Production Considerations (II)

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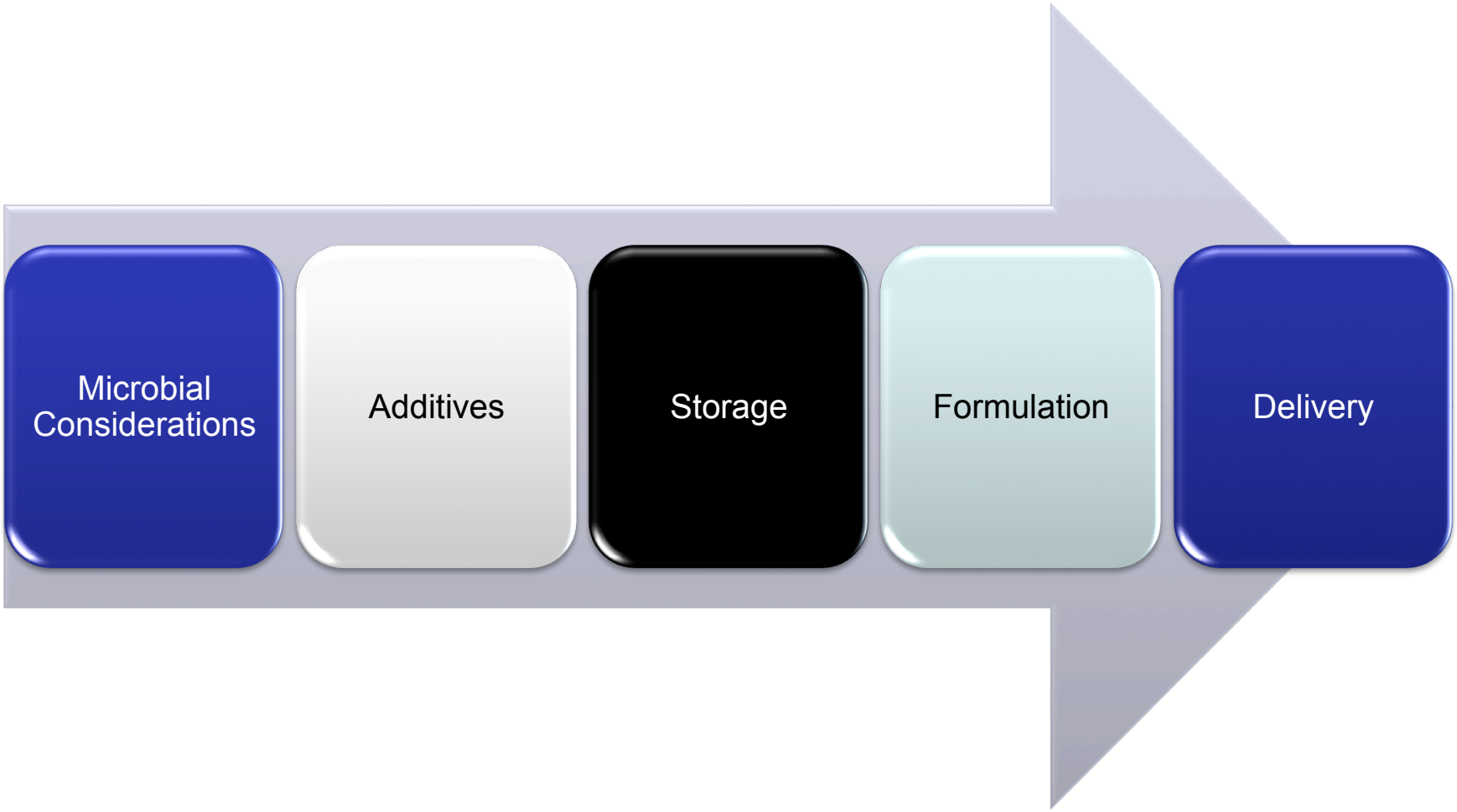
Objectives of this lecture

