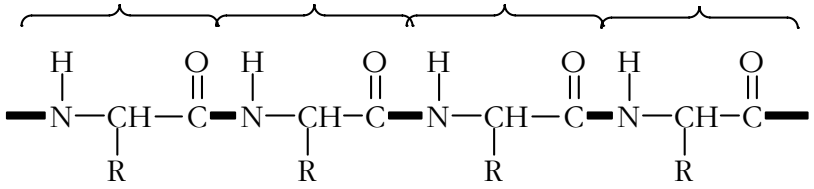
EXPERIMENT #4 – DETERMINATION OF VITAMIN C

Introduction

Protein molecules are composed largely, and in some cases exclusively, of *amino acid residues* that are joined to each other through a bond between a carbonyl carbon and an amine nitrogen known to biochemists as a *peptide bond*.

Amino acid residues with peptide bonds highlighted.

Amino acid residue portion of a protein molecule.



R may be any of about 20 small groups such as: -H, $-CH_3$, $-CH_2OH$.

Protein molecules can be divided into two categories based on their gross morphology (structure viewed on a large scale, not in detail): *fibrous* and *globular*. The names are descriptive. Fibrous proteins are essentially linear in shape and form fibers. These are structural proteins like keratin (found in hair and nails), myosin (found in muscle) and collagen (found in bones, tendons, *etc.*). They are not easily dispersed in water. Globular proteins are more or less spheroidal in shape and disperse in water. Many of the globular proteins function as *enzymes*. Enzymes are catalysts for biochemical reactions.

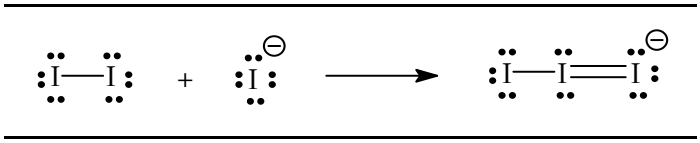
More than 2000 enzymes are known and most of them are globular proteins. In addition to the protein part, most enzymes also have a small (but crucially important for the enzyme's biological function) *nonpeptide* (not made of amino acid residues) part. This part may be covalently bonded to the rest of the protein, in which case it is called a *prosthetic group*. If it is not covalently bonded to the protein it is called a *cofactor*. The protein part of an enzyme that has a cofactor is called an *apoenzyme* and the combination of apoenzyme plus cofactor is called the *holoenzyme*. In these two-part enzymes it is the holoenzyme that is biologically active; neither the apoenzyme nor the cofactor can function on its own. A cofactor might be an inorganic ion, such as Zn^{+2} or Cu^{+2}, or it may be a small organic molecule called a *coenzyme*. Many coenzymes are *vitamins* that must be present in our diets in small amounts.

Among these coenzymes is vitamin C, a reducing agent. Vitamin C, also known as *ascorbic acid*, helps to keep the iron in the enzyme prolyl hydroxylase in reduced form, thereby maintaining the enzyme's activity. Prolyl hydroxylase is essential for the synthesis of normal collagen; in its absence abnormal collagen forms, resulting in scurvy. Since humans cannot synthesize ascorbic acid, it must be present in our diets: 60 mg/day is the recommended minimum for adults. Citrus fruits, apples, broccoli, sweet peppers and tomatoes are rich sources. British sailors are sometimes called "limeys" owing to the practice of British naval sailing vessels carrying citrus fruits to prevent scurvy.

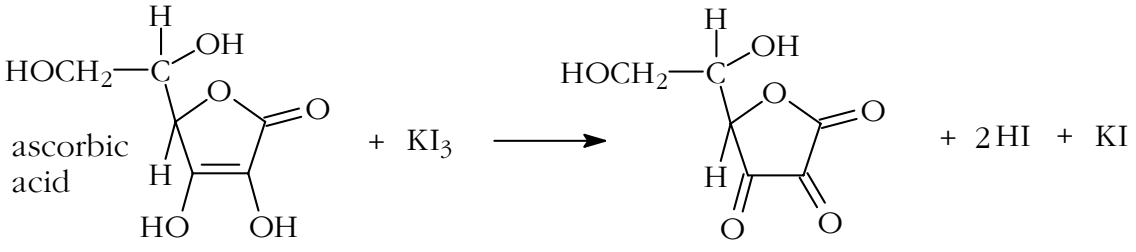
Experimental Objective and Background

You will determine the amount of ascorbic acid in a fruit drink by means of a titration. In order to see the endpoint in the titration clearly, the fruit drink should be light colored like apple, orange or grapefruit. Dark purple grape drink would not be suitable. You may provide your own sample or use one available in the laboratory.

