Experiment 15 – Vitamins

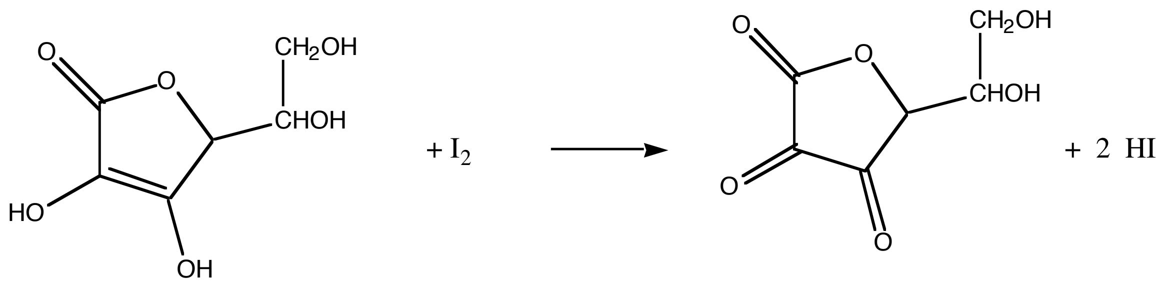
Part 1: Solubility of Vitamins

Vitamins are organic compounds that are required as cofactors for specific enzymes. They are not synthesized in the body and therefore must be obtained from the diet. Some vitamins are water-soluble, and others are fat-soluble. Excesses of water-soluble vitamins are excreted in the urine, but excesses of fat-soluble vitamins are stored in body fat and levels that are too high can be toxic. Deficiencies in any vitamins cause specific symptoms. Refer to your textbook for a list of vitamins and the corresponding deficiency symptoms.

Titration of Vitamin C

Vitamin C is an antioxidant vitamin. It is useful in the body as a "free-radical scavenger" – that is, it reacts with any free radicals that it comes into contact with. A free radical is a molecule that has an odd number of electrons, and therefore is extremely reactive. Because they are so reactive, free radicals can actually cause damage to biological molecules such as DNA and proteins. Vitamin C can serve to protect DNA and proteins from attack by free radicals. Since vitamin C is very easily oxidized, free radicals will react with vitamin C before they will react with DNA and proteins.

We will be analyzing several samples to determine their vitamin C content. We will take advantage of the fact that vitamin C is very easily oxidized to do our analysis. The technique we will be using is a redox titration. Elemental iodine (I_2) reacts with vitamin C according to the following reaction:



Vitamin C (Ascorbic acid)

I_2 forms a blue complex with starch

Oxidixed vitamin C

In this reaction, the vitamin C is oxidized and the iodine is reduced to iodide ion. We will be using starch as our indicator. Iodine forms a blue-black complex with starch. However, if iodine is added to a solution that contains both starch and vitamin C, the iodine will react with the vitamin C first. Therefore, in this experiment, you will add an iodine solution slowly to a solution containing both vitamin C and starch. When the vitamin C is completely used up, the next drop of iodine reacts with starch, turning the solution blue or gray. The appearance of this color will be your indicator that the vitamin C has been completely consumed.

Part 2: Standardization of an Iodine Solution

In this part, you will titrate a vitamin C tablet that contains a known amount of vitamin C with a solution of iodine. You will determine what volume of iodine is needed to react with your mass of vitamin C. You will then calculate the number of milligrams of vitamin C oxidized per milliliter of iodine solution used. This quantity will be used as a conversion factor to determine the vitamin C content of other samples.

Part 3: Analysis of Vitamin C in Fruit Juices and Fruit Drinks

In this part of the experiment, you will titrate two samples of different juices with iodine. Based on the amount of iodine needed to react with each one, you will calculate the vitamin C content of each type of juice.

Part 4: Heat Destruction of Vitamin C

In this part, you will use one of the same juices that you used in part 3, but this time you will heat the juice before titrating it. One sample will be heated for 10 minutes and the other sample will be heated for 30 minutes. The samples will then be titrated with iodine to determine their vitamin C content. You will then compare the vitamin C content of the unheated sample to the heated samples.

Safety Precautions:

- Wear your safety goggles.
- Keep the iodine solution away from skin and clothing.

Waste Disposal:

- Waste that contains methylene chloride (CH_2Cl_2) must go into the **organic** waste containers (which have a **pink** label) in one of the fume hoods.
- All other waste must be placed in the **inorganic** waste containers (which have a **blue** label) in one of the fume hoods.

