DETERMINATION OF VITAMIN C IN A PRODUCE PROTECTOR

*Iodometric method - **TEACHER***

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 suggest 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]

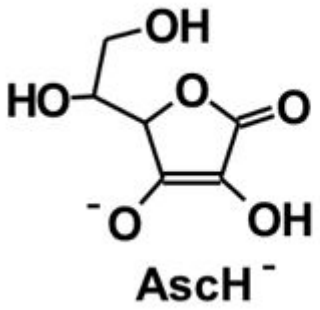

***Figure 1 -** ascorbate monoanion*

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

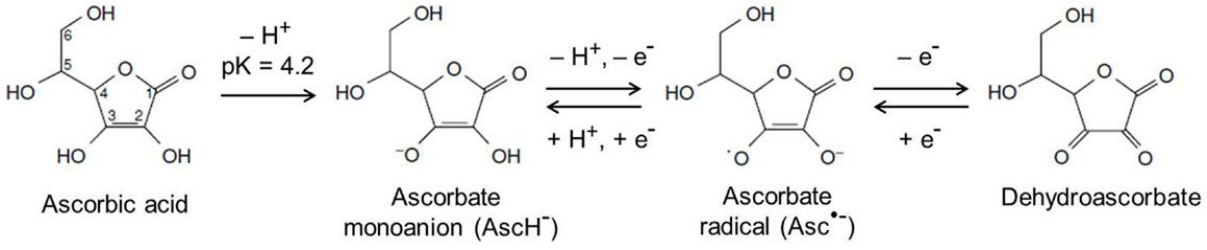

***Figure 2** - sequence of reactions which Vitamin C undergoes during its oxidation*

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 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

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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.*

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].

Figure 3: *equipment used for the determination of Vitamin C in a produce protector*

The Vitamin C clock reaction published in the Journal of chemical education[6] inspired this activity. That reaction is very impressive because of the sudden color change. Besides that, the chemistry behind the reaction is simple yet interesting. It is a useful demonstration since students really see when one of the reactions involved is over and the other one steps in. It is easily doable with household items (Vitamin C, hydrogen peroxide, starch and tincture of iodine).

Expanding on the idea of the Vitamin C clock reaction, we will determine the amount of Vitamin C as well, going over the methods that can be used to determine the concentration of that compound in several products (juices, candies etc). In order to determine the amount of Vitamin C by titration, *iodometry* steps in. In this kind of process, iodine I_2 is titrated with sodium thiosulfate through a redox reaction:

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

[5] *Advances in Nutrition*, Volume 5, Issue 1, 1 January 2014, Pages 16–18
[6] *J. Chem. Educ., 2002, 79 (1), p 41*

Neither the standardization of sodium thiosulfate nor the actual titration of iodine involve the use of dangerous chemicals (except for quite concentrated hydrochloric acid); in addition, the method is easy, quick and accurate.

Iodine is readily reduced by Vitamin C and by knowing the initial amount of iodine in the solution and that one reduced by sodium thiosulfate, it is possible to determine the content of Vitamin C in a specific product.

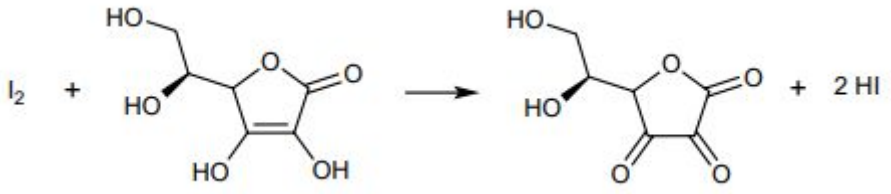
***Time required*:** one 50 minutes class period if the instructor prepares the standardized $Na_2S_2O_3$ solution.

Here are the reactions involved:

1. First reaction takes place between iodate (IO^{3-}) with excess of iodide (I^-) in acid environment with following formation of iodine (I_2). Solution turns dark-brown.

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

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)*:



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

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

By doing some common stoichiometric calculations, it is possible to determine the iodine titrated and therefore the amount of Vitamin C present in the sample you are analysing.
Equipment used is shown in Figure 3.

EXPERIMENTAL RESULTS

The first step is standardization of sodium thiosulphate. That involves preparation of a 0.01 M solution of potassium iodate, KIO_3 which is going to be our standard (*record the actual mass of potassium iodate in order to calculate the actual concentration after the addition of water*). KIO_3 is a primary standard so it only requires to be dried in a oven at 100 °C for a couple of hours.

A 0.05 M solution of sodium thiosulphate, $Na_2S_2O_3$ is prepared as well; while preparing that solution, add a teaspoon of $NaHCO_3$ (which will act as a preservative) to that. Store the solution in a dark-bottle to avoid light exposure. Solutions made like that, should be stable for 3-4 days.

