Vitamin C Analysis[1]

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Introduction

Vitamins are a group of small molecular compounds that are essential nutrients in many multi-cellular organisms, and humans in particular. The name "vitamin" is a contraction of “vital amine”, and came about because many of the first vitamins to be discovered were members of this class of organic compounds. And although many of the subsequently discovered vitamins were not amines, the name was retained. In this exercise you will be studying vitamin C, also known as ascorbic acid.

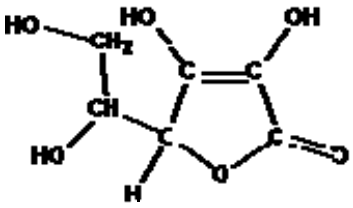


Figure 1. Structure of vitamin C (ascorbic acid).

Ascorbic acid ($C_6H_8O_6$) is a water-soluble vitamin, whose structure is shown in Fig. 1. Vitamin C is easily oxidized, and the majority of its functions *in vivo* rely on this property. It plays a key role in the body’s synthesis of collagen and norepinephrine by keeping the enzymes responsible for these processes in their active reduced form.[2] Vitamin C may also play a role in detoxifying by-products of respiration. Occasionally during respiration O_2 is incompletely reduced to superoxide ion (O_2^-) instead of being reduced completely to its -2 oxidation state (as in H_2O). Normally an enzyme called superoxide dismutase converts O_2^- to H_2O_2 and O_2, but in the presence of Fe^{2+} the hydrogen peroxide may be converted into the highly-reactive hydroxyl radical (•OH). The hydroxyl radical can initiate unwanted and deleterious chemistry within a cell when it removes a hydrogen atom (H•) from an organic compound to form H_2O and a new, potential more reactive free radical. Ascorbic acid can donate a hydrogen atom to a free radical, and thus stop these reactions from occuring.[2]

The human body cannot produce ascorbic acid, and so it must be obtained entirely through one’s diet. A vitamin C deficiency in humans results in the disease called scurvy, whose symptoms include hemorrhaging (especially in the gums), joint pain and exhaustion.[2] In its final stages scurvy is characterized by a profound exhaustion, diarrhea, and then pulmonary and kidney failure, which result in death.[3] A very small daily intake of vitamin C (10-15 mg/day for an adult) is required to avoid deficiency and stave off scurvy.[4] However, there has been, and continues to be, vigorous debate on what the optimum daily intake of vitamin C is. Some have argued that 200 mg/day is an optimal daily intake for adult humans. Others have suggested 1-2 g/day is best,[3] this

despite studies that show that the blood is saturated with vitamin C at 100 mg/day, and any excess is excreted in the urine. In an attempt to balance the competing claims, and ensure the general population's good health, the Federal Food and Drug Administration has adopted a the recommended dietary allowance (RDA) of 60 mg/day for adults (aged 15 or older), less for children, and more for pregnant and lactating women.[2]

Fruits, vegetables, and organ meats (e.g., liver and kidney) are generally the best sources of ascorbic acid; muscle meats and most seeds do not contain significant amounts of ascorbic acid.[5] The amount of ascorbic acid in plants varies greatly, depending on such factors as the variety, weather, and maturity.[6] But the most significant determinant of vitamin C content in foods is how the food is stored and prepared. Since vitamin C is easily oxidized, storage and the cooking in air leads to the eventual oxidation of vitamin C by oxygen in the atmosphere. In addition, ascorbic acid's water-solubility means that a significant amount of vitamin C present in a food can be lost by boiling it and then discarding the cooking water.

