Determination of the Amount of Ascorbic Acid (Vitamin C) in a Commercial Product by Redox Titration

Goal and Overview

The ascorbic acid (vitamin C) content of a commercial product will be determined by redox titration with 2,6-dicholorindophenol (DCP). In the first set of titrations, a DCP solution will be standardized against samples containing a known amount of ascorbic acid. To determine the ascorbic acid content of a commercial product, aliquots of the commercial product will be titrated with the standardized DCP. The experimentally-determined ascorbic acid content will be compared to that listed on the product's label.

Objectives and Science Skills

Perform redox titrations for standardization and for analysis.
Evaluate experimental data to determine the concentration of vitamin C in a commercial product.
Identify and discuss factors or effects that may contribute to deviations between theoretical and experimental results and formulate optimization strategies.

Suggested Review and External Reading

Reference materials and textbook section on oxidation-reduction reactions and redox titration

Introduction

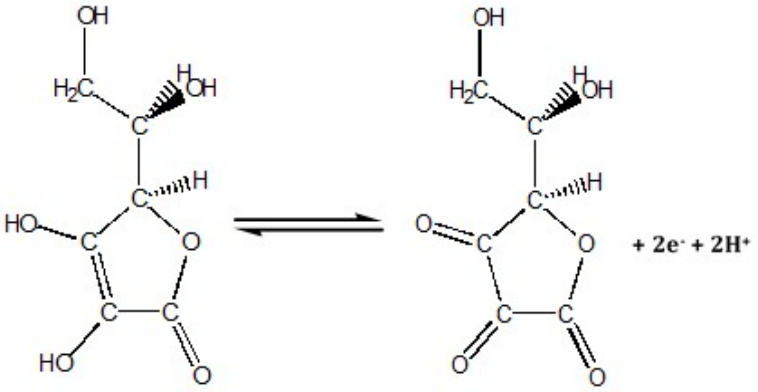
Ascorbic acid (vitamin C) is an essential nutrient, performing a role in numerous biological functions and biosynthetic pathways. These include tissue repair, collagen synthesis, wound healing, enzymatic production of certain neurotransmitters, and immune system function.

Humans cannot biosynthesize ascorbic acid, and the human body can only store a certain amount (about 300 mg at the near-scurvy level up to about 2 grams). If adequate fresh supplies are not consumed, ascorbic acid deficiency can lead to a host of health problems, not the least of which is scurvy. Although daily intake recommendations vary depending on a number of factors, such as age, smoking, and pregnancy, a value of 60.0 mg/day for humans age 4 and up is a common value (newer guidelines suggest 90.0 mg/day).

A major biochemical role of ascorbic acid is as an antioxidant (electron donor), meaning that its effective action requires that it is in the reduced form. Quantifying the amount of ascorbic acid in an analyte solution often involves its titration with 2,6-dichloroindophenol (DCP) as the titrant. DCP serves as the oxidizing agent and as the indicator.

Under neutral/basic conditions, DCP in its oxidized state appears intense blue; in acidic conditions, light pink. When the light pink species is reduced, the resulting compound is colorless. In the redox titration, the DCP added to the analyte will remain colorless until all of the reduced ascorbic acid has been oxidized. If additional DCP is added, it will remain in its oxidized (faint pink) form.

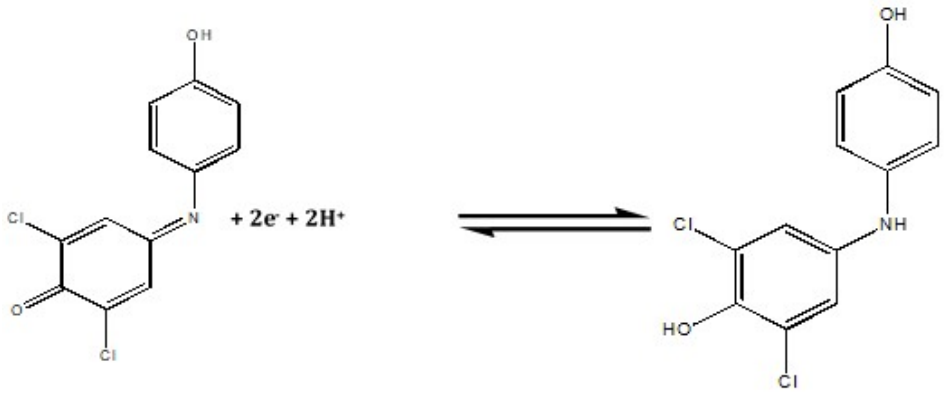
Ascorbic half-reaction (oxidation)