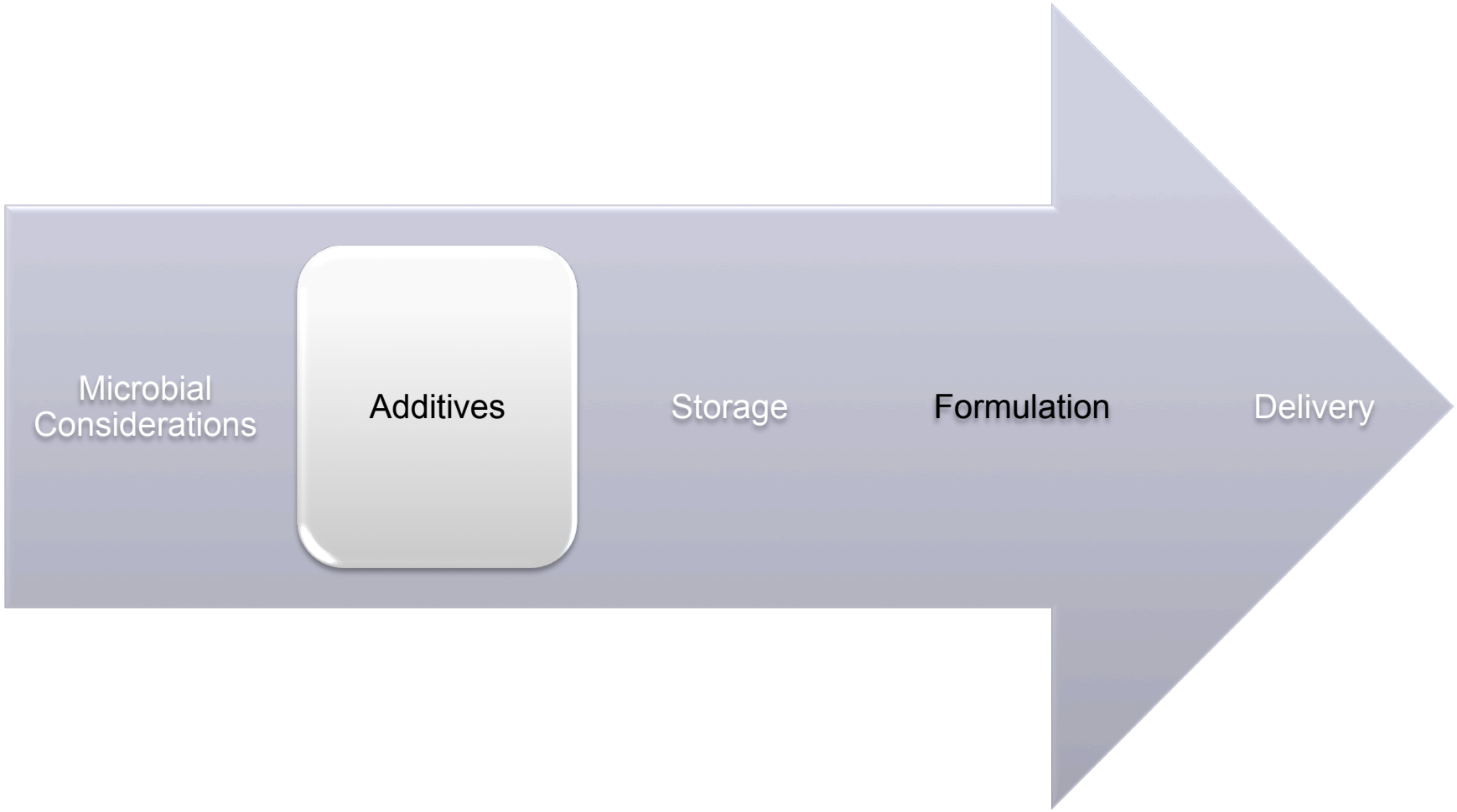
By the end of this lecture you will be able to:

1. Describe the problems associated with protein formulations
2. Numerate strategies to improve protein formulations
3. Understand the difficulty of scaling up pharmaceutical protein industry

Solving the problems



Solving the problems



Additives

1. Active ingredient
2. Solubility enhancer
3. Anti-adsorption/aggregation agent
4. Buffer components
5. Preservative/anti-oxidant
6. Lyoprotectant/cryoprotectant
7. Osmotic agents
8. Delivery systems

Solubility enhancer

- **Problem:**
  - Aggregation and precipitation especially with non-glycosylated proteins

- **Solution:**
  - Proper pH and ionic strength
  - Cationic amino acids (Lys and Arg)
  - Surfactants (e.g. SDS)

Anti-adsorption Anti-aggregation

- **Problem:**
  - Hydrophobic sites causes adhesion and adsorption to solid interfaces and leads to unfolding and aggregation

- **Solution:**
  - Proper pH and ionic strength
  - Surfactants (e.g. phospholipids and SDS)
  - Competitor protein (e.g. Albumin)

