ESTIMATION OF L-ASCORBIC ACID BY REDOX TITRATION METHOD

Principle: The method depends on the stoichiometric reduction of the dye (2,6-dichlorophenol indophenol) to a colourless compound by L-ascorbic acid (vitamin C). The titration is conducted in the presence acetic acid and metaphosphoric acid solution. Metaphosphoric acid solution is used to inhibit aerobic oxidation of ascorbic acid.

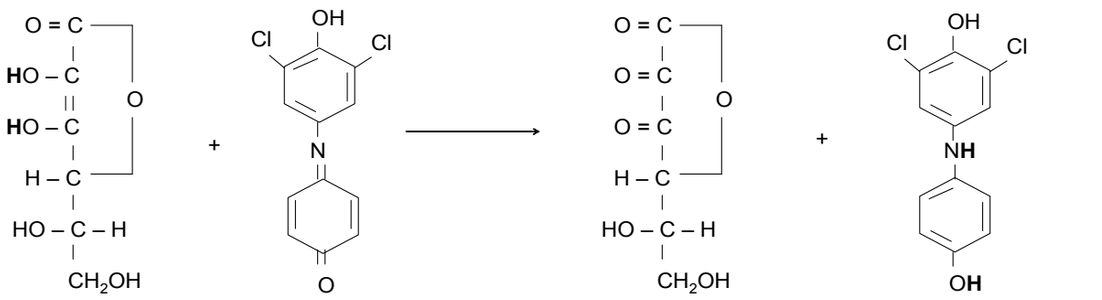
Reagent preparation:

1. **Dye solution**: 260 mg of dye (2,6-dichlorophenol indophenol) and 210 mg sodium carbonate dissolved in 1000 ml of distilled water.
2. **Metaphosphoric acid solution:** 30 gm of metaphosphoric acid [$(HPO_3)n$] and 800 cc of glacial acetic acid diluted to 1000 ml of distilled water
3. **Standard L-ascorbic acid solution (5 mg%):** 50 mg of crystalline L-ascorbic acid was dissolved in metaphosphoric acid solution to make the volume to 1000 ml.

Standardization of dye: Take 5 ml of the standard ascorbic acid solution in a conical flask and the dye in a 50 ml burette. Titrate ascorbic acid with the dye. End point is denoted by the appearance of persistent pink colour. Calculate and express the strength of dye as mg ascorbic acid equivalent per ml of the dye.

Procedure: Follow the procedure as same as that done for the standardization of the dye with the ascorbic acid solution with unknown concentration. Express the concentration as mg percent (mg solute present in 100 ml of the solution)

Reaction:



Extraction and estimation of L-ascorbic acid from fresh plant sample:
Extraction: Take a weighed amount (5 g) of freshly collected sample in a beaker with 30 ml of metapsosphoric acid solution. Grind the sample in a wiring blender or in a mortar-pestle. Filter the mashed sample through a filter paper (Whatman No. 1) or centrifuge at 5000 rpm for 20 minutes. Collect the supernatant in a 100 ml volumetric flask. Repeat the extraction twice by adding 20 ml of metaphosphoric acid solution each time. Make the volume of the supernatant to 100 ml by adding metaphosphoric acid solution.

Estimation: Follow the same procedures for standardization of dye and estimation of L-ascorbic acid as were discussed above.

Experimental data -1 (For known ascorbic acid solution)

No. of observation	Vol. of L-ascorbic acid solution (ml)	Burette reading for the amount of dye required (ml)		Amount of dye required (ml)	Mean (ml)
		Initial	Final		
1.	**5**	**10**	**11.1**	**1.1**	**1**
2.	**5**	**11.1**	**12.1**	**1**	
3.	**5**	**12.1**	**13**	**0.9**	

Experimental data -2 (For unknown ascorbic acid solution)

No. of observation	Vol. of L-ascorbic acid solution (ml)	Burette reading for the amount of dye required (ml)		Amount of dye required (ml)	Mean (ml)
		Initial	Final		
1.	**5**	**12.2**	**14.2**	**2**	**2**
2.	**5**	**14.2**	**16.3**	**2.1**	
3.	**5**	**16.3**	**18.1**	**1.9**	

Calculation:

Standardization of dye:

