Research Project
Ascorbic Acid Titration

“If we knew what it was we were doing, it would not be called research, would it?”
—Albert Einstein
Physicist

Abstract
The technique is a redox (oxidation-reduction) titration for the presence of ascorbic acid in solution. The ascorbic acid is reduced to dehydroascorbic acid with dichlorophenol-indophenol (DCPIP). The DCPIP also functions as the indicator with the endpoint being the appearance of color due to the accumulation of unreacted DCPIP. The reaction is one-to-one: at the endpoint, the moles DCPIP consumed equals the moles ascorbic acid originally present.

Application
Ascorbic Acid, better known as vitamin C, is a controversial chemical and important nutrient. This is one of many techniques commonly used to determine the amount present in naturally occurring food stuffs and in dietary supplements. Some areas of study are the effect of cooking on the ascorbic acid content of natural foods and the effect of ultraviolet light on anything used as a source of ascorbic acid.

Sample Issues
The reaction is straight forward and not susceptible to very many direct interferences. The presence of other oxidizing agents in the sample can produce incorrect results (these would also react with the titrant). Since the endpoint is the retention of a pink color in the titration solution, colored samples are also difficult, if not impossible to evaluate.

Because this is a titration, care must be taken to avoid unintended dilution errors. Intensely colored samples will not work because it will not be possible to “read” the indicator. Suitable samples include:
- fruit juices
- colorless commercial products
- water samples
Background
Ascorbic acid (C\textsubscript{6}H\textsubscript{8}O\textsubscript{6}), a small organic molecule closely related to carbohydrates, has a number of vital physiological functions. It helps to make collagen, a major structural component of blood vessels, bone and skin structures. It is involved in the metabolism of amino acids and in the absorption of iron. It also acts as a cellular anti-oxidant (reductant) by reacting with potentially harmful oxidants before they can react with other molecules. Scurvy is the name given to patients with severe ascorbic acid deficiency. Symptoms are related to the failure of the body to make collagen and include hemorrhaging, bleeding gums, muscle degeneration, joint pain, and failure of wounds to heal.

Ascorbic acid is classified as a vitamin because humans need it for good health but are not capable of manufacturing it in their bodies. A steady dietary source is required. In recent years there has been considerable discussion of the value of ascorbic acid as a cold preventative medication. Dr. Linus Pauling, a Nobel prize winning chemist, was one of the first to recommend mega-doses of ascorbic acid to ward off the common cold. Although this has not been proven, research has shown that vitamin C does increase the body's immune defenses.

DCPIP (M.W. 290.12) is an organic dye with both acid/base and redox properties. In basic solution it is blue in color. In acid, it is red (pink in dilute solutions).

\[
\text{DCPIP, blue, } pK_a = 5.5 \quad \text{HDCPIP, red}
\]

The acid form can is easily reduced by ascorbic acid to a colorless form

\[
\text{HDCPIP, red } \quad \text{DCPIPH}_2, \text{ colorless}
\]

As the red HDCPIP is added to a solution of ascorbic acid in the presence of an acid, a reaction will occur and the color will go away. The reaction between ascorbic acid and HDCPIP is one-to-one.

\[
\text{ascorbic acid + HDCPIP } \rightarrow \text{DCPIPH}_2 + \text{dehydroascorbic acid}
\]
Once all the ascorbic acid is gone, further addition of HDCPIP will produce a faint pink color that does not go away. This is the endpoint of the titration.

**Overview**
Although this is primarily a measurement lab, there are many observations you can make to help you with your analysis. In particular, you should be noting what changes occur in your samples every time you perform an operation. Unusual observations often explain unusual numbers. Remember that to be able to describe any changes that occur, you will need to have accurate descriptions of the starting conditions as well as the ending conditions.

**Reagents**
All chemicals are provided as solids. The main reagent, the HDCPIP titrant, will have to be made. No indicator is required as the HDCPIP also fulfills this function. The procedure DOES require a phosphoric acid solution, discussed below.

**Design Issues**
The titration is very straightforward and easy to perform. Design issues relate to sample preparation. Solid samples need to be made into aqueous solutions. The solution must be free of other oxidants that would also oxidize the HDCPIP.

**Procedure**
1. **Preliminary analysis.** There are three quantities you will need to decide upon before beginning. You will need to know the sample volume, the titrant volume and the titrant concentration. The first two need to be appropriate for the glassware available to you. Values less than 10 mL or greater than 50 mL will reduce precision. The third value, the titrant concentration, will depend on the anticipated ascorbic acid concentration in your sample and your desired volumes. It is recommended you check your quantities with your instructor before beginning.

2. **Preparation of titrant.** Due to its cost, you will be provided with roughly one gram of the DCPIP titrant. This is sufficient to titrate 0.6 grams of ascorbic acid. You should plan accordingly. Should you use up this allotment, your instructor can obtain a second (and final) one for you. For most food samples a suitable DCPIP titrant concentration is on the order of 2.0 x 10^{-4} M. Once you have made your titrant, it is recommended you check its concentration by titration against a primary standard such as pure ascorbic acid (which will be provided).

3. **Preparation of acid.** The reaction between DCPIP and ascorbic acid works best in the presence of 0.4 M phosphoric acid, H₃PO₄. There are a number of ways to introduce the acid into your samples. Perhaps the easiest is to prepare a 0.8 M phosphoric acid solution, then add it 50/50 to your samples at the time you titrate them. You will be provided with concentrated phosphoric acid, 18 M H₃PO₄. A 0.8 M solution can be prepared using a quantitative dilution. Because it is a concentrated mineral acid you will need to follow all the appropriate precautions for using a concentrated acid. Avoid all
contact and avoid breathing the fumes. Immediately clean up any spills. Immediately notify your instructor if you come in contact with the concentrated acid.

4. **Preparation of Samples.** The following issues need to be addressed.
   
   A. If the expected [ascorbic acid] is not in the effective range (see Application above) the sample will have to be concentrated or diluted as appropriate. If the sample is a solid, it needs to be dissolved to an appropriate approximate concentration.
   
   B. If the ascorbic acid is not free to enter solution (For example, bound up inside cells) a digestion will have to be performed. A procedure is given below.

5. **Performing the titration.** Follow the general procedure given in chapter 10 of your technique book.

**Sample Digestion** The following is a description of a digestion designed to break down cell walls and dump the contents into solution.

1. Obtain a mortar and pestle. Put a small amount of the sample in the mortar. Add some sand. Generally this should be about half the volume of the sample.

2. Add a few mLs of the appropriate solvent. This could be water, but usually is a weak acid solution such as 0.1 M acetic acid or 0.1 M hydrochloric acid.

3. Grind the sample with the pestle.

4. Gravity filter the sand from the solution..