DETERMINATION OF VITAMIN C IN A PRODUCE PROTECTOR
(Iodometric method) - STUDENT

Since its discovery in the late 1920s[1], no other chemical has ever been as celebrated as Vitamin C. The beneficial effect of Vitamin C is almost universally recognized[2]. It is a water-soluble keto-lactone with two ionizable hydroxyl groups; ascorbate monoanion (Figure 1), $AscH^-$, is the dominant form at physiological pH and is is an excellent reducing agent as well. Research suggests that Vitamin C-rich foods, play an essential role against development of cancer; in addition plasma concentrations of ascorbate have been shown inversely associated with cancer risk[3],[4]

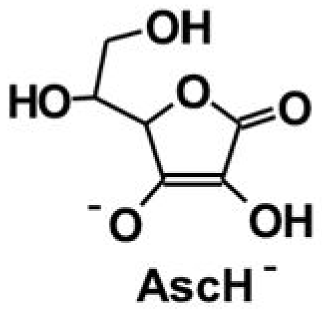

***Figure 1**: ascorbate monoanion*

Ascorbate monoanion undergoes two consecutive oxidations to form ascorbate radical ($Asc^{\cdot -}$) and dehydroascorbic acid (DHA) as shown in Figure 2:

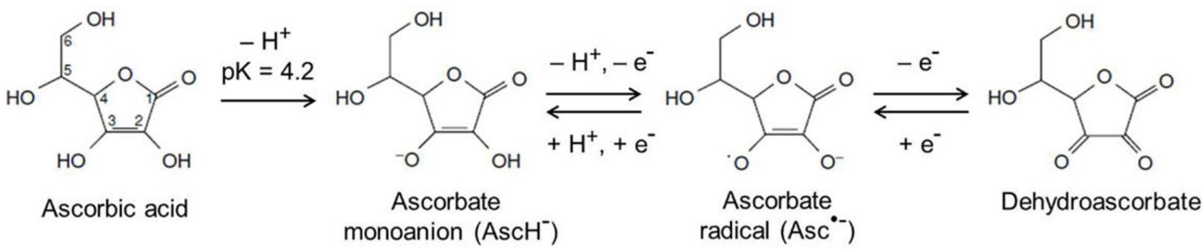

***Figure 2 -** ascorbate monoanion*

Fruit and vegetables are good sources of vitamin C, and ~90% of the daily intake in the general population comes from these sources. The content varies between species, but citrus fruit, kiwi, mango, and vegetables such as broccoli, tomatoes, and peppers are all rich sources of Vitamin C. Since it degrades when heated and during storage, processing and preparation

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[1] *J.L. Svirbely, A. Szent-Györgyi, The chemical nature of vitamin C, Biochem. J. 27 (1933) 279–285.*

[2] *Biochim Biophys Acta. 2002 Jan 15;1569(1-3):1-9.*

[3] *C.A. Gonzalez, E. Riboli, Diet and cancer prevention: contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, Euro. J. Cancer 46 (2010) 2555–2562.*

[4] *F. Musil, Z. Zadák, D. Solichová, R. Hyšpler, M. Kaška, L. Sobotka, J. Manák, Dynamics of antioxidants in patients with acute pancreatitis and in patients operated for colorectal cancer: a clinical study, Nutrition 21 (2005) 118–124.*

procedures should be considered when estimating dietary intake of vitamin C. In the small intestine, Vitamin C reduces dietary iron and allows for efficient transport across the intestinal epithelium. In general, Vitamin C is safe and well tolerated, even in large doses. The U.S. Institute of medicine set the Tolerable Upper Intake Level for oral vitamin C ingestion at 2 g daily for adults. High amounts of Vitamin C intake have been associated with an increased risk of kidney stones, although the evidence is mixed and inconsistent. The current recommendation is to avoid Vitamin C supplementation in those susceptible to kidney stone formation. Vitamin C consumed with iron could increase the risk of iron overload in susceptible individuals[5].

In this lab activity you are going to determine the content of Vitamin C in a produce protector (in this case, *Ball® Fruit Fresh*) by an Iodometric method. Equipment used is illustrated in the picture on the right.

Figure 3 - Equipment and Chemicals required for the activity.

