Industrial Pharmacy - I

Experiment No. 7

Aim: Preparation of Ascorbic Acid Injection

Objective

The aim of this experiment is to prepare a sterile aqueous solution of ascorbic acid suitable for parenteral administration, ensuring the correct concentration, pH, and sterility, and adhering to pharmaceutical quality standards.

Introduction

Ascorbic acid (Vitamin C) is a water-soluble vitamin with potent antioxidant properties. It plays a critical role in collagen synthesis, wound healing, immune function, and as a cofactor in enzymatic reactions. Deficiency of ascorbic acid results in scurvy, characterized by weakness, gum bleeding, and poor wound healing.

Therapeutic Uses:

- Treatment of Vitamin C deficiency
- Adjunct therapy in wound healing
- Antioxidant in critical illnesses or sepsis
- Supportive therapy in certain cancer or viral infections

Chemical Profile:

- **Chemical Name:** L-Ascorbic Acid
- **Molecular Formula:** $C_6H_8O_6$
- **Molecular Weight:** 176.12 g/mol
- **Appearance:** White to slightly yellow crystalline powder
- **Solubility:** Freely soluble in water, insoluble in ethanol and ether
- **Stability:** Sensitive to light, heat, and alkaline conditions; hence, preparation must be done under controlled conditions.

Parenteral administration is preferred when rapid vitamin C supplementation is needed, as oral absorption is limited at high doses.

Materials and Equipment

Materials

- Ascorbic acid (USP grade)
- Sterile distilled water for injection
- pH adjusting agents: Sodium bicarbonate or Sodium hydroxide (to adjust pH if needed)
- Preservatives (optional, e.g., benzyl alcohol 0.9% w/v for multi-dose vials)

Equipment

- Analytical balance
- Glass beakers, graduated cylinders, and volumetric flasks
- Magnetic stirrer
- pH meter or pH indicator strips
- Sterilizing-grade filter (0.22 µm) for filtration
- Autoclave (121°C, 15 psi) if heat-stable
- Laminar airflow chamber for aseptic filling
- Sterile ampoules or vials for injection
- Hot air oven (optional for drying ampoules)

Formulation Example (100 mL Injection)

Ingredient	Quantity	Function
Ascorbic acid	1–2 g	Active pharmaceutical ingredient
Sterile distilled water	q.s. to 100 mL	Vehicle / solvent
Sodium bicarbonate/NaOH	q.s. to pH 3–4	pH adjustment
Benzyl alcohol (optional)	0.9 g	Preservative (for multi-dose vials)

Remarks:

- Typical parenteral concentrations: 1–2% w/v
- pH adjustment is critical; ascorbic acid is acidic (pH 2–3 in water), and too low a pH can cause vein irritation.

Theoretical Calculations

Step 1: Determine weight of ascorbic acid required

For 1% w/v in 100 mL:

$$\text{Amount required} = 1\% \times 100\ \text{mL} = 1\text{g}$$

For 2% w/v in 100 mL:

$$\text{Amount required} = 2\% \times 100\ \text{mL} = 2\text{g}$$

Step 2: pH Adjustment

- Ascorbic acid solutions are naturally acidic (pH 2–3).
- Slowly add sodium bicarbonate or NaOH to adjust pH to 3–4 for parenteral safety.
- Avoid excessive alkalinity, as it degrades ascorbic acid.

Procedure

Preparation of Solution

1. Accurately weigh the required amount of ascorbic acid.
2. Dissolve in 80% of the total volume of sterile distilled water using a magnetic stirrer.
3. Check pH using a pH meter. Adjust to 3–4 with dilute sodium bicarbonate or NaOH.
4. Make up to the final volume with sterile distilled water.
5. Mix gently to avoid oxidation.

Sterilization

- **Filtration (preferred for heat-sensitive solutions):** Pass the solution through a 0.22 µm sterilizing filter into a sterile container under laminar airflow.
- **Autoclaving:** Only if using heat-stable formulations and properly sealed containers (rarely used due to thermal degradation).

Filling and Sealing

- Fill sterile solution into ampoules or vials under aseptic conditions.
- Seal ampoules by flame or cap vials with sterile rubber stoppers.
- Label with drug name, concentration, batch number, and expiry date.

Quality Control Tests

1. **Appearance:** Clear, colorless to slightly yellow solution, free of particulates
2. **pH:** 3–4
3. **Sterility Test:** As per USP <71>
4. **Assay of Ascorbic Acid:** Titrimetric (using 2,6-dichlorophenolindophenol) or HPLC
5. **Pyrogen Test:** LAL assay or rabbit pyrogen test
6. **Particulate Matter:** According to USP <788>

Storage Conditions

- Store in tight, light-resistant containers to prevent degradation.
- Temperature: 2–8°C (refrigeration) is preferred for prolonged stability.
- Avoid exposure to air, light, and heat.
- Use freshly prepared solutions if possible.

Precautions

- Perform all steps under aseptic conditions to avoid microbial contamination.
- Avoid prolonged exposure to air, as ascorbic acid oxidizes rapidly.
- Adjust pH carefully; avoid over-alkalinization to prevent degradation.
- Use amber-colored vials to protect from light-induced decomposition.
- Label vials clearly, including concentration, preparation date, and expiry.

References

1. United States Pharmacopeia (USP 43-NF 38), Ascorbic Acid Injection Monograph.

2. Indian Pharmacopoeia (IP), 2020, Volume II.