Buffer components

- **Problem:**
  - Protein solubility and stability depend to a great extent on the pH of the surrounding environment. Temporary change in the pH can cause aggregation

- **Solution:**
  - Add buffer components
  - Citrate (pH 3-7), acetate (pH 3-7), and phosphate (pH 7-11) buffers
  - Choose buffer systems that do not crystallize during freezing

Preservatives and Anti-oxidants

- **Problem:**
  - Oxidation occurs to (Met, Cys, Trp, Tyr, and His)
  - Contamination with microorganisms expecially in multiple-dosing dosage forms

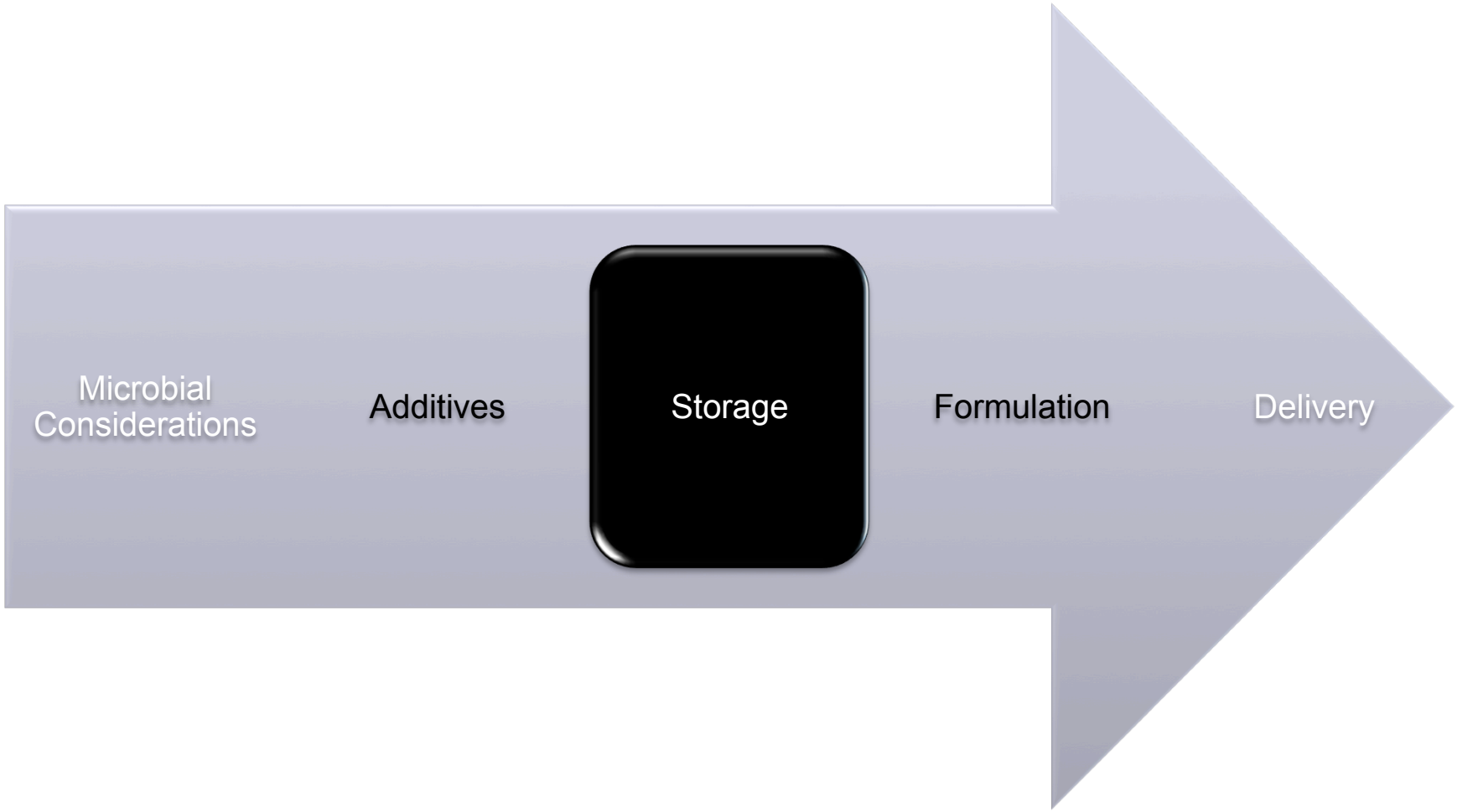
- **Solution:**
  - Replace oxygen in the vial with inert gas
  - Ascorbic acid
  - Preservatives at bacteriostatic concentrations (e.g. p-hydroxybenzoic acid and thimerosal “thiomersal”)