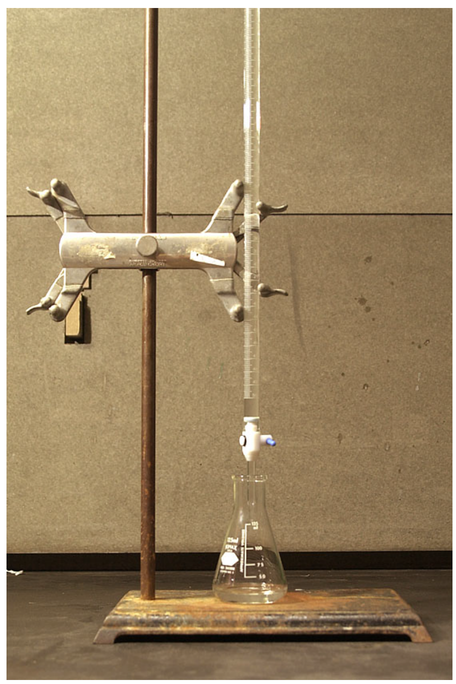
Procedure

Part 1: Solubility of Vitamins

1. You will test each vitamin available (vitamins A, B, C, D, E, folic acid, and possibly others) to see if it is soluble in water or in methylene chloride (CH_2Cl_2). Be sure to use very small amounts of each vitamin (an entire pill is too much). Place a small amount of each vitamin in separate clean, dry, labeled test tubes. (If you are using pills of vitamins A or E, be sure to puncture or cut the pill to get a sample of the liquid inside the pill.) Add 20 drops of methylene chloride to each sample, and mix each tube well by shaking it vigorously from side to side. Observe whether each vitamin sample is soluble or insoluble in the methylene chloride, and record your observations. Dump the contents of the tubes in the organic waste container and wash your tubes with plenty of soap. Make sure the tubes are clean and have no more remnants of methylene chloride for the next part.
2. Place new samples of each vitamin into clean, labeled test tubes. This time, the tubes do not have to be dry. Add 2 mL of deionized water to each tube and mix thoroughly by shaking each tube. Observe whether each vitamin is soluble or insoluble in water. Record your observations. Which vitamins are “water-soluble” and which are “fat-soluble”?

Part 2: Standardization of an Iodine Solution by Titration with Vitamin C

3. Get a 100-mg vitamin C tablet from the hood. If 100-mg tablets are not available, record the actual number of milligrams of vitamin C specified on the label. Crush the tablet with a mortar and pestle and transfer it to a 250-mL Erlenmeyer flask. (Do not use a beaker!) Rinse the mortar with deionized water and transfer the rinse water to the same flask. Add about 50 mL of deionized water to the flask and 2 mL of 0.1 M acetic acid. Mix well by swirling the flask vigorously, and try to dissolve the vitamin C completely. Add 10 drops of 1% starch solution and swirl the flask again.
4. At your desk, set up a ring stand with a buret clamp. (The setup is shown on the next page.) Collect about 75 mL of iodine solution in a small beaker, and rinse a 50-mL buret and its tip with a small amount of the iodine solution (5-10 mL). Clamp the buret in place, and using a funnel, fill the buret with the iodine solution to above the 0 mL mark. Remove the funnel and drain the solution into a waste beaker until the level of the solution is below the 0 mL mark. Make sure there are no air bubbles in the tip of the buret.



Titration Setup

5. Record the initial buret reading (to the nearest 0.1 mL). Begin adding the iodine solution from the buret to the flask of vitamin C solution. Keep swirling the flask as you add the solution from the buret. When you see an abrupt color change (to blue or gray), STOP adding the iodine solution and read the final buret reading. The volume of iodine solution added is the difference between the final and the initial readings.
Caution: do not let the level of the iodine solution in the buret go below the 50 mL mark, because there are no markings below that level. If it goes beyond the 50 mL mark, you won't be able to measure how much solution has been added, and you will have to start over. If you get near the 50 mL mark and you have not yet reached the endpoint, stop adding iodine, read and record the buret reading, then

re-fill the buret with iodine solution and read and record the new initial reading. Continue the titration, and when the solution changes color, stop adding iodine and read and record the final buret reading. The total volume of iodine solution added to the vitamin C flask is then: [(final reading 1 – initial reading 1) + (final reading 2 – initial reading 2)].

