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ASCORBIC ACID

VITAMIN C

Quantitative Assay

Redox Titration

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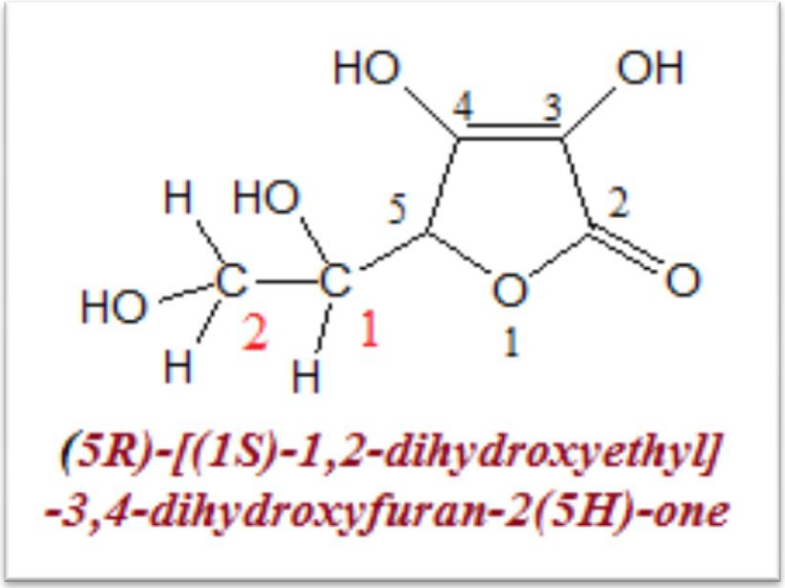
L – Ascorbic Acid :

- Chem. formula, $C_6H_8O_6$
  M.Wt, 176.13 gm /mol
- **It is a naturally occurring organic compound.**
- **White to slightly yellowish crystals or powder.**
- **It dissolves well in water to give mildly acidic solutions.**
- **In the dry state, it is reasonably stable to air.**
- **In solution, it's rapidly oxidized in the presence of air , the reaction is catalyzed by traces of some metals especially copper.**
- **If the medium is alkaline on exposure to air and light, it gradually darkens.**



(5R)-[(1S)-1,2-dihydroxyethyl] -3,4-dihydroxyfuran-2(5H)-one

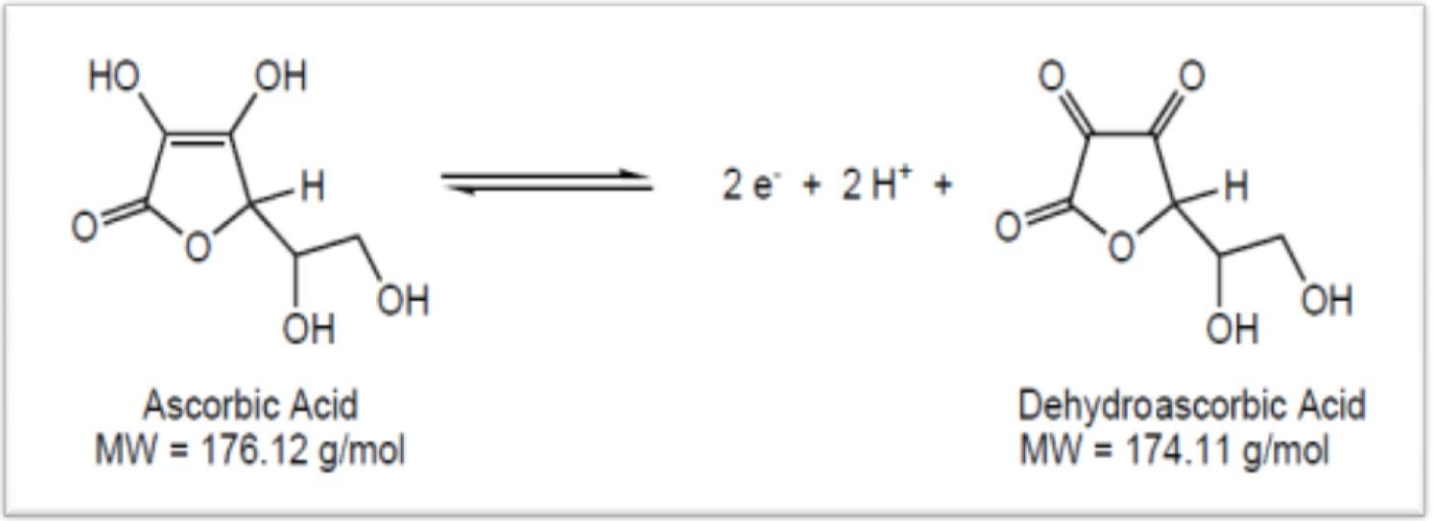
Theoretical aspect :

The determination of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical, clinical & food application. A large number of methods have been reported for the determination of ascorbic acid.

