**QUANTIFICATION OF VITAMIN C**

**INTRODUCTION**:

Ascorbic acid otherwise known as Vitamin C is antiscorbutic. It is present in citrus fruits, gooseberry, bittergourd etc. in high amount. Generally, it is present in all fresh vegetables and fruits. It is water soluble and heat-labile vitamin. Due to its vast biological applications this vitamin has got both medical commercial importance. Hence, the knowledge of quantifying this vitamin in biological sample plays an important role. The method described below is easy, rapid and a large number of samples can be analyzed in a short time.

**Aim**

To estimate the amount of vitamin C in a biological sample.

**Principle:**

Ascorbic acid reduces the 2, 6-dichlorophenol indophenol dye to a colorless leuco- base. The ascorbic acid gets oxidized to dehydroascorbic acid. Though the dye is a blue-coloured compound, the end point is the appearance of pink colour. The dye is pink colour in acidic medium. Oxalic acid is used as the titrating medium.

**Materials Required:**

**Glassware:** 100 ml Volumetric conical flask, Pipette, Burette, Burette stand and 100 ml standard flask.

**Preparation of Reagents:**

**DCIP Dye solution**:

Separately weigh 26 mg of the dye and 21 mg of sodium bicarbonate powder. Transfer both of them into a 100 mL volumetric flask, dissolve slowly and make up the volume to 100 ml with distilled water. Filter the reagent and use (store in dark colored glass bottle).

**Oxalic acid solution 4%** (w/v): Weigh 4g of oxalic acid crystals and dissolve in 100 ml of distilled water.

**Vitamin C stock solution (1 mg/ml)**:

Take 100 mg of vitamin C (Tablet), and dissolve it with 4% oxalic acid in 100 ml volumetric flask. Later, up the final volume to 100 mL with oxalic acid solution.

**Vitamin C working standard solution (0.1mg/mL):** Take 10 mL of vitamin C stock solution and makeup to 100 ml, with 4% oxalic acid in a 100 mL volumetric flask.

**Preparation of Test sample:** Follow the steps as shown in (Fig. 9.1), in brief take 10g of citrus fruit, juice into a 100 ml beaker. Transfer juice into 100 mL volumetric flask and bring the final volume to 100 ml with 4% oxalic acid solution. Dilute the citrus solution ten times with oxalic acid solution before titration. 5 mL of diluted juice is used for titration and the titration is repeated thrice and average value is obtained (V2).

**PROCEDURE:**

1. Transfer 5 mL of the vitamin C working standard solution into a 100 ml conical flask.
2. Later, add 10 ml of oxalic acid solution and mix well.
3. Titrate the contents against the DCIP solution, taken in a burette. Add dye solution drop by drop into the conical flask and mix the contents thoroughly.
4. The appearance of a pale pink colour from blue, indicates the endpoint (appears for few minutes). Repeat the procedure thrice to obtain an average value of dye consumed.

**Result:** The amount of vitamin C present in 100 grams of fruit is \_\_\_\_\_\_ mg

**Table : Quantitative Estimation of Vitamin C**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Sample for**  **Titration** | **Volume of Working Standard (ml)** | **Volume of Oxalic acid Solution (ml)** | **Volume DCIP dye consumed**  **(burette, ml)** | | |
| **initial** | **final** | **Initial - final** |
| 1 | Blank (5ml Distilled water) | - | 10 |  |  |  |
| 2 | Vit. C Standard  Titration 1 | 5 | 10 |  |  |  |
|  | Titration 2 | 5 | 10 |  |  |  |
|  | Titration 3 | 5 | 10 |  |  |  |
| 3 | Test sample  Titration 1 | 5 ml of  diluted  juice | 10 |  |  |  |
|  | Titration 2 | 5 | 10 |  |  |  |
|  | Titration 3 | 5 | 10 |  |  |  |

**Calculation:**

Vitamin C content (mg/100g sample) is calculated by the following equation:

Vitamin C (mg/100g) =

0.5mg x V1ml x 100ml x 10 x 100

V2ml 5ml weight of the sample (g)

Where,

V1 = Volume of the dye consumed for standard vitamin C (ml).

V2 = Avarage Volume of dye consumed for the sample (ml).

X 10 = Dilution factor.

Note: 10 g of citrus fruit is estimated to contain 0.5-1.0 mg of vitamin C