Lab 4. Analysis of Vitamin C

Prelab Assignment

Before coming to lab:

- This exercise does *not* require a report in your lab notebook. Record your experimental results, calculations, analysis and conclusion in the spaces provided on the <u>Lab 4 Report Sheet</u> (a separate handout). The Lab 4 report sheet will be collected in place of a formal lab report.
- Read the lab thoroughly and answer the pre-lab questions that appear at the end of this lab exercise and hand in at the start of your lab period.

Purpose

In this lab you will learn to use the back titration procedure to analyze an unknown and a juice sample for the concentration of Vitamin C.

Introduction

Vitamin C (or ascorbic acid) is a vitamin commonly found in fruits and vegetables that serves a wide variety of biochemical functions. In addition to preventing scurvy (no longer a problem in the developed world), Vitamin C is known to assist in the absorption of iron and to improve resistance to infection. The well-known chemist Linus Pauling, who won the 1954 Nobel Prize in Chemistry as well as the 1962 Noble Peace Prize, firmly believed in the ability of Vitamin C to aid in the prevention of the common cold.
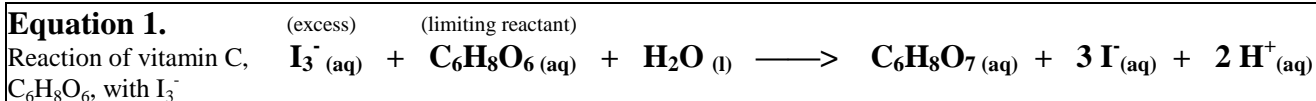
Vitamin C is a compound that is readily *oxidized*, and is therefore a good *reducing agent*. Because it is preferentially oxidized in the body it serves as an *antioxidant*, protecting other substances in the body from oxidation. Recent studies suggest that the antioxidant properties of Vitamin C are important part of its cancer fighting ability by reducing oxidative damage from highly reactive free radicals, normal (but highly reactive and destructive!) byproducts of cellular respiration. Its formula is $C_6H_8O_6$.
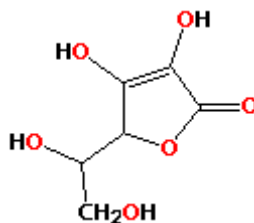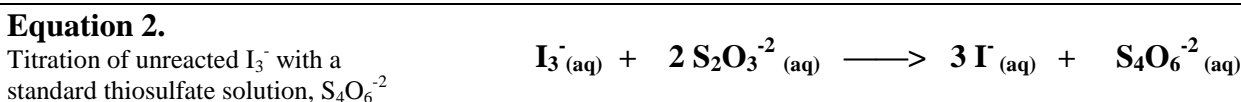

Figure 1. The structure of Vitamin C, $C_6H_8O_6$.

Since Vitamin C is an acid it would seem a simple matter to use an acid–base titration to measure its concentration. But juices, fruits and vegetables contain other acids besides Vitamin C, so an acid–base titration would detect not only the Vitamin C, but also the citric and other acids that are present. To circumvent this obstacle we will use a method that relies on Vitamin C's properties as a *reducing agent.*

To analyze for Vitamin C content a sample is titrated with a solution of triodide ion, I_3^-. The triodide ion oxidizes the Vitamin C to dehydroascorbic acid, $C_6H_8O_7$, as shown in equation 1:

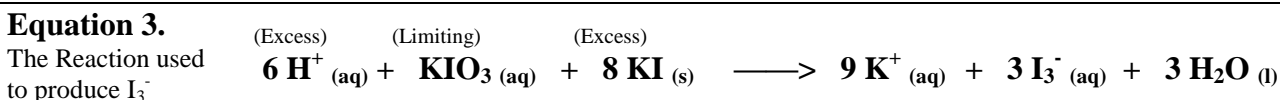
Equation 1.
Reaction of vitamin C, $C_6H_8O_6$, with I_3^-

(excess)   (limiting reactant)

$$I_3^-{}_{(aq)} \; + \; C_6H_8O_{6\,(aq)} \; + \; H_2O_{(l)} \; \longrightarrow \; C_6H_8O_{7\,(aq)} \; + \; 3\,I^-{}_{(aq)} \; + \; 2\,H^+{}_{(aq)}$$

In this experiment, you will add an ***excess amount of I_3^-*** to the solution containing the ascorbic acid. You will then determine the amount of unreacted I_3^- by titrating with a solution of thiosulfate, $S_2O_3^{-2}$, of known concentration as shown in equation 2.