Reduced L-Ascorbic Acid

Oxidized L-Ascorbic Acid

2,6-dichloroindophenol half-reaction (reduction)



Oxidized DCP

Dark blue in base

Light pink in acid

Reduced DCP

Colorless

Oxidation half-reaction	$\text{Ascorbic acid}_{red} \rightarrow \text{Ascorbic acid}_{ox} + 2\ e^- + 2\ H^+$
Reduction half-reaction	$DCP_{ox} + 2\ e^- + 2\ H^+ \rightarrow DCP_{red}$
Overall redox equation	$\text{Ascorbic acid}_{red} + DCP_{ox} \rightarrow \text{Ascorbic acid}_{ox} + DCP_{red}$

Equipment List

Ring stand, buret, and buret clamp
250-mL Erlenmeyer flask and stopper
100-mL volumetric flask
10-mL volumetric pipet and pipetter
Analytical balance and weigh boat or paper
Follow your TA's instructions regarding the use (or not) of a magnetic stir bar and plate
Miscellaneous labeled glassware

Procedure

You will carry out two sets of titrations. First, you will determine the number of milligrams of ascorbic acid oxidized per mL of the DCP titrant. In the second part of the experiment, you will determine the ascorbic acid content of a commercial product and compare your experimental result with the value listed on the product's label.

Ask your TA how many titration trials you will do for each part.

For each trial, you will need about 10 mL of pH 3 buffer. Take the required amount in a labeled beaker.

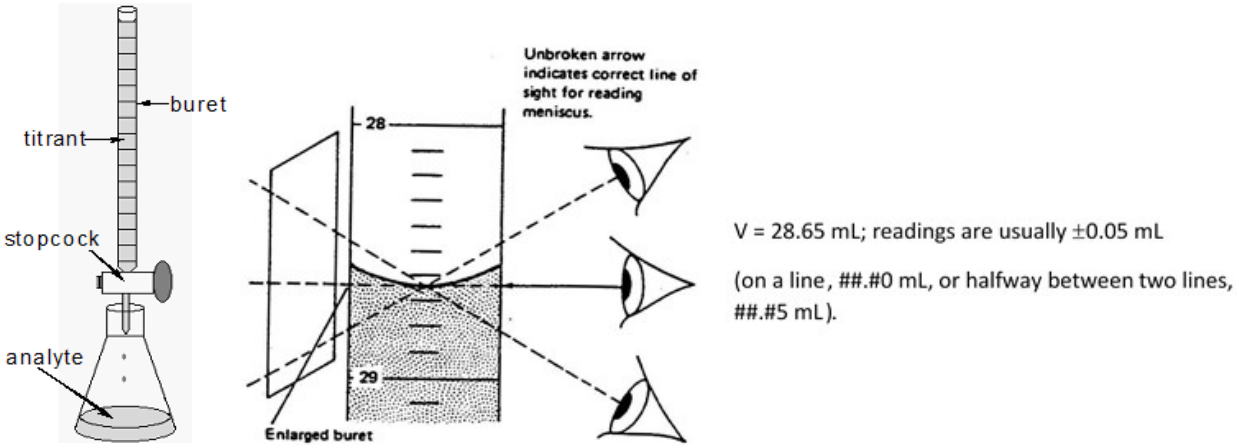
For the DCP, take ~50 mL in a labeled beaker to start. This is enough to fill your buret one time. Once you see how much DCP a single titration requires, you can take more if you need it. Please minimize the amount you waste (you cannot pour excess back into the reagent bottle).

Part 1. Standardization of the DCP titrant $\left(\frac{\text{\# mg ascorbic acid oxidized}}{\text{mL DCP}}\right)$

1. Carefully fill your buret with DCP (see Caution below). Record the initial volume reading, V_{in}, at the bottom of the meniscus to 2 decimal places. V_{in} does NOT need to be 0.00 mL.

Caution

Make sure that the buret is secure and straight in the buret clamp that is fastened to the stand. There should always be a labeled container under the buret large enough to capture the liquid contents.
Do not pour chemicals above eye level. You can carefully set the stand on the floor long enough to fill the buret. Use a funnel; have a labeled waste beaker beneath the buret; and, make sure that the stopcock is closed.
Carefully set the buret stand back on your lab bench. Allow a small amount of DCP to pass through the tip into the waste beaker. Record the initial volume, V_{in}, to two decimal places.



2. Mass ~50 mg (0.0500 g) ascorbic acid powder on the analytical balance (use a weigh boat and keep the balance area clean). Record the mass to four decimal places.