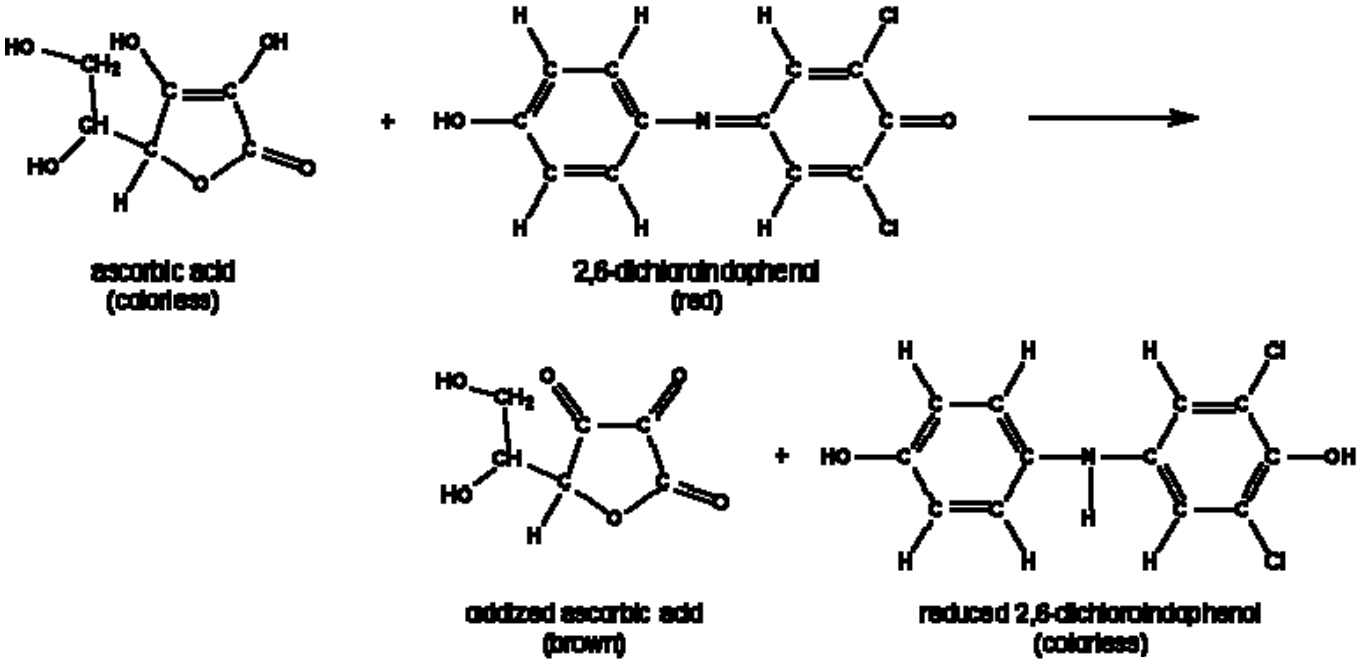
In this exercise you are to develop a testable hypothesis concerning the amount of vitamin C in a sample, design a procedure to test your hypothesis and then execute your plan in the laboratory. You and your laboratory partner will need to develop this hypothesis and submit a one-page typed proposal, which must be approved by your instructor before you can begin work. Include in your proposal a list of the samples that you will analyze (you will supply the samples) and outline any sample preparation that is not described in the experimental section of this exercise. If you need to analyze many samples, you may collaborate with another group in the laboratory (submit one proposal and one report for everyone involved).

Please remember that certain activities are not easily performed in the laboratory (i. e., cooking food). Therefore, you may need to perform some sample preparations, such as cooking, before coming to the laboratory. Also remember that if you wish to look at the change in vitamin C over time then you must plan ahead to allow for this.

Some possible topics are given below, but you are not limited to this list (creativity will be rewarded). In general, projects that seek to only measure the amount of vitamin C in two or more samples (for example, comparing apple and orange juice) will not be graded as highly because they have a weak hypothesis. The only restriction on your choice of topic is that your work may **not** involve vertebrate animals or any samples of human origin.

- What factors are important in decomposition of ascorbic acid in a particular system?
- What are the effects of different food preparation methods on ascorbic acid content?
- Are there differences in ascorbic acid content in various parts of a fruit or vegetable?

The amount of vitamin C in a sample will be determined by redox titration using the reaction (shown in Scheme 1) between ascorbic acid and 2, 6-dichloroindophenol (DCIP).[7,8] DCIP is used as the titrant because it should 1) only oxidize ascorbic acid and not other substances that might be present, and 2) because it will act as a self-indicator in the titration. To be a self-indicator a substance must be one color in the presence of excess analyte (i. e., ascorbic acid) and another color when the analyte has all reacted.



Scheme 1. Redox reaction between ascorbic acid (vitamin C) and 2, 6-dichloroindophenol (DCIP).

In acidic solutions DCIP is red, but if ascorbic acid is present, it will be reduced to a colorless substance. The solution will remain colorless as more DCIP is added until all of the ascorbic acid has reacted. As soon as the next drop of DCIP solution is added at the solution will be light red, due to the excess DCIP and the end point of the titration has been reached.

There are several limitations to this method that you must consider when you are designing and performing your experiment. First, the presence of particles (as in fruit juice) can interfere with your ability to see the end point, and so cloudy juices will need to be filtered first. Second, when analyzing solid substances you will need to dissolve the vitamin C before you can perform the titration. This may require blending or crushing the material and it may also require the addition of known amounts of water to the material. And finally, red materials cannot be analyzed by this method because it is impossible to see the end point.

There are several other things to consider as you design your experiment. First, you will need to have an estimate of how much titrant (DCIP) will be used in your titration. Look up values for the amount of vitamin C in your sample and from the known concentration

of DCIP (approximately 250 mg/L), determine what volume of DCIP will be required to titrate your sample. To achieve the most precise and accurate results, it is preferable that you do not use more than one full buret (40 mL) of your titrant (DCIP) for a single titration. Conversely, a sample that requires only a few milliliters of titrant to reach an endpoint can also have very imprecise results (your instructor will not accept endpoints which occur with less than 1 mL of DCIP). If you find that you need to completely empty and refill the buret during a single run of you sample or your endpoint is reached too soon, you may want to change your titration procedures for that sample so that a more reasonable volume (between 10 and 20 mL) of titrant is required. Finally, remember that all solutions must be prepared using volumetric glassware!