Equation 2.
Titration of unreacted I_3^- with a standard thiosulfate solution, $S_4O_6^{-2}$

$$I_3^-{}_{(aq)} \; + \; 2\,S_2O_3^{-2}{}_{(aq)} \; \longrightarrow \; 3\,I^-{}_{(aq)} \; + \; S_4O_6^{-2}{}_{(aq)}$$

Since the original amount of I_3^- is known (i.e. it can be easily calculated), and the excess is determined by titration with thiosulfate, the amount needed to react with the ascorbic acid can be found by subtraction. This procedure is called a ***back titration***. You will prepare your own solution of sodium thiosulfate, and standardize it with a known quantity of I_3^-.

There is just one other problem. Solutions of I_3^- cannot be directly prepared from any solid reagent. In other words, you cannot go to the shelf and find, for example, NaI_3. In order to generate I_3^-, a solution of KIO_3 is reduced with solid KI in acidic conditions, as shown in equation 3.

Equation 3.
The Reaction used to produce I_3^-

(Excess)   (Limiting)   (Excess)

$$6\,H^+{}_{(aq)} \; + \; KIO_{3\,(aq)} \; + \; 8\,KI_{(s)} \; \longrightarrow \; 9\,K^+{}_{(aq)} \; + \; 3\,I_3^-{}_{(aq)} \; + \; 3\,H_2O_{(l)}$$

Since KI is in excess, the amount of I_3^- produced can be calculated from the stoichiometry of equation 3 and the amount of the limiting reactant, KIO_3, used. This amount (i.e. the amount of I_3^- produced), minus the excess titrated with the thiosulfate (i.e. the amount of unreacted I_3^-), represents the amount of I_3^- that reacted with the ascorbic acid.

Your task will be to analyze two samples. One will be a sample of juice and the other will be an unknown sample that you will attempt to analyze to within 5% of the true value. After completing calculations for the juice sample, you will report your results to the instructor who will post them in a spreadsheet for the class. You will then be able to analyze the class results for precision, and can compare the class average for each juice with the value on the product label.

Procedure

In this lab exercise you will work individually.

Part I — Preparation and Standardization of Sodium Thiosulfate

1. Prepare 500 mL of ~0.08 M solution of sodium thiosulfate, $Na_2S_2O_3 \cdot 5H_2O$. Stopper and label the flask.

2. Clean a buret. Flush and fill with the sodium thiosulfate solution—don't forget to ensure that all air is expelled from the tip of the buret.

3. Obtain in a clean and dry beaker about 150 mL of 0.0200 M KIO_3 from the lab cart.

4. Pipet 25.00 mL of the KIO_3 solution into a 250 mL Erlenmeyer flask.

5. Add to the flask ~20 mL of 0.5 M H_2SO_4 and ~2.0 g of KI. Stir to ensure that all of the KI dissolves. The solution should become a red–brown color due to the presence of I_3^- ion.

6. Begin titrating the solution with the thiosulfate solution. The red–brown color should fade as the I_3^- is consumed. When the solution takes on a yellow color, add a few drops of the <u>starch indicator</u>. The solution should turn a deep blue, or possibly a green–brown. Continue titrating until the blue color has disappeared. Record the amount of thiosulfate used.

7. Repeat the titration one more time, or as needed to ensure precision—two to three trials within +/- 0.2 to 0.3 mL.

8. Calculate the concentration of the thiosulfate solution. You already know the approximate concentration; this calculation will give you the exact value.

Part II — Analysis of an Unknown Sample

> **IMPORTANT NOTE**!! Vitamin C oxidizes readily! If you start your analysis on one day, and return to finish it on another, it is likely that your sample will have changed. Try to start and finish the unknown on the same day.

1. Select an unknown sample from the lab cart, *record its number in your lab book and report your unknown number to the instructor*. Pipet 25.00 mL of the solution into a 250 mL Erlenmeyer flask. Add ~20 mL of 0.5 M H_2SO_4.

2. Add 1.00 g of KI and 25.00 mL of 0.0200 M KIO_3 to the flask. Begin titrating with the thiosulfate solution. As before, add the starch solution when the red–brown color fades and continue titrating until the blue color disappears.

9. Repeat the experiment as needed for precision—two to three trials within +/- 0.2 to 0.3 mL.

Part III — Analysis of Vitamin C in Juice

1. Use a graduated cylinder to measure out a 25.0 mL sample of either pineapple or grapefruit juice. Add the juice to a 250 mL Erlenmeyer flask, and then add 20 mL of 0.5 M H_2SO_4.

