6.1 Determination of ascorbic acid by titration

**Principle**

Ascorbic acid may be determined by titration with the dye 2,6-dichlorophenolindophenol (DCP) which is reduced by the ascorbic acid to a colourless form.

**Apparatus**

- Volumetric flasks
- Pipettes
- Boiling tubes
- Burettes
- Macerator or liquidiser
- Potato peeler
- Centrifuge or funnel
- Muslin
- Thermometers
- Conical flasks
- Filter funnels and paper

**Reagents**

(a) *Fruit and vegetables*

*2,6-Dichlorophenolindophenol dye solution.* Dissolve 800 mg of the dye in about 500 ml hot, previously boiled and cooled distilled water. Filter if necessary and make up to 1 l with distilled water. Keep cool and in a dark bottle. Use within 7 days.

*Metaphosphoric–acetic acid mixture (MPA).* Dissolve 15 g metaphosphoric acid, HPO$_3$, in 40 ml glacial acetic acid and 200 ml water. Dilute to 500 ml and filter. This will last in a refrigerator for about 7 days.
Standard ascorbic acid solution. Dissolve 200 mg L-ascorbic acid in 10 ml MPA solution and make up to 100 ml with distilled water. Dilute 1 ml to 100 ml with distilled water and store in a refrigerator. This is solution A and contains 2 mg ascorbic acid per 100 ml.

Sand

(b) Milk

2, 6-Dichlorophenolindophenol dye solution. Dissolve 0.125 g of the dye in warm distilled water, filter and make up to 50 ml with distilled water. Dilute 5 ml to 100 ml with distilled water. Use within 7 days.

Metaphosphoric acid (MPA) solution. Dissolve 25 g MPA sticks in distilled water and make up to 500 ml. Filter and store in a refrigerator. Use within 7 days.

Standard ascorbic acid solution. Dissolve 100 mg of ascorbic acid in 5% m/v metaphosphoric acid solution and dilute to 500 ml in a volumetric flask using more metaphosphoric acid solution.

Procedure

A. Fruit and vegetables

(a) Standardisation of reagents. Standardise the dye solution by pipetting 5 ml of the ascorbic acid solution into a boiling tube and titrating rapidly with the dye solution. The blue dye is first decolorised by the ascorbic acid. Continue running in the dye, with shaking, until a faint pink colour persists for 15 s. Calculate the number of milligrams of ascorbic acid that are equivalent to 1 ml of dye solution. Since ascorbic acid solutions are unstable, this standardisation should be carried out each day.

(b) Determination of ascorbic acid in fruit juice. Pipette 10 ml of the fruit juice into a conical flask and add 2 ml of the metaphosphoric–acetic acid mixture. If the juice is concentrated it may require dilution, e.g. pipette 20 ml into a 100 ml volumetric flask and make up to volume with metaphosphoric–acetic solution. Titrate 10.00 ml against the dye as before.

(c) Determination of ascorbic acid in fruit and vegetables. Weigh out 10 or 20 g of the fruit or vegetable (depending on the amount of ascorbic acid likely to be present) and either:

(i) Grind it using a pestle and mortar with a little clean sand and 10 to 20 ml metaphosphoric–acetic acid mixture. This mixture will reduce oxidation