Chemical methods have replaced almost entirely the earlier biological methods since they are :

1- **Less time consuming.**
2- **More precise.**
3- **Less expensive.**

Most chemical methods are based on the reducing capacity of ascorbic acid (antioxidant) being readily oxidized to dehydroascorbic acid.



Name of experiment : **ASSAY OF ASCORBIC ACID**

Aim of experiment : Determination of the amount of Ascorbic acid in an unknown sample by Redox titration.

Procedure :-

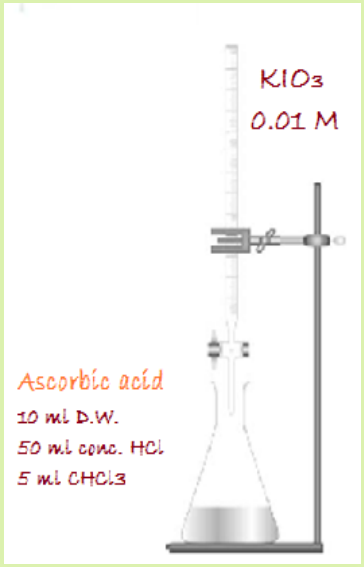
1- In a ***glass stoppered - iodine flask***, dissolve the unknown powder of ascorbic acid in 10 mL D.W., then add 50 mL of concentrated HCl

2- Cool with constant swirling till ascorbic acid dissolves .

3- Run in 30 mL of M/100 KIO_3 solution from a burette , cool , add 5 mL of $CHCl_3$.

4- Stopper the flask and shake vigorously.

5- Continue titration alternatively adding small volume of M/100 KIO_3 & shake vigorously until the layer of $CHCl_3$ is just discharged, (colorless) .



6- Calculate the weight of ascorbic acid by using the chemical factor .

Discussion:-

In our analysis today ascorbic acid is titrated with 0.01 M KIO_3 primary standard solution under strongly acidic medium.
Potassium iodate is a powerful oxidizing agent , but the course of the reaction is governed by the conditions under which it is employed.

KIO_3 solution under Strongly acidic medium

$$KIO_3 + HCl \longrightarrow HIO_3 + KCl$$

Potassium iodate — *Iodic acid*

Ascorbic acid reduces the iodate into iodide.

$$6\,C_6H_8O_6 + 2\,HIO_3 \rightleftharpoons 6\,C_6H_6O_6 + 2\,HI + 6\,H_2O \quad \text{---- 1}$$

Ascorbic acid — *Iodic acid* — *Dehydro-ascorbic acid*

Iodide is oxidized by more iodate to free iodine

$$5\,HI + HIO_3 \longrightarrow 3\,I_2 + 3\,H_2O \quad \text{---- 2}$$

Iodine

In Andrew's Condition, HCl > 5 M

$$3\,I_2 + 3\,HCl \longrightarrow 3\,ICl + 3\,HI \quad \text{---- 3}$$

Iodine monochloride Colorless

Summation of eq. 2 & 3

$$2\,HI + HIO_3 + 3\,HCl \longrightarrow 3\,ICl + 3\,H_2O \quad \text{---- 4}$$

Hydroiodic acid — *Iodic acid*

Summation of 1 & 4

$$6\,C_6H_8O_6 + 3\,HIO_3 + 3\,HCl \longrightarrow 6\,C_6H_6O_6 + 9\,H_2O + 3\,ICl$$

Division on 3 gives:

$$2\,C_6H_8O_6 + HIO_3 + HCl \longrightarrow 2\,C_6H_6O_6 + 3\,H_2O + ICl$$

2 moles *1 mole*

Chemical Factor:

$$2\ C_6H_8O_6 + HIO_3 + HCl \longrightarrow 2\ C_6H_6O_6 + 3\ H_2O + ICl$$

2 moles 1 mole

2 Moles of Ascorbic Acid ≡ 1 Mole of KIO_3 standard solution
Wt. of Asc. A. = No. of moles * M.wt ≡ 1L of 1 M KIO_3 std. solution
Wt. of Asc. A. = 2 moles * 176.13 g/mol ≡ 1000 mL of 1 M KIO_3 std. solu.
Wt. of Asc. A. = 352.26 g / 100 ≡ 1000 mL of 0.01 M KIO_3 std. solu.
Wt. of Asc. A. = 3.5226 g / 1000 ≡ 1 mL of 0.01 M KIO_3 std. solution
Wt of Ascorbic Acid = 0.0035226 g ≡ 1 mL of 0.01 M KIO_3 std. solu.