<u>PROCEDURE 1</u>**: If your instructor has not standardized the $Na_2S_2O_3$ solution, you will follow these steps.**

Equipment and Chemicals

- *Distilled water*
- *Sodium thiosulphate pentahydrate - $Na_2S_2O_3 \times 5H_2O$*
- *Potassium iodate - KIO_3*
- *Potassium iodide - KI*
- *6 M hydrochloric acid - HCl*
- *Starch solution*
- *Burette*
- *250 ml Erlenmeyer flask*
- *Balance*

[5] *Advances in Nutrition*, Volume 5, Issue 1, 1 January 2014, Pages 16–18

1. Using a 500 ml volumetric flask, prepare a 0.05 M solution of $Na_2S_2O_3$ by dissolving 6.20 g of the solid in 400 ml of distilled water. Add a teaspoon of sodium bicarbonate in order to create an alkaline environment to preserve the solution. As soon as the solid dissolves, bring the volume up to 500 ml. Solutions should be stored in a dark bottle to avoid light exposure. Solutions prepared like that, should be stable for 3-4 days.
2. Using a 250 ml volumetric flask, prepare 0.01 M KIO_3 by adding 0.54 g of the solid into 200 ml of distilled water. Stir the mixture until complete dissolution and bring the volume up to 250 ml.
3. Obtain a burette and wash it (at least three times) with small volumes of the sodium thiosulphate solution.
4. Fill the burette with the sodium thiosulphate solution over the zero mark; discard part of the liquid into a waste beaker and record the volume on the scale of the burette. This is going to be your starting point.
5. Into an Erlenmeyer flask, pour 20 ml of 0.01 M KIO_3 solution; then add 3 g of KI and swirl the flask until complete dissolution of the solid.
6. Add 2 ml of 6 M HCl; the solution will turn dark-brown due to the formation of iodine. Swirl the flask in order to have an homogeneous mixture.
7. Slowly add sodium thiosulphate solution to the flask. You will gradually notice a decolorization of the solution. In case, wash the walls of the flasks with distilled water.
8. As soon as the solution is getting to a yellowish kind of color, slow down the titration. Add the sodium thiosulphate solution until you get a light yellow liquid in the flask.
9. At this point, add 10-15 drops of starch solution into the flask. Solution will turn dark-purple.
10. Slowly adding sodium thiosulphate solution until you get a colorless liquid in the flask. That will be your endpoint. Record the volume added from the burette.

The sequence of colors you will see is shown in the picture below:

***Figure 4 -** from left to right: solutions of KIO3 after the addition of KI and HCl - solution immediately before the endpoint - solution after the addition of starch - end of titration*

In order to determine the concentration of the $Na_2S_2O_3$ solution, I would suggest you to look the reactions involved in the process:

Reaction 1: First reaction takes place between iodate (IO^{3-}) with excess of iodide (I^-) in acid environment. Iodine (I_2) is produced. The solution turns dark-brown.

$$IO_3^- + 5\,I^- + 6\,H^+ \rightarrow 3\,I_2 + 3\,H_2O$$

Reaction 2: The iodine is titrated with sodium thiosulfate:

$$2S_2O_3^{2-} + I_2 \longrightarrow 2\,I^- + S_4O_6^{2-}$$

Here are some questions and calculations to do in order to complete this first step:

A. How many moles of KIO_3 are there in 0.54 g of it? (Molar mass of KIO_3 is 214 g mol^{-1})
B. Calculate the concentration of KIO_3 obtained if you dissolve 0.54 g of that in 250 ml of distilled water.
C. How many moles of KIO_3 there are in 20 ml of the solution prepared according to points A and B?
D. Look at **Reaction 1** and at its stoichiometry; how many moles of I_2 do we get after the addition of KI and HCl to 20 ml of KIO_3 solution?
E. Consider the stoichiometry of **Reaction 2** and figure out how many moles of $Na_2S_2O_3$ are titrated at the endpoint.
F. Looking back at point E, which is the additional parameter you would need to calculate the actual concentration of sodium thiosulphate?

Table 1 - Report all data here.

Trial	Volume of KIO_3 (ml)	Volume of $Na_2S_2O_3$ (ml)	Concentration of $Na_2S_2O_3$ (M)	Concentration of $Na_2S_2O_3$ (M) - average value
1				
2				
3				

<u>PROCEDURE 2</u>: determination of Vitamin C in a produce protector

Equipment and Chemicals

- *Ball® Fruit Fresh*
- *Distilled water*
- *Sodium thiosulphate standardized solution*
- *Potassium iodate 0.01 M solution*
- *Potassium iodide - KI*
- *6 M hydrochloric acid - HCl*
- *Starch solution*
- *Burette*
- *250 ml and 125 ml Erlenmeyer flasks*
- *Balance*

1. Obtain some Fruit Fresh with a teaspoon; record its mass and dissolve it into 100 ml of distilled water. Vigorously stir the mixture until it is adequately clear. This is going to be our Fruit Fresh stock solution. In case it is too opaque, set up a filtration.
2. Into a 250 ml Erlenmeyer flask, pour 20 ml of 0.01 M potassium iodate solution. Add 3 g of KI and swirl the flask until complete dissolution of the solid.
3. Add 3 ml of 6 M HCl; solution will turn dark-brown.
4. Into the same flask, add 5 ml of Fruit Fresh previously prepared solution. You may notice a light decolorization of the dark-brown liquid due to the reduction of I_2 to I^-.
5. Fill a burette with sodium thiosulphate standardized solution over the zero mark; discard part of the liquid into a waste beaker. Record the actual volume of the solution in the burette. This is going to be your *titrant solution.*
6. Place the flask underneath the burette and start the titration; continuously swirl the flask while adding the titrant. In case, wash the walls of the flask with distilled water.
7. As soon as the liquid in the flask becomes clearer, getting to a yellowish color, slow down the titration. Continue adding titrant solution until you get a light yellow liquid.
8. At this point, add 10-15 drops of starch solution into the flask and swirl it. Solution will turn dark-purple.
9. Continue adding the titrant solution until complete decolorization of the liquid in the flask. That is your endpoint.
10. Record the added volume of sodium thiosulphate solution