2. Add 1.00 g of KI and 25.00 mL of 0.0200 M KIO_3 to the flask. Begin titrating with the thiosulfate solution. As before, add the starch solution when the red–brown color fades and continue titrating until the blue color disappears. However, note that the solution will not turn completely clear, due to the color of the juice. You will have to make your best estimate as to when to stop the titration.

3. Repeat the experiment a second time.

On the lighter side...
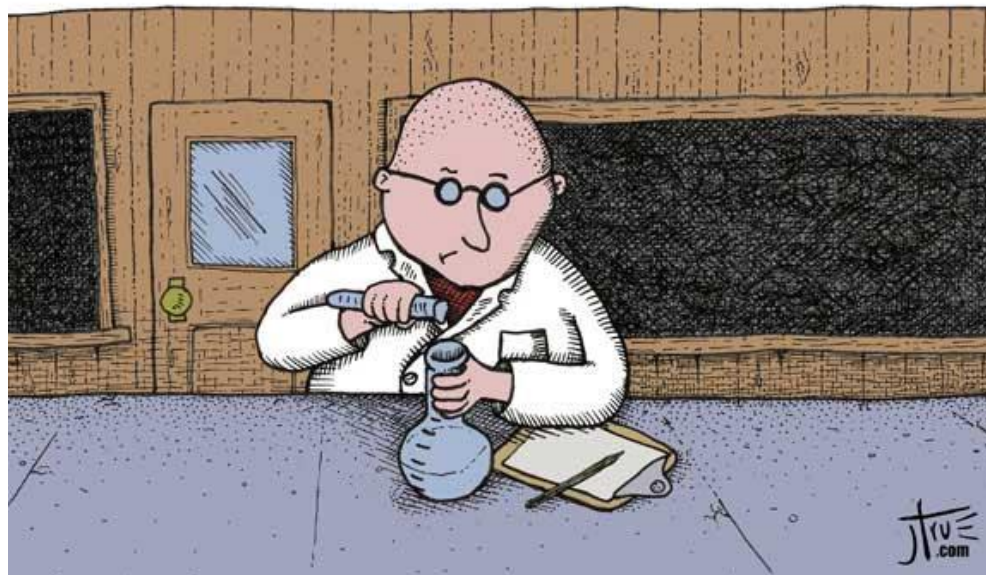
Lab 4. Analysis of Vitamin C
Prelab Questions   Name_____

Instructions: Complete the following questions and hand in at the start of your lab period. Show your work with units and correct significant figures for all questions that involve a calculation.

1. Consult the amounts used in part 2 of the procedure. Prove that KI is in excess and that KIO3 is the limiting reactant in equation 3. By how many <u>millimoles</u> is KI in excess? No work, no credit.

2. How many grams of sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$, are needed to make up 500.0 mL of 0.080 M $Na_2S_2O_3$? *Circle your answer and then* ***record this mass next to <u>step 1 of Part 1</u> of the procedure on <u>page3</u>.***

3. Why is it necessary to standardize the sodium thiosulfate solution made in part 1 of the procedure? <u>Hint</u>: It's for the same reason we had to standardize the EDTA solution in the *Analysis of Hard Water* lab performed last quarter.

4. The following results were obtained when a sample of pineapple juice was analyzed to determine its vitamin C content. A 19.8 mL sample of pineapple juice was placed in a flask with 20 ml of 0.5 M H_2SO_4, 1.0 g of potassium iodide, KI, and 25.00 mL of 0.01988 M potassium iodate, KIO_3. Titration with sodium thiosulfate required the use of 23.85 mL of 0.08014 M $Na_2S_2O_3$. Calculate the concentration of vitamin C in the sample in mg vitamin C per 100 mL juice. Parts a – e below take you step by step through this calculation.
    a. How many moles of I_3^- are produced from the reaction between KI and KIO_3? *Circle your answer.*

(Continued on the next page)

b.   How many moles of I_3^- are consumed from the reaction with thiosulfate? *Circle your answer.*

c.   How moles of I_3^- reacted with the ascorbic acid in the pineapple juice? *Circle your answer.*

d.   How many grams of ascorbic acid were in the juice sample? *Circle your answer.*

e.   What is the concentration of Vitamin C in the juice sample in mg Vitamin C per 100 mL juice? *Circle your answer.*