3. Fill a labeled 100-mL volumetric flask ~1/4 full with deionized water. Add the solid ascorbic acid and dilute to just below the line around the flask's neck with deionized water. Use a disposable pipet to add drops of deionized water so that the bottom of the meniscus is on the line.

4. Stopper the flask and invert it until the solid dissolves. Make sure to hold the stopper in securely while you do this, and leave the stopper in place as much as possible to reduce the oxidation of the ascorbic acid by oxygen in air.

5. Prepare your analyte solution. Volumetrically pipet 10.00 mL of the ascorbic acid solution into a 250-mL Erlenmeyer flask.

Caution

Incorrect use of the pipet and pipetter can result in **serious injury**.
Please **do NOT force or jam the pipet** into the pipetter.

If you push too hard, the pipet can break and **cut your hand**.
Use **just enough force** to create a seal. The pipet does not "click" into place.
Hold both the pipet and pipetter at all times.
If you pull any solution into the pipetter, please give it to your TA so that it can be cleaned.

If you have trouble, **ask your TA for help**.

6. Add ~20 mL deionized water and ~10 mL pH 3 buffer to the Erlenmeyer flask. These volumes do not need to be exact.

7. Place a light colored sheet or paper or paper towel under your flask. Titrate the analyte solution, swirling the flask as you add DCP.

When you see temporary light pink-beige that disappears readily upon swirling, decrease the volume of DCP per addition, going to dropwise as you approach the endpoint. At the endpoint, the light pink-beige should persist for a given amount of time (30 seconds or more; be as consistent as possible trial-to-trial).

Record the final volume, V_f, to two decimal places, and calculate V_{eq} to two decimal places.

$$V_{eq} = V_f - V_{in}$$

8. Your TA will tell you how many titrations to do; generally, three consistent trials are sufficient.

9. For each trial, calculate the number of milligrams of ascorbic acid (AA in the equation below) oxidized per mL DCP. The mass of ascorbic acid in each analyte solution equals the number of milligrams in 10.00 mL of the solution you prepared; the milliliters of DCP used is V_{eq}.

$$\frac{\text{\# mg AA oxidized}}{\text{mL DCP}} = \frac{\text{10.00 mL AA solution}}{V_{eq}\text{ of DCP in mL}} \times \frac{\text{\# mg solid AA massed on balance}}{\text{100.00 mL AA solution}}$$

Also find the average and standard deviation. You will use this ratio in part 2.

Part 2. Ascorbic acid content of a commercial product

The commercial product will be provided, along with a label that lists its theoretical ascorbic acid content per serving as a percentage of 60.0 mg ascorbic acid per day, an allowance at the low end of current daily recommendations.

10. Each titration uses 10.00 mL of the commercial product. In a labeled beaker, take just enough to complete the number of trials your TA specifies.

11. Record the information regarding the ascorbic acid content per serving from the label. As an example, it might list that a 10.00-mL serving contains 3.0% of the 60.0-mg recommended daily value, or 60.0 mg ascorbic acid $\times \frac{3.0\%}{100\%} = 1.8$ mg ascorbic acid.

12. Perform each titration as you did in part 1.
Volumetrically pipet 10.00 mL of the commercial product into a 250-mL Erlenmeyer flask; add ~20 mL deionized water and ~10 mL pH 3 buffer.
Record the DCP V_{in} from the buret to two decimal places.
Titrate until the color change persists for approximately the same length of time as your part 1 trials (the color at the endpoint depends on the color of the original commercial product, but there should be an observable difference pre-endpoint and at endpoint).
Record V_f to two decimal places and calculate V_{eq}.

13. For each trial, calculate the number of milligrams of ascorbic acid in the 10.00-mL aliquot that you used, per mL, and per one serving of the commercial product.

$$\frac{\text{\# mg AA in product}}{\text{10.00 mL product}} = \frac{V_{eq} \text{ of DCP in mL}}{\text{10.00 mL product}} \times \frac{\text{\# mg AA oxidized}}{\text{mL DCP}} \xrightarrow{\div \text{by } 10.00} \frac{\text{\# mg AA in product}}{\text{mL product}}$$

14. Find the average and standard deviation.

Follow your TA's instructions regarding clean-up and waste disposal.

Results / Sample Calculations

Complete the online inlab or write a lab report as directed by your TA.

DCP standardization
Ascorbic acid in commercial product

Discussion Questions and Review Topics

What did you find out and how?
How did the experimentally-determined ascorbic acid content in the commercial product compare to the value listed on the label? If they differ, what might be possible explanations?
How might you modify the procedure to improve the accuracy of the results?