Experimental 

Preparation of the 2, 6-Dichloroindophenol Solution

The DCIP solution will be prepared for you by dissolving 0.250 g of 2, 6-dichloroindophenol in about 500 mL of water. Sodium bicarbonate (0.21 g) is then added and dissolved. The resulting solution is finally diluted to 1 L with distilled water. The approximate concentration of this solution is 250. mg DCIP/L. Because the exact concentration, and can vary from day-to-day, you will need to determine the actual [DCIP] by the standardization procedure given below.

Standardization of the 2, 6-Dichloroindophenol Solution

Before using DCIP to quantitatively measure vitamin C, you must know the concentration of the DCIP solution. You can use the reaction of the DCIP solution with a solution of ascorbic acid with a known concentration to find the concentration of the DCIP solution. This is known as “standardizing” the solution, and it must be done once a day for the DCIP solution.

Each pair will separately standardize the DCIP. Carefully pipet 5.00 mL of a standard ascorbic acid solution into a 250-mL Erlenmeyer flask. Be sure to record the concentration of the standard ascorbic acid (it should be about 0.500 g of vitamin C per 1.000 L). Add 2 mL of the sulfuric acid mixture and about 25 mL of distilled water to the flask (More Info). Swirl the flask to mix the solution. Fill a 50-mL buret with the DCIP solution. Use the DCIP to titrate the ascorbic acid until a permanent (lasting more than 30 sec) light red or pink color appears. Record the volume of DCIP needed to oxidize all of the ascorbic acid. Repeat the procedure on two additional samples of standard ascorbic acid. If you are working with another group, or groups, check that all [DCIP] are in agreement.

Using the balanced equation for the oxidation-reduction reaction between ascorbic acid and DCIP, determine the concentration (in mg/L) of the DCIP solution found with each of your titrations. If the results from your three runs are not within 5% of each other, you should repeat the standardization until you have three runs that are.

Determination of Vitamin C in a Sample

The procedure for determination vitamin C in a sample is identical to that for standardizing the DCIP solution. Simply substitute your sample for the standard ascorbic acid solution. You may need to adjust the volume that you pipet in, based on your calculations of the approximate amount of vitamin C present (pipets with the following volumes will be available for you to use: 1.00, 2.00, 3.00, 4.00, 5.00, 10.00, 25.00 mL). If you use a different volume of sample, you will also need to change the amount of the sulfuric acid solution used such that the proportion between the solutions remains the same. Perform at least three titrations, and repeat as necessary until at least three runs are within 5% of each other.

Results and Analysis

Determine the average and the standard deviation for the DCIP concentration and for the amount of vitamin C in your sample. Calculate the uncertainty in the amount of vitamin C in your sample at the 95% confidence limit. Collect your values, and those of other groups that worked with you, in a single table in your laboratory notebook.

Conclusions

The *Discussion and Conclusions* section of your notebook for this exercise should follow that of a measurement experiment.

In addition to your laboratory notebook, you will also prepare a short written report (one report per group) on your experimental results. Detailed instructions for writing a formal laboratory report can be obtained from the Laboratory Reports section of the Truman ChemLab page. Also available from the ChemLab web page are a Laboratory Report Template and an example of a completed formal laboratory report. The following are some helpful hints for you to consider as you prepare your report.

> In your report's *Introduction* section you should explain the problem under investigation and how you tried to solve it. Include enough background that a person who knows something about chemistry, but not this particular problem, can understand why your problem is important/interesting and how you planned on solving it. Your proposal is a good starting point for your report's *Introduction*.
>
> The *Experimental* section is a relatively detailed description of the procedures that you used. A helpful hint, when you write the *Experimental* section be concise; it should not be a step-by-step recounting of everything that you did.
>
> The report's *Results* section presents what you found. This should succinctly summarize your data and any results calculated from your data; tables and figures may be the best way to present this information.

In the report's *Discussion* section you will try to make sense of your results. This section is almost identical to the *Discussion and Conclusions* section of your notebook, although it is often longer. For this exercise, use the outline for a measurement experiment as a guide to writing your formal discussion.

The *Conclusions* section of a formal laboratory report may be very short and simply summarizes what you found.

The final section that you should write, although it will be the first thing your reader sees, is the *Abstract*, which is a short summary of the problem to be addressed, how it was addressed and what was learned.

Part of your grade will be based on the originality and/or difficulty of your proposal. Include any modifications, especially ones prompted by your experience in the laboratory. Include all data in a table. Part of your grade will be based on the accuracy and precision of your data, or at least whether you attempted to obtain accurate and precise data.

References

1. Click here to obtain this document in PDF format.

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