When all the materials are ready, fill a burette with the $Na_2S_2O_3$ solution. Pour 20 ml of 0.01 M KIO_3 into an Erlenmeyer flask; add 3 grams of potassium iodide, dissolve it and add 2 ml of 6 M HCl solution. The reaction shown in point 1 will take place.
Start adding the $Na_2S_2O_3$ solution, drop by drop; the mixture in the flask will eventually become clearer, going from dark-brown to a yellowish kind of color. When the solution is light yellow, add a couple of drops of starch solution; you will get a dark-purple color.

Continue adding sodium thiosulphate until the solution is colorless. That means that titration process is over so keep track of the volume added.

Color changes happening during the process are 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*

Here are the calculations to do in order to get the actual concentration of $Na_2S_2O_3$ (*Suggestion - look at the reactions involved and at their stoichiometry*)

Calculations are as follows:

A. *Moles of KIO_3 in 0.54 g (Molar mass of KIO_3 is 214 g mol^{-1})*: 0.54 g / 214 g mol^{-1} = 0.00252 moles (KIO_3)
B. *Therefore, its concentration in 250 ml of water is*: 0.00252 moles (KIO_3) / 0.250 L = 0.0100 M
C. *In 20 ml of this solution*, we have 0.0100 M x 0.020 L = 0.0002 moles of KIO_3
D. *By looking at **Reaction 1,** after the addition of KI and HCl*, we can say that we get 0.0002 (KIO_3) x 3 = 0.0006 moles of I_2
E. ***Reaction 2** states that titrated moles of $Na_2S_2O_3$ are*: 0.0006 moles (I_2) x 2 = 0.0012 moles ($Na_2S_2O_3$)
F. *If we add 25.6 ml of sodium thiosulphate*, concentration of that will be: 0.0012 moles ($Na_2S_2O_3$) / 0.00256 L = 0.0472 M

In the following table you will find sample results collected for the standardization of sodium thiosulphate (*concentration of KIO_3 was 0.0103 M*):

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	20	25,5	0,0485	0,0485
2	20	25,6	0,0483	
3	20	25,6	0,0483	

As soon as we are aware of the concentration of the sodium thiosulphate solution, we can move on to the determination of Vitamin C in our produce protector. *Ball® Fruit Fresh* is used in this activity(in Italy this product is not available).

Titration process of Vitamin C in Fruit Fresh is basically the same as sodium thiosulphate standardization. 1.15 grams (a full plastic teaspoon) of produce protector is added to 100 ml of distilled water and vigorously stirred. The solution might need to be filtered if not clear. In an Erlenmeyer flask, pour 20 ml of 0.01 M KIO_3 then add 3 grams of potassium iodide. Swirl until dissolution and add 2 ml of 6 M HCl. Solutions will turn dark-brown. Add 5 ml of Fruit Fresh solution; you may notice a light decolorization of the dark-brown solution in the flask due to the reduction of iodine to iodide by Vitamin C. In any case, during the titration, color changes will be the same as those ones shown in Figure 4.

Start adding the sodium thiosulphate solution following the *exact same procedure* as for the standardization of that. After recording the added volume of titrating agent, you can do these calculations:

A. *Same as points A,B and C of $Na_2S_2O_3$ standardization section.* That will give you the moles of iodine in solution.
B. Let's suppose we added 23.7 ml of 0.0472 M $Na_2S_2O_3$ solution: that means we added 0.00112 moles of that.
C. Therefore we can say that the moles iodine titrated by sodium thiosulphate solution are 0.00112 moles ($Na_2S_2O_3$) / 2 = 0.00056 moles (I_2) (*see reaction 3*)
D. Moles of iodine that reacted with vitamin C are: 0.0006 moles - 0.00056 moles = 0.0004 moles
E. Since the reaction ratio between vitamin C and iodine is 1:1 (*see reaction 2*) and the molar mass of ascorbic acid is 176.12 g/mol, we can say that its amount in solution is 0.0004 moles x 176.12 g/mol = 0.007 grams (or 7 mg).

Sample results can be found in the table below (*concentration of KIO_3 was 0.0103 M*).

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	23,7	0,0485	0,00115	0,000618	0,000575	0,0000433	0,00762	0,00734
2	23,9		0,00116	0,000618	0,000580	0,0000384	0,00677	
3	23,7		0,00115	0,000618	0,000575	0,0000433	0,00762	

By looking at the table, the amount of Vitamin C in 5 ml of Fruit Fresh solution is 0.00734 grams; since we added 1.15 grams of produce protector in 100 ml of distilled water, it means that we have 0.147 grams of ascorbic acid in 1.15 grams of Fruit Fresh.

This method was tried with a defined amount of pure ascorbic acid from Sigma-Aldrich and results turned out to be consistent. You can experiment with other substances such as orange juice, vitamin supplements and other household items.

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 reactions which Vitamin C undergoes during its titration process (structural formulas are not necessary. Just identify their names).
   [Figure 2]
2. Explain why as soon as you add the acid to the KIO_3 - KI solution, that it turns dark-brown. ***[Formation of iodine]***
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. ***[0.185 M -using sample data]***
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. ***[0.042 g; 0.024 M]***
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. ***[0.000582 mol or 0.582 mmol]***