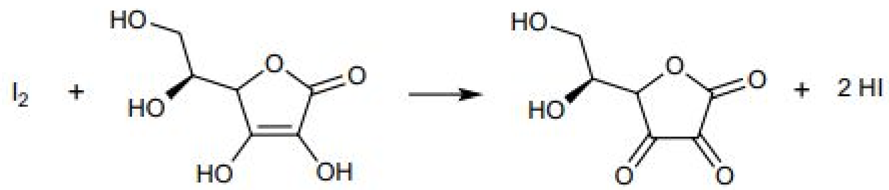
Sequence of colors you will see during the process is the same as figure 4.

In order to determine the concentration of the Vitamin C in the solution, consider the reactions involved in the whole process:

Reaction 1: First reaction takes place between iodate (IO^{3-}) with excess of iodide (I^-) in acid environment. Iodine (I_2) is produced. The solution turns dark-brown.

$$IO_3^- + 5\,I^- + 6\,H^+ \rightarrow 3\,I_2 + 3\,H_2O$$

Reaction 2: In presence of Vitamin C, part of the iodine I_2 is reduced to iodide I^- which is colorless compared to iodine. On the other hand, Vitamin C is oxidized to *dehydroascorbic acid (reagents ratio is 1:1)*:



Reaction 3: The rest of the iodine is titrated with sodium thiosulfate:

$$2S_2O_3^{2-} + I_2 \longrightarrow 2I^- + S_4O_6^{2-}$$

Now, look at these question and calculations; they will be useful to complete this second and last step.

Let's suppose that the concentration of KIO_3 is 0.0100 M and the actual concentration of $Na_2S_2O_3$ titrant solution is 0.0472 M.

A. *Calculate of how many moles of KIO_3 there are in 20 ml of the 0.0100 M solution and how many moles of iodine are produced after the addition of KI and HCl to that solution (same as point D of Procedure 1)*
B. *After the execution from point 4 to 9 of the instructions, let's suppose we added 23.7 ml of titrant solution. How many moles of that did we actually add? Record this number.*
C. *Look at **Reaction 3** and at its stoichiometry; figure out how many moles of iodine have been titrated by sodium thiosulphate.*
D. *Therefore, calculate how many moles of iodine have reacted with Vitamin C (Hint: consider the total number of moles of iodine calculated in point B).*
E. *Look at **Reaction 2**; what is the mole ratio between Vitamin C and iodine? By knowing that, figure out how you can use that number in order to calculate the amount of Vitamin C in the solution you have analyzed.*

F. *(Optional) - since you have determined the amount of Vitamin C in 5 ml of the Fruit Fresh stock solution, how many grams are there in that?*

Table 2 - *Report all data from calculations below.*

Trial	Volume of $Na_2S_2O_3$ (ml)	Concentration of $Na_2S_2O_3$ (M)	Moles of $Na_2S_2O_3$ added	Moles of iodine in solution	Moles of iodine titrated	Residual moles of iodine	Amount of Vitamin C (g)	Amount of Vitamin C (g) - average value
1								
2								
3								

DISPOSAL NOTES: All titrated solutions have to be collected into a waste beaker and diluted with plenty of water. Let the solution sit for 30 minutes in order to make sure there is no iodine left (in case the liquid turns dark-purple, add either some sodium thiosulphate or Fruit Fresh until complete decolorization). After that, the mixture can be poured down the drain.

QUESTIONS:

1. Write the sequence of reaction which Vitamin C undergoes during its titration process (structural formulas are not necessary. Just identify their names).
2. Explain why as soon as you add the acid to the KIO_3 - KI solution, that it turns dark-brown.
3. At the endpoint, a standardization of a sodium thiosulphate solution requires 24.2 ml of it in order to reduce all the iodine contained in a 25 ml of a 0.03 M solution of KIO_3 which has been previously added of KI and HCl. Calculate the actual concentration of sodium thiosulphate.
4. A label of a food supplement states that 2.5 g of that, contains 1.67% of Vitamin C. Determine how many grams of ascorbic acid there are in the supplement and its molar concentration if that were dissolved in 10 ml of water.
5. Into a 16.2 ml solution of KIO_3 with a concentration of 0.017 M, has been added a sample which contains 0.043 g of ascorbic acid. Explain why the solution could decolorize after its addition and calculate how many moles how iodine have been reduced.