Osmotic Agents

- **Problem:**
  - Most proteins are given parenterally. Therefore, they must be administered as isotonic solutions. However, excipients used in this regard may influence protein structural stability

- **Solution:**
  - Sugars (e.g. sucrose) and polyhydric alcohols i.e. sugar alcohol e.g. glycerol and PEG improves protein stability through ***preferential exclusion***

Solving the problems



Storage

1. Aqueous solutions:

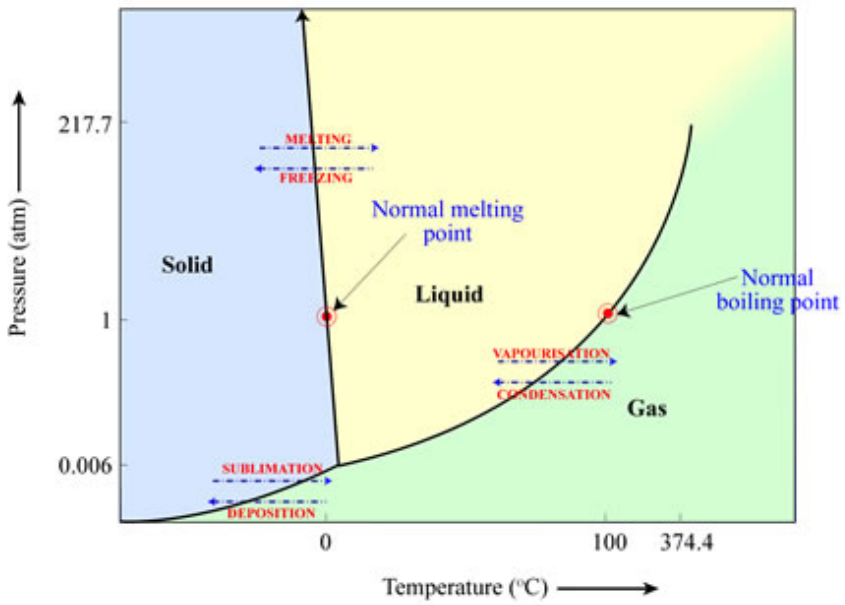
- Stability of protein solutions depends on pH, ionic strength, temperature, and stabilizers
- Smooth walled glass
- Air-tight container
- Dark

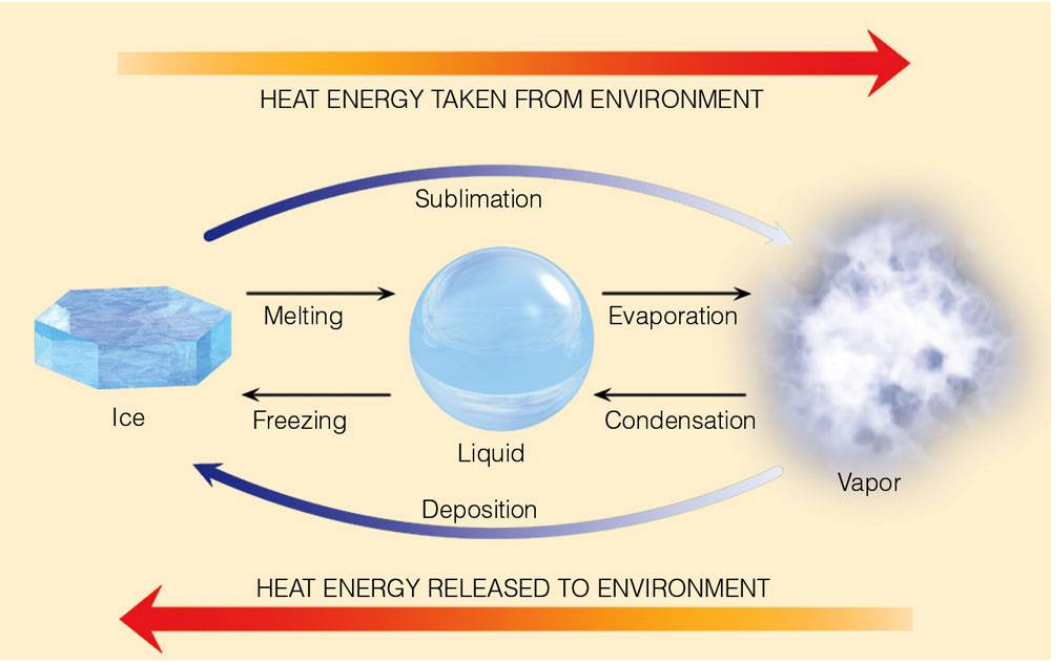
2. Freeze-dried form (Lyophilized)

