pill


Materials Needed:
- vitamin C tablet
- iodine solution
- 100 mL volumetric flask with stopper
- orange, lemon, grapefruit juices
- small beaker
- Beral pipets
- 10 mL reference ascorbic acid
- wellplate
- 1-mL volumetric pipet and pipet bulb
- starch solution

Additional Reading Assignment

McMurry, chap 19.10, 10.15

Introduction

Vitamins are organic compounds that are required in trace amounts for normal human health and functioning. If a certain vitamin is not provided by the diet then the resulting vitamin deficiency normally results in symptoms of an associated illness. For example, a deficiency of vitamin A, the vitamin formed from the enzymatic reaction of β-carotene, can lead to night blindness. A lack of vitamin D₃ causes rickets, a disease characterized by skeletal deformations and vitamin C deficiency can lead to the development of scurvy. Vitamins A and D₃ are classified as "fat-soluble vitamins". Clearly, the large hydrocarbon sections of these molecules make them overall hydrophobic and, thus, water insoluble. Vitamin C, on the other hand, with its four hydroxy groups is very soluble in water and, thus, classified as a "water soluble vitamin".

Interestingly, humans are one of few species that require ascorbic acid in the diet. However, as with all vitamins, only a trace amount of it is required. Ascorbic acid is present in all citrus fruits as well as many other fruits and vegetables. The recommended daily allowance (RDA) of vitamin C is 60 mg for the average adult. This is the amount supplied by a single glass of orange juice. To put it into perspective, a teaspoon of ascorbic acid would be a large enough supply to last 12 weeks. However, because ascorbic acid is water soluble, any taken in excess is excreted rather than stored in our bodies. Therefore, it is crucial that an appropriate amount be ingested daily.
Ascorbic acid acts as a reducing agent in our bodies. That is, it is able to donate electrons to other molecules/atoms in a chemical reaction. It is this action that is beneficial in digestion and metabolism. The reducing action of ascorbic acid causes it to react with iodine (I$_2$) to form iodide ion (I$^-$) (eq 1).

\[
\begin{align*}
\text{I}_2 & \quad \text{ascorbic acid} \quad \rightarrow \quad 2 \text{I}^- \\
\end{align*}
\]  

(eq 1)

Starch reacts with iodine (I$_2$) to form an easily observed starch-iodine complex with a deep blue-black color. Therefore, starch can be used as an indicator for the presence of iodine (I$_2$).

\[
\begin{align*}
starch & \quad + \quad \text{I}_2 \quad \rightarrow \quad \text{starch-iodine complex} \\
& \quad \text{(deep blue-black)}
\end{align*}
\]  

(eq 2)

In this experiment, the amount of ascorbic acid in a food sample will be determined by titrating a weighed amount of the sample with iodine. The iodine will immediately react with the ascorbic acid (eq 1) until all of the ascorbic acid has been exhausted. The next drop of iodine cannot be reduced to iodide (I$^-$) and, thus, reacts with the starch (eq 2) causing the solution to turn blue-black. Thus, the amount of iodine necessary to bring about the color change is an indicator of the amount of ascorbic acid present in the sample.

In a titration procedure a solution of unknown analyte concentration is mixed with a solution with a known concentration of a compound that reacts with the analyte. (The analyte is the compound being analyzed, in this experiment it is ascorbic acid.) Measuring the amount of known solution required to just completely use up the analyte allows the calculation of the concentration of analyte in the unknown solution. (The known solution is called the "titrant". In this experiment, the titrant is the iodine solution.) Usually a buret is used to measure the amount of the known solution required. However, in this experiment you will be using a special small-scale item called a microtip Beral pipet.

**LAB PROCEDURES**

Because the size of a drop may vary, it is important to maintain consistency throughout the titration procedure. You should practice using the Beral pipet before using it for the titration procedure.

Take an empty Beral pipet, fill it with water, and try dispensing drops into a well of the wellplate. Your goal is to be able to confidently add a known number of drops to a well, one drop at a time. Squeeze the bulb gently while the pipet is held vertically and directly over the center of the well. You may find it helpful to use two hands to steady the pipet. Microtip Beral pipets easily acquire air bubbles in their stems that lead to "half-drops" and "quarter-drops" that can lead to errors. It is good practice to reserve a well for solution waste for any titration drops not added to the titration well to be counted.

**Preparation of the Vitamin C Tablet**

1. Weigh a vitamin C tablet and record the weight to the nearest milligram (0.001 g).
2. Place the tablet in a clean 100-mL volumetric flask. Fill the flask about half full with deionized water. Stopper the flask and shake it until the tablet is broken down. Label the flask, set it aside and then go on to the next procedure.