Mean burette reading of standard dye = X ml
Mean burette reading of unknown dye = Y ml
Concentration of standard L- ascorbic acid (vitamin C) solution = 5 mg %

Here,
X ml standard dye ≡ 5ml L- ascorbic acid (vitamin C) solution ≡ Y ml unknown dye

100 ml of known L- ascorbic acid (vitamin C) solution contain = 5 mg of L- ascorbic acid (vitamin C)

∴ 1 ml of known L- ascorbic acid (vitamin C) solution contain = $\frac{5}{100}$ mg of L- ascorbic acid (vitamin C)

∴ 5 ml of known L- ascorbic acid (vitamin C) solution contain = $\frac{5 \times 5}{100}$ mg of L- ascorbic acid (vitamin C)

= 0.25 mg of L- ascorbic acid (vitamin C)

Concentration of ascorbic acid in unknown sample/fresh extract:

X ml standard dye required to oxidized ≡ 0.25 mg L- ascorbic acid (vitamin C)

∴ Y ml unknown dye required to oxidized ≡ $\frac{0.25 \times Y}{X}$ mg L- ascorbic acid (vitamin C)

= Z mg L- ascorbic acid (vitamin C)

5 ml unknown L- ascorbic acid (vitamin C) sample contained = Z mg L- ascorbic acid (vitamin C)

∴ 100 ml unknown L- ascorbic acid (vitamin C) sample contained = $\frac{Z \times 100}{5}$ mg L- ascorbic acid (vitamin C)

= P

∴ **Result:** The concentration of L-ascorbic acid in the supplied/collected sample was P mg% L- ascorbic acid (vitamin C)

Experimental data -1 (For known ascorbic acid solution)

No. of observation	Vol. of L-ascorbic acid solution (ml)	Burette reading for the amount of dye required (ml)		Amount of dye required (ml)	Mean (ml)
		Initial	Final		
1.	**5**	**10**	**11.1**	**1.1**	**1**
2.	**5**	**11.1**	**12.1**	**1**	
3.	**5**	**12.1**	**13**	**0.9**	

Experimental data -2 (For unknown ascorbic acid solution)

No. of observation	Vol. of L-ascorbic acid solution (ml)	Burette reading for the amount of dye required (ml)		Amount of dye required (ml)	Mean (ml)
		Initial	Final		
1.	**5**	**12.2**	**14.2**	**2**	**2**
2.	**5**	**14.2**	**16.3**	**2.1**	
3.	**5**	**16.3**	**18.1**	**1.9**	

Calculation of the sample result:

Standardization of dye:

Mean burette reading of standard dye = 1 ml
Mean burette reading of unknown dye = 2 ml
Concentration of standard L- ascorbic acid (vitamin C) solution = 5 mg %

Here,
1 ml standard dye ≡ 5ml L- ascorbic acid (vitamin C) solution ≡ 2 ml unknown dye

100 ml of known L- ascorbic acid (vitamin C) solution contain = 5 mg of L- ascorbic acid (vitamin C)

∴ 1 ml of known L- ascorbic acid (vitamin C) solution contain = $\frac{5}{100}$ mg of L- ascorbic acid (vitamin C)

∴ 5 ml of known L- ascorbic acid (vitamin C) solution contain = $\frac{5 \times 5}{100}$ mg of L- ascorbic acid (vitamin C)

= 0.25 mg of L- ascorbic acid (vitamin C)

Concentration of ascorbic acid in unknown sample/fresh extract:

1 ml standard dye required to oxidized ≡ 0.25 mg L- ascorbic acid (vitamin C)
∴ 2 ml unknown dye required to oxidized ≡ (0.25 X 2)/ 1 mg L- ascorbic acid (vitamin C)
= 0.50 mg L- ascorbic acid (vitamin C)

5 ml unknown L- ascorbic acid (vitamin C) sample contained = 0.50 mg L- ascorbic acid (vitamin C)
∴ 100 ml unknown L- ascorbic acid (vitamin C) sample contained = (0.50 X 100)/ 5 mg L- ascorbic acid (vitamin C)
= 10 mg L- ascorbic acid (vitamin C)

∴ **Result:** The concentration of L-ascorbic acid in the supplied/collected sample was 10 mg% L- ascorbic acid (vitamin C)