Each 1 mL of M/100 KIO_3 is equivalent to 0.003523 g of ascorbic acid.

Notes:

Iodate solutions are best expressed in terms of molarity instead of the more usual normality, since KIO_3 solution is often used in the titration of solution containing both iodide and free iodine. The equivalence of KIO_3 in it's reaction with KI defers from it's equivalence when reacted with the free iodine.

$$IO_3^- + 6\,H^+ + 6\,e^- \rightleftharpoons I^- + 3\,H_2O \qquad Eq.w.\ of\ KIO_3 = M.wt/6$$

$$IO_3^- + 5\,I^- + 6\,H^+ \longrightarrow 3\,I_2 + 3\,H_2O \qquad Eq.w.\ of\ KIO_3 = M.wt/5$$

$$IO_3^- + 2\,I_2 + 6\,H^- \longrightarrow 5\,I^+ + 3\,H_2O \qquad Eq.w.\ of\ KIO_3 = M.wt/4$$

A few milliliters of an immiscible solvent (Chloroform) were added to the solution being titrated contained in a glass - stoppered iodine flask. Chloroform serves as a solvent for iodine & as an indicator , so shaking vigorously to extract the free iodine, I_2 , into the $CHCl_3$ layer which will be stained violet due to the presence of the free iodine.
The end point is marked by the disappearance of the last trace of violet color (due to free iodine , I_2) from the solvent ($CHCl_3$) ; iodine monochloride, ICl, is not extracted & imparts a pale yellowish color to the aqueous phase.

The Advantage of this method :

Is that the extraction end point is very sharp.

The disadvantage of this method:

Is that the inconvenience of vigorous shaking with the extraction solvent in a glass_stoppered iodine flask after each addition of the reagent near the end point.

In Andrew's titration, the medium must be strong acidic , since in moderate & low acidity the reaction between KIO_3 and the reducing agent stops at the stage when the iodate is reduced to iodine, (The reaction is not completed).

$$IO_3^- + 6\,H^+ + 6\,e^- \rightleftharpoons I^- + 3\,H_2O$$

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

While in strong acidic medium, HCl at or above *5 M*, (Andrew's Condition), the free iodine is further reduced to iodine monochloride.

$$I_2 + HCl \longrightarrow \underset{\text{Iodine monochloride}}{ICl} + HI$$

Post Lab Exercises:

1- For each of the following molecules;
Write the molecular formula, Draw the chemical structure & Mention the oxidation state of iodine atom in the molecule:

a- Iodic acid.
b- Potassium iodate.
c- Hydroiodic acid.
d- Potassium iodide.
e- Free iodine.
f- Iodine monochloride.

2- How could you prepare 1 L of 0.01 M KIO_3 standard solution knowing that the molar mass of KIO_3 is 214 g/mol .

3- Prepare a solution of KIO_3 from a 0.25 M primary standard solution , using a 10 mL volumetric pipette & a calibrated 250 mL volumetric flask & Find the molarity of the prepared solution .

4 - A 1.49 g sample of ascorbic acid powdered tablets was dissolved in a 100 mL calibrated volumetric flask, A 10 mL aliquot was taken with a calibrated pipette & required 39.4 mL of a 0.01 M KIO_3 standard solution. Calculate the % assay of the sample.

Hint:

%assay = (Wt. of vit. C in sample) / (Wt. of powdered tablets) x 100%

5- Determine the sample weight needed for a 35 mL titration with 0.009 M KIO_3, knowing that the % assay was 75% ?

6- The amount of vitamin C in orange juice was determined by oxidizing the ascorbic acid to dehydroascorbic acid with KIO_3, under Andrew's condition.
A 5 mL sample of filtered orange juice required 50 mL of 0.01 M KIO_3.
Report the concentration of ascorbic acid in mg / 100 mL.

7 - What is the purpose of using the following in our assay experiment:

a- $CHCl_3$?

b- Concentrated HCl ?

c- Glass – stoppered iodine flask ?

8 - Describe the color changes observed throughout the titration and explain them with the aid of equations.

Give, also, the overall equation of the reaction.

References :

* J . Mendham , R . C. Denney , J . D. Barnes , M . Thomas , *Oxidation with potassium iodate* , **Vogel's Textbook of Quantitative Chemical Analysis,** 6th edition, 2000 .

* Samira Finjan Hassan , Amer Nadem , May Mohammed Jawad , *Assay of Ascorbic Acid* , **A Laboratory manual on Practical Medical Chemistry for 4th year students,** University of Baghdad , College of Pharmacy , Department of Pharmaceutical Chemistry , 2010 .