3. Dried form in compact state (pills)

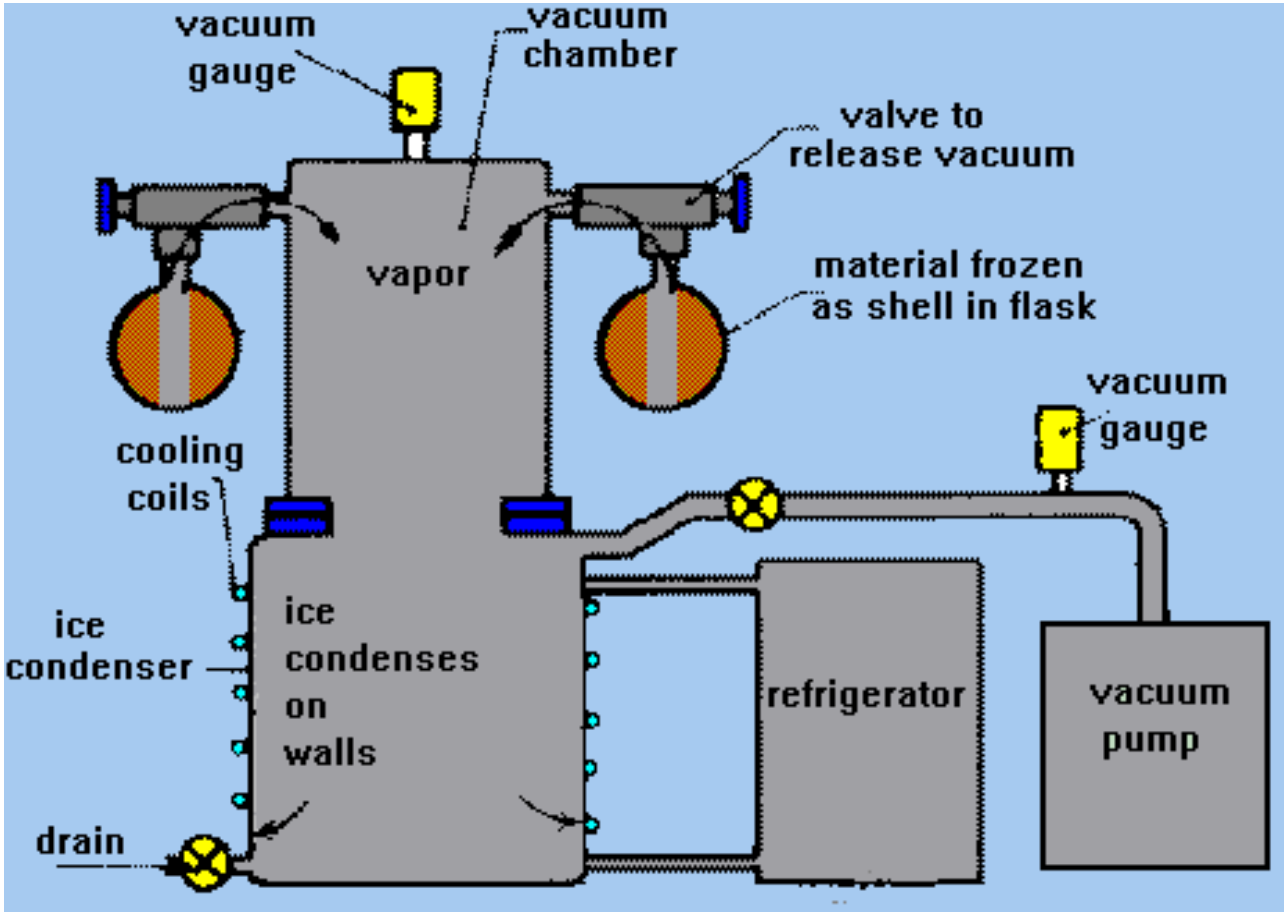
Freeze Drying

- Presence of water in the protein solution promotes chemical and physical degradation, which reduces the expected shelf life
- Freeze drying removes water through sublimation and not evaporation