6. Calculate the mass (in mg) of vitamin C that reacts with 1 mL of iodine solution. You can do this by dividing the number of milligrams of vitamin C in the tablet by the number of milliliters of iodine solution needed to reach the endpoint.

Part 3: Analysis of Vitamin C in Fruit Juices and Fruit Drinks

7. In this part, you will test two different types of fruit juice or drink. The experiment works best if the juice is colorless or has a light color. Obtain the first type of juice. Record the type of juice or drink.
   If the juice has a lot of pulp, pour it through a piece of cheesecloth in a funnel. Use a pipet or a graduated cylinder to transfer 20 mL of juice into a clean 125-mL Erlenmeyer flask. (If you use a pipet, the volume of juice will be 20.0 mL. If you use a graduated cylinder, record the actual volume of juice used to the nearest 0.1 mL.) Add 30 mL of deionized water to the juice and swirl to mix well.
   If you are using a powdered drink (such as Tang or Kool-Aid), weigh out 1.0 g of the powder and transfer it to a 125-mL Erlenmeyer flask. Add about 50 mL of deionized water to the powder and swirl vigorously until the powder is completely dissolved. (Do not use a dark colored drink powder, because the endpoint of the titration will be too difficult to see.)
   If you are using a vegetable, weigh out about 10 g of the vegetable, recording the actual mass used to the nearest 0.1 g. Puree the vegetable in a blender with 20 mL of water. Filter the puree through cheesecloth over a funnel. Add water to give a total volume of 50 mL (measured with a graduated cylinder), and transfer the sample to a 125-mL Erlenmeyer flask.
8. Once your sample is prepared, add 2 mL of 0.1 M acetic acid and 10 drops of 1% starch indicator and mix well. Re-fill the buret with iodine solution (remember that the initial level should be below the 0 mL mark).
9. Record the initial volume reading of the iodine solution in the buret. Titrate your sample with the iodine solution (add iodine slowly from the buret) until one drop of the iodine solution causes an abrupt color change in the solution (to blue or gray, as before). Record the final buret reading. Subtract to calculate the volume of iodine solution added.
10. Calculate the number of milligrams of vitamin C in the fruit juice sample. Use your value from part 2 (# mg vitamin C / 1 mL of iodine solution) as a conversion factor, and multiply this conversion factor by the number of milliliters of iodine added.
11. Repeat the titration with a sample of a different type of juice, and calculate the number of mg of vitamin C in this new sample.

Part 4: Heat Destruction of Vitamin C

12. In this part of the experiment, you will test two different samples of one of the juices you used in part 3. Choose the juice that had the highest vitamin C content. With a graduated cylinder, measure out 20 mL of the juice sample, recording the actual volume used to the nearest 0.1 mL. Pour this sample in a clean 250-mL Erlenmeyer flask. Label this flask "10 min." Measure out another 20-mL sample of the same juice in the same way, and put this sample in a second clean Erlenmeyer flask. Label this flask "30 min."
13. Add 50 mL of water to each of these samples and mix well. Boil the first sample for 10 minutes, and boil the second sample for 30 minutes.

14. When you take the 10-minute sample off of the heat, place it in an ice-water bath. Add 2 mL of 0.1 M acetic acid and 10 drops of the 1 % starch indicator and titrate the sample with the iodine solution in the buret. (Read the initial volume, add iodine until the color changes, and read the final volume.)
15. After the second sample has been boiling for 30 minutes, repeat step 14 with this second sample.
16. Calculate the volume of iodine solution used in each titration.
17. Calculate the number of milligrams of vitamin C present in each of the heated samples.
18. Calculate the amount of vitamin C (in mg) that was <u>destroyed</u> by heating the juice. You will need to use the amount of vitamin C in the unheated sample of the juice that you determined in part 3.

<u>Questions</u>

1. Which of the vitamins you tested were water-soluble? Which were fat-soluble?
2. Which vitamins would be excreted daily?
3. Which of the juices that you analyzed had the highest vitamin C content?
4. If the daily requirement of vitamin C is 75 mg, how many milliliters (or grams) of each sample would you need to consume to get 75 mg? Show your calculations.
5. How does heating affect the vitamin C content of a fruit juice?
6. If vitamin C tablets are stored in a warm, humid bathroom cabinet, what might happen to the vitamin C content after a while?
7. If you wanted to keep most of the vitamin C content of your vegetables, how would you prepare them for dinner?