

Ascorbic Acid Injection IP

Experiment No. 12

Date: _____

Aim : To prepare and submit 2 ampoules, each containing 2 ml of ascorbic acid injection IP.

Synonyms : Vitamin C injection, L-Ascorbic acid injection.

Formula : As per IP

| Ingredients | Official formula | Working formula | Overages |
|--------------------------|------------------|-----------------|----------|
| Ascorbic acid | 25.00 g | | 3.75 g |
| Sodium bicarbonate | 14.58 g | | — |
| p-chloro meta cresol | 0.1 g | | — |
| Water for injection q.s. | 100 ml | | — |

Manufacturer's Definition (IP) : Ascorbic acid injection is a clear, colourless, sterile solution of ascorbic acid or sodium ascorbate prepared with the aid of sodium hydroxide or sodium carbonate or sodium bicarbonate in water for injection.

Standards (IP) : Ascorbic acid injection contains not less than 95.0 per cent and not more than 115.0 percent of the amount of ascorbic acid, $C_6H_8O_6$.

Usual Strength : 0.5 g in 2 ml (25% w/v of ascorbic acid).

Type : Monophasic liquid dosage form—solution.

Theory

Ascorbic acid closely resembles monosaccharides in structure. It is a strong antioxidant (reducing agent). Ascorbic acid in solution is rapidly oxidized in air and alkaline media. L-ascorbic acid undergoes oxidation to form dehydro ascorbic acid and this reaction is reversible. Both ascorbic acid and dehydro ascorbic acid are biologically active. On hydration, dehydroascorbic acid is irreversibly converted to 2,3-diketo-gulonic acid, which is inactive.

Ascorbic acid is freely soluble in water. Ascorbic acid solutions exhibit maximum stability at about pH 5.4. As it undergoes oxidation in acidic pH, sodium bicarbonate is added to adjust the pH to 5.7 (limit is 5.5 to 6.4).

Sterilisation by autoclaving is not possible owing to its decomposition. Hence aseptic filtration and filling is advisable. However, steaming of ampoules is done terminally. To prevent decomposition of ascorbic acid during steaming, a preservative is added.

Oxidation of ascorbic acid is rapid in the presence of copper. Hence the solution of ascorbic acid should not be prepared in copper vessels. In order to avoid atmospheric oxidation, the injection is filled and maintained under nitrogen environment. Alternatively, 0.5% sodium hydrosulfite is used as an antioxidant. However, 15% overages are added to ascorbic acid injection to maintain shelf life of preparation.

Ascorbic acid gradually darkens on exposure to light. A slight colour developed during storage does not impair the therapeutic activity. To avoid excessive pressure inside the ampoules, they should be stored in the refrigerator and not allowed to stand at room temperature before use.

Intramuscular injection of ascorbic acid is preferred, but it may also be given subcutaneously or intravenously. Intravenously, it can be added to a large volume of a compatible diluent and infused slowly.

Ascorbic acid is compatible with substances such as dextran, dextrose, sodium chloride, fructose, amikacin sulphate, aminophylline, bleomycin sulfate, calcium gluconate, and chlorpromazine HCl. However it is incompatible with drugs such as oestrogens, bleomycin sulfate, chlorothiazide sodium, methenamine, phenylephrine hydrochloride, pyrilamine maleate, salicylamide, kanamycin sulfate, etc.

Apparatus and Chemicals

| | | |
|---------------------------|---|----------------------|
| Ampoules, 2 ml | 3 | Ascorbic acid |
| Beaker, 250 ml | 1 | Sodium bicarbonate |
| Beaker, 100 ml | 1 | p-chloro meta cresol |
| Measuring cylinder, 50 ml | 1 | Water for injection |
| Syringe with needle, 2 ml | 1 | |
| G-4 filter | 1 | |
| pH paper/pH meter | 1 | |

Procedure

Depending on the number of injections to be submitted, the working formula is calculated. A model calculation is given as an example.

Cleaning of ampoules : Three ampoules of Type I (or Type II) glass are cleaned thoroughly with detergent solution, then with tap water and finally with distilled water. The ampoules are kept in an inverted position in a beaker containing distilled water. The water is boiled for 15 minutes. The ampoules are removed and dried in a hot-air oven.

Preparation of ascorbic acid solution : Ascorbic acid solution is prepared as follows:

1. $\frac{2}{3}$ rd of the total volume of water is placed in a beaker.
2. Ascorbic acid is weighed and suspended in water with continuous stirring.
3. Sodium bicarbonate is slowly added with vigorous stirring. Effervescence is produced.
4. After subsiding the effervescence, remaining sodium bicarbonate is added. This process is continued till ascorbic acid completely dissolves.
5. Para chloro meta cresol is added and stirred to dissolve.
6. The pH is adjusted to 5.7.
7. The solution is filtered through G-4 filter.

Filling of ampoules : The syringe is rinsed with water for injection followed by rinsing with drug solution. The syringe is loaded with the drug solution. Air bubbles (if any) are removed. This can be achieved by tapping the syringe with finger, while holding it in perfectly vertical position. (After expelling the air, at least one drop of drug solution should be expelled. After this adjustment, the volume indicates the desired quantity of liquid in ml.) Then the drug solution is transferred into the ampoules slowly by inserting the needle up to the bottom of the container. Therefore, a needle larger than the length of ampoule is necessary. This prevents the rim (or neck) from contamination by drug solution and also prevents the charring of the solution.

Sealing of ampoules : Ampoules can be sealed by two methods, namely pull sealing method and tip sealing method. Proper and improper seals of ampoules are shown in Figure 13.