Freeze Drying



Freeze Drying

Freeze Drying Steps

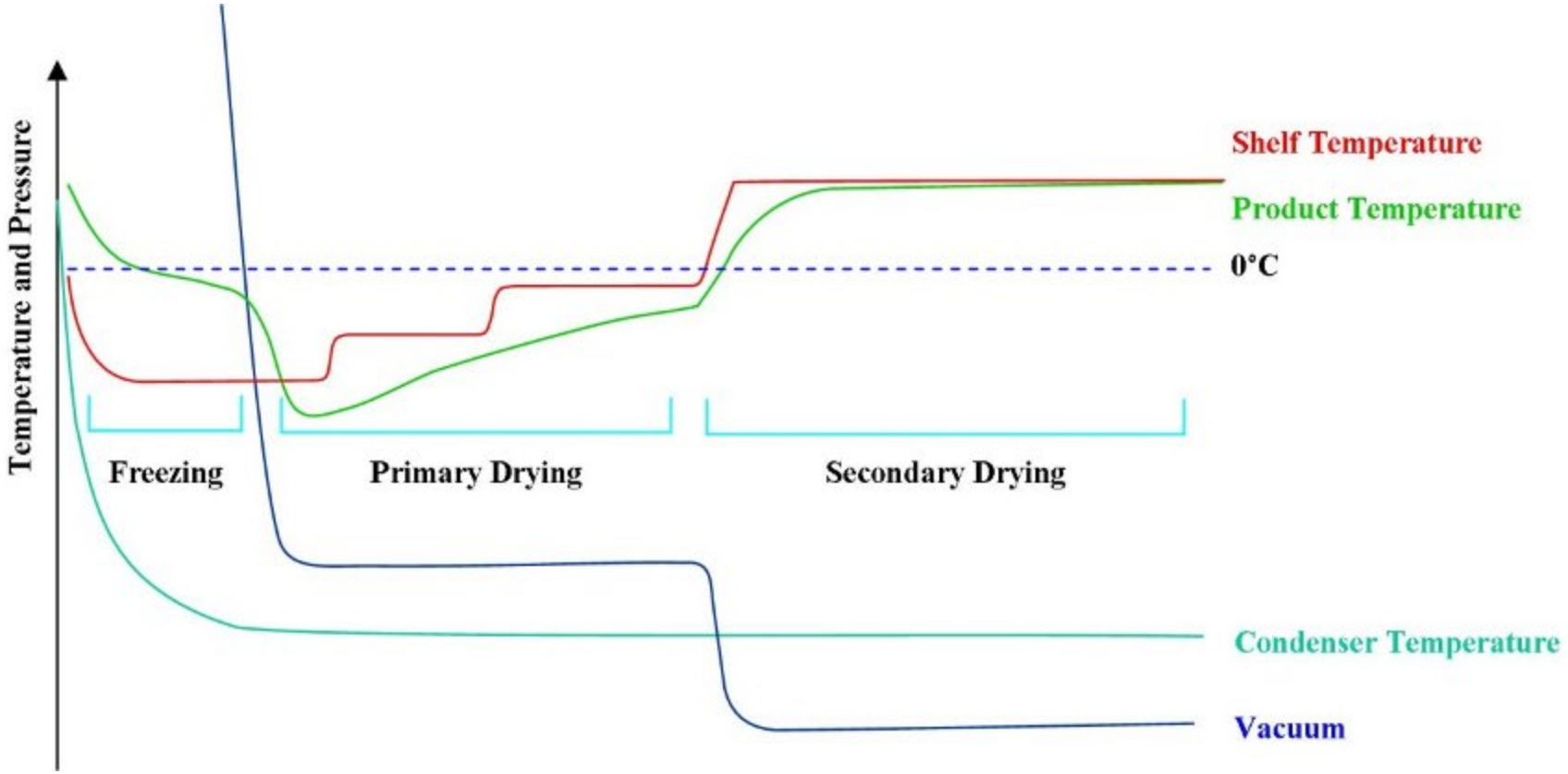
- **The freeze drying process consists of three steps:**
  1. **Freezing:**

     Crystallization of water molecules (bound and unbound to protein/excipients)
  2. **Primary drying:**

     Removal of unbound water molecules by sublimation
  3. **Secondary drying**

     Removal of protein/excipient bound water by sublimation

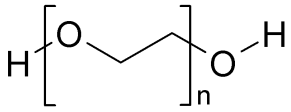
Freeze Drying Steps