The titration that is used to determine the amount of ascorbic acid present in a sample is based on its being a reducing agent. An appropriate oxidizing agent will oxidize a reducing agent as the reducing agent reduces the oxidizing agent; oxidation and reduction always occur together. So, you will employ an oxidizing agent in the titration, determine how much oxidizing agent is reduced by the ascorbic acid, and thereby determine how much reducing agent (ascorbic acid) was in the sample. The oxidizing agent you will use is potassium triiodide which is made by dissolving iodine in an aqueous solution of potassium iodide. Iodine itself could be used as the oxidizing agent except that it is almost insoluble in water because it is nonpolar and water is highly polar. We might note, in passing, that the two iodine atoms joined by the double bond in the triiodide ion have valence shell occupancies of 10 electrons; this is OK for atoms in the third and higher rows of the periodic table, like iodine, but is not possible for atoms in the first two rows.



Potassium triiodide reacts *quantatively* with ascorbic acid according to the following balanced chemical equation. By quantatively we mean that the "reaction goes to completion." In other words, the equilibrium between reactants and products favors the products to such an extent that any unreacted ascorbic acid remaining after a stoichiometric equivalent of potassium triiodide has been added will be minuscule.



Starch is added to the sample of fruit juice being tested as an indicator. As potassium triiodide solution is added to the *analyte* (the fruit juice sample) from the buret the triiodide anion reacts with the ascorbic acid oxidizing it, and the triiodide is reduced to iodide anion. When the ascorbic acid is exactly used up, the next drop of *titrant* from the buret will have no ascorbic acid to react with and will remain as potassium triiodide. This potassium triiodide will complex with the starch that was added producing a dark blue color. The appearance of this color is the end-point of the titration.

Procedure

1. Pour 60 to 70 ml of the fruit drink that you will be analyzing into a clean, dry 125 ml Erlenmeyer flask. The fruit drink should not be a dark colored one which would make seeing the end-point difficult. Record the kind of fruit drink you are going to analyze on the report sheet.

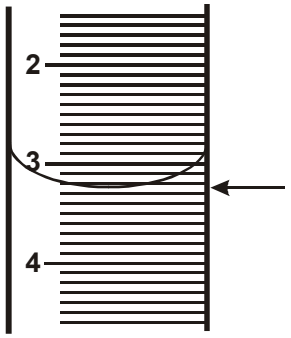
2. If the fruit drink is extremely cloudy or contains a lot of suspended particles, it can be clarified by adding about 0.5 gram of Celite filter aid, stirring, and filtering the material through a wad of glass wool placed in the bottom of a conical funnel.

3. Using a 10.00 ml transfer pipet transfer 10.00 ml of the fruit drink into a second 125 ml Erlenmeyer flask. Touch the tip of the pipet to the inside of the flask above the liquid surface for a few seconds to allow the pipet to drain. Do not blow out the last drop in the tip of the pipet. The pipet you are using is graduated *to deliver* (as opposed to *to contain*) and will deliver the stated amount without removing the drop in the tip. Using a graduated cylinder, add 20 ml of deionized water and then 10 drops of 2% aqueous starch solution to the flask.

4. Attach a buret clamp to a ring stand and clamp a clean 25 ml buret to the buret clamp. Rinse the buret in twice with 5 ml portions of potassium triiodide solution. Let the rinses run through the tip of the buret and discard them. Fill the buret with the potassium triiodide solution. Open the stopcock, then close it, <u>making sure that the tip of the buret is full of titrant and there are no air bubbles in the tip</u>. You may have to open and close the stopcock several times and/or shake the buret to insure this. If the liquid level is below the 5 ml mark on the buret, top it off but do not attempt to get the liquid level to exactly 0.00 ml – real chemists don't waste their time this way. Record the initial buret reading. Record the molarity of the potassium triiodide solution.

<u>Reading the Buret</u>

You should take your readings at the bottom of the liquid meniscus and you should estimate to 0.01 ml as shown in the graphic to the right. You may find reflections from the laboratory surroundings distracting as you try to take a reading. If so, it is usually helpful to place an object of uniform color (white is most popular) behind the buret to eliminate or reduce this problem.



5. Place the flask that contains the analyte under the buret and add the titrant slowly while swirling the contents of the flask. Make sure all of the titrant you use winds up in the flask. When the blue-black color of the iodine-starch complex begins to persist as you swirl, slow the addition of the titrant to a dropwise rate. When the flask contents turn blue-black, stop – don't add another drop of titrant. If the blue-black color does not persist for 20 seconds with swirling, add another drop of titrant and continue in this fashion until the color does persist for 20 seconds. Record the final buret reading. Dispose of the flask contents.

6. Rinse out the 125 ml Erlenmeyer flask with deionized water and repeat the above titration procedure twice more, but use 20 ml and then 30 ml of the fruit drink. Remember to record the initial and final buret readings each time.