(a) **Pull sealing method :** Ampoules can be sealed by this method using twin-jet burner. LPG (liquefied petroleum gas) cylinder and oxygen gas cylinder are connected to the burner. Oxygen gas increases the heat of fusion of flame making tip sealing easier and faster. Sealing can be done by keeping tip of the ampoule in the flame while holding the ampoule in the left

hand. Grip the end of the ampoule with blunt nosed forceps held in the right hand. The tip of the glass is allowed to melt. The tip of the ampoule is pulled out when the tip turns red-hot (becomes soft). The tip is left in the flame for one or two seconds and removed. The tip should be smoothly and evenly rounded with a small flat glass in the center (Figure 13 a).

(b) **Tip sealing method :** Ampoules can be sealed by this method using Bunsen burner connected to LPG cylinder. The ampoule is rotated almost horizontally (slight inclination) with about 1 cm of the neck in the non-luminous flame. The ampoule is slowly withdrawn from the flame when the tip starts bending. Ampoule should be appropriately rotated to correct the bending. The resulting seal is elegant and very strong (Figure 13 d). This method of sealing requires considerable practice to perfect. In industries, usually 2 or 4 station tip sealing is done using a good flame system.

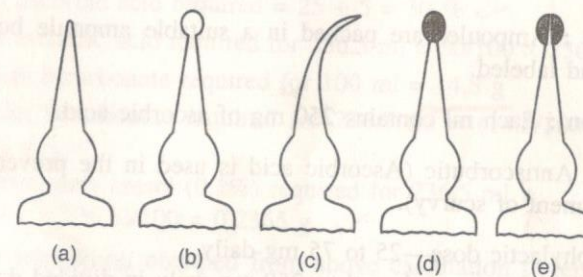


Figure 13. Proper and improper seals of ampoules

Problems encountered during sealing : Sealing of ampoules is associated with both pull sealing and tip sealing techniques.

1. **Charring of tip** is possible when organic substances present at the tip undergo decomposition during sealing. Therefore, the drug solution should never be allowed to come in contact with the neck at the time of filling or sealing.
2. **Fracture of the neck** of ampoules often occurs during sealing, if wetting had occurred at the time of filling. This is also possible if the drug solution comes in contact with hot tip immediately after sealing. Therefore, ampoule should not be inverted immediately after sealing.
3. **Bubble formation (bulbing) :** It is the process of formation of fragile bubbles at the tip. This is due to excessive heating of air and gases in the neck resulting in more expansion (Figure 13 b).

Wet glass at the neck increases the frequency of bubble formation.

4. *Sharp tips* : This is a problem with pull sealing method. This is due to pulling off the tip too vigorously. It results in the formation of fine sharp tips (Figure 13 c) that are easily broken and can give a painful prick when the ampoules are handled.
5. *Capillary formation* : This is a problem with tip sealing method (Figure 13 e). This can often be detected by a small depression at the tip.

The sealing of ampoules can be grossly checked by shaking the ampoules with right hand against palm of left hand. Improperly sealed ampoules leak out the contents that can be felt. However, sealing can be perfectly determined using leakage test.

Sterilization : The sealed ampoules are sterilised by steaming for 30 minutes.

Packing : Ampoules are packed in a suitable ampoule box (light-resistant) and labeled.

Composition : Each ml contains 250 mg of ascorbic acid.

Category : Antiscorbutic (Ascorbic acid is used in the prevention and the treatment of scurvy).

Dose : Prophylactic dose—25 to 75 mg daily.

Therapeutic dose—not less than 250 mg daily in divided doses.

Storage : Store in a cool and dark place not exceeding temperature of 25°C.

Auxiliary Label : Discard if any particulate matter is present.

Precaution : Since pressure may develop on long storage, precaution should be taken to wrap the container in a protective covering while it is being opened.

Route of Administration : Intramuscular route.

Date of Expiry : 2 years from the date of manufacture.

Quality Control :

1. *Particulate matter* : absent / present
2. *Leaking* : no / yes
3. *Tip* : sharp / round
4. *Charring* : absent / present

Report :

Example : Prepare and submit 100 ampoules of ascorbic acid injection IP each containing 2 ml. Give the necessary calculations and working formula.

Volume of contents in each ampoule = 2 ml ✓

Excess volume in each ampoule (overflow volume) = 0.15 ml ✓

Total volume required for each ampoule = 2.15 ml ✓

Number of ampoules to be submitted = 100

Number of extra ampoules required = 10

Total number of ampoules required = 100 + 10 = 110

Total volume of drug solution required = 2.15 × 110 = 236.5 ml ✓

Ascorbic acid required = 25% ✓

Overages added = 20%, i.e., $(20/100) \times 25 = 5\%$ ✓

Total ascorbic acid required = 25 + 5 = 30% ✓

Total ascorbic acid required for 236.5 ml = $30/100 \times 236.5 = 70.95$ g ✓

Sodium bicarbonate required for 100 ml = 14.8 g

Sodium bicarbonate required for 236.5 ml = $(14.8/100) \times 236.5 = 35$ g ✓

p-chloro meta cresol (0.1%) required for 236.5 ml = $(0.1 \times 236.5)/100 = 0.2365$ g ✓

The composition obtained from above calculation is reported in the below table.

| Ingredients | Working formula* |
|--------------------------|------------------|
| Ascorbic acid | 70.95 g |
| Sodium bicarbonate | 35.0 g |
| p-chloro meta cresol | 0.2365 g |
| Water for injection q.s. | 236.5 ml |

* for 110 ampoules

QUESTION BANK

1. Write formulation and method of manufacture of ascorbic acid injection (25% w/v) of 2 ml each.
2. Write the use of sodium bicarbonate in the preparation of ascorbic acid injection.