**Titration of the Reference Sample**

3. Using a clean dry beaker, obtain about 10 mL of the reference ascorbic acid solution. Make sure to record the exact concentration given (mg/mL).
4. Use a volumetric pipet to dispense exactly 1.00 mL of ascorbic acid solution in to a clean, dry well in the wellplate.
5. Add a drop of starch solution to the acid solution.
6. Fill a 2-mL microtip Beral pipet with the iodine solution provided.
7. Titrate the sample with the iodine solution; counting drops and stirring as you go. The end point is when one drop gives a pale blue color that remains upon stirring. Record the number of drops required.
8. Repeat steps 4-6 above and titrate at least 3 more ascorbic acid reference samples.
Titration of the Vitamin Tablet Solution
Now that the vitamin C tablet in your volumetric flask should be mostly dissolved (except for the insoluble “filler” material, typically starch), you will titrate the solution to determine the amount of ascorbic acid in it.
9. Add deionized water to fill the flask up to the line in the neck of the flask. The bottom of the meniscus should just touch the line.
10. Stopper the flask and mix it well by repeatedly turning the flask upside down.
11. Using a volumetric pipet, add exactly 1.00 mL of the vitamin solution to a clean well.
12. Add a drop of starch solution.
13. Titrate as in step 7 above using the same iodine solution you used before.
14. Repeat steps 11-13 at least 3 more times.

Titration of a Juice Sample – do two samples
15. Obtain a sample of fruit juice. Make sure to record which type of juice you use.
16. Rinse your pipet with some of the juice.
17. Use the volumetric pipet to add exactly 1.00 mL of juice to a well in the wellplate.
18. Add a drop of starch solution
19. Titrate using the same iodine solution and record your data.
20. Repeat steps 17-19 at least 4 more times.
IN-LAB OBSERVATIONS/DATA/CALCULATIONS

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Name ___________________________________________  Section ____________  Date ___________
Partners_________________________________  Instructor __________________________

Concentration of reference ascorbic acid _________________

Titration of the reference sample
Sample number  Number of drops of iodine solution

1
2
3
4
5
6
average

mass of ascorbic acid per drop of iodine __________mg

Titration of the vitamin tablet solution

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Number of drops of iodine solution</th>
<th>Mass of tablet _______g</th>
<th>Mass of ascorbic acid in one tablet as stated on the bottle ________mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>2</td>
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<tr>
<td>average</td>
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Experimental mass of ascorbic acid in 1.00 mL of solution ________mg

Experimental mass of ascorbic acid in one tablet (remember the tablet was dissolved in 100 mL of solution) ________mg
**Titration of a juice sample**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Number of drops of iodine solution</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>average</td>
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Juice used ________________________________

Experimental mass of ascorbic acid in 1.00 mL of juice __________ mg

Experimental mass of ascorbic acid in one 6-oz serving of juice (1 oz = 30 mL) __________ mg

OBSERVATIONS - record your observations on each part of the experiment here

**Titration of the reference sample**

**Titration of the vitamin tablet solution**

**Titration of a juice sample**
REPORT

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Determination of Vitamin C Content in Fruit Juice and in a Vitamin Pill

Name ___________________________________________  Section ____________  Date ___________

Partners_________________________________  Instructor __________________________

RESULTS TABLE

<table>
<thead>
<tr>
<th>Sample</th>
<th>experimental mass ascorbic acid per serving in mg (1 tablet or 6 oz juice)</th>
<th>literature (or stated) mass ascorbic acid per serving in mg (1 tablet or 6 oz juice)</th>
<th>% error (see calculation below)</th>
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</table>

Percent Error Calculation

\[
\text{error} = \text{experimental value} - \text{literature value}
\]

\[
\% \text{ error} = \left| \frac{\text{error}}{\text{literature value}} \right| \times 100
\]

QUESTIONS

1. Accuracy is defined as the closeness of a measurement to the "true" value. Which of your analyses, the vitamin C tablet or the juice, was more accurate? Discuss thoroughly and suggest reasons why your results might be inaccurate. Why do you think we used the average of several trials in these determinations?
2. Precision is defined as the reproducibility of a measurement. In another words, the closer the individual trials of a measurement are to each other, the more precise the measurement is. Which determination was more precise, the vitamin C tablet or the juice? Explain and use numbers to back up your answer.

3. How much of the juice that you analyzed would you have to drink to meet the U.S. RDA for vitamin C?

4. Explain why vitamin C is considered a water-soluble vitamin.

6. (a) Draw the structure and give the name of another water-soluble vitamin.

   b.) Label the functional groups in the structure.

   c.) Explain why the vitamin you chose is water-soluble.