Estimation of ascorbic acid content of lemon

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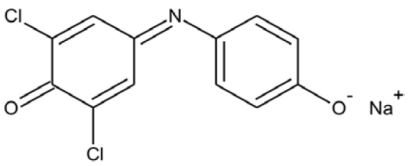
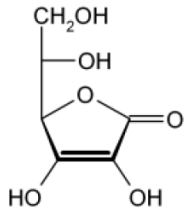
Background:

Ascorbic acid (vitamin C) is a water-soluble vitamin found in abundance in fresh vegetables and fruits; namely, citrus. Ascorbic acid plays important role in a number of organismic physiological processes, such as: collagen biosynthesis, iron absorption, immune response activation, wound healing and osteogenesis. Ascorbic acid is also a powerful antioxidant. However, an ascorbic acid excess can lead to gastric irritation. Further, oxalic acid (the metabolic product of ascorbic acid) can cause renal problems. Ascorbic acid can easily be degraded enzymatically, and even by atmospheric oxygen, making it a highly labile substance. Its oxidation can further be enhanced by excessive heat, light, and heavy metal cations. Thus ascorbic acid contents of food and beverages act as indicators of their quality; as it can vary during manufacturing, transport and storage of these food items.

The ascorbic acid content of a sample can be determined by redox titration method. The dichlorophenolindophenol (DCPIP) (a blue solution in oxidised state) solution is reduced by ascorbic acid to a colourless solution ($DCPIPH_2$). During this process, the ascorbic acid in-turn is oxidized to dehydro-ascorbic acid, which in acidic medium, gives a light pink colour.



(DCPIP)



(Ascorbic acid)

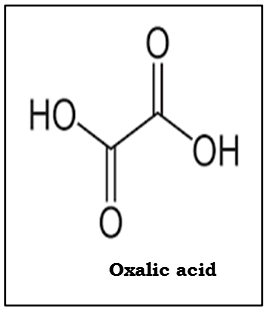
The aim of the experiment is to make a standard graph by titrating DCPIP against known concentration of ascorbic acid. Subsequently, this can then be used to estimate the ascorbic acid content of lemon juice, by using the standard graph.

Materials supplied to you:

1. 1mg/ml ascorbic acid solution (in centrifuge tubes)
2. Oxalic acid solution (in centrifuge tubes)
3. DCPIP solution in the burette
4. Lemon juice (undiluted) in a centrifuge tube
5. Conical flask (1)
6. Funnel (1)
7. Distilled water (in a beaker)
8. Grid (graph) paper
9. 1 ml syringe (2)

Procedure:

1. Using the ascorbic acid stock supplied to you, calculate the required volumes of ascorbic acid (from stock) and volume of oxalic acid (diluent), to make different working solutions of ascorbic acid as indicated in ' Table A' and write these volumes in the respective columns.



Oxalic acid

[Oxalic acid is usually associated with many anti-oxidant rich food materials and is known to protect oxidation of these antioxidants. Oxalic acid is the metabolic product of Vitamin C. However, excess of oxalic acid in the body may lead to formation of kidney stones]

2. For the first set '1' draw required volume of ascorbic acid and oxalic acid as per table A and pour them into the conical flask. Mix well.
3. Fill the burette with DCPIP solution.
4. Titrate till appearance of light pink colour.
5. Record the reading in the space provided.
6. Wash the conical flask and repeat step 2 to 6 for each set from 2-6. Also repeat the titration step for the lemon juice sample.
7. Plot a standard graph by taking volume of DCPIP on the X axis and concentration of ascorbic acid on the Y axis. [Do not forget to label the axes].

8. From the standard graph, determine the concentration of ascorbic acid in the lemon juice.

Table A

SN	Conc. Of ascorbic acid (µg/ml)	Volume of ascorbic acid from stock (ml)	Volume of oxalic acid (ml)	Final volume (ml)	Volume of DCPIP solution consumed
1	0			1	
2	0.2			1	
3	0.4			1	
4	0.6			1	
5	0.8			1	
6	1.0			1	
Lemon Juice		-	-	1	

Answer the following questions:

a. If one single lemon gives 2 ml lemon juice, calculate the total amount of ascorbic acid content of the lemon.
b. If the lemon weighs 10g then calculate the ascorbic acid content per gram wet weight of the lemon.
c. Give one liner reason for the use of oxalic acid as a diluent in the present experiment. What will happen if water is used as a diluent?

(Standard answers)

a. From the standard graph the ascorbic acid concentration can be determined in the lemon juice (say Z µg/ml). So the total ascorbic content in the lemon will be 2xZ µg.
b. Ans: 2xZ/10g (unit µg/g)

c. Oxalic acid protects spontaneous oxidation of ascorbic acid. Water will promote oxidation of ascorbic acid. [If ascorbic acid is diluted in water, it will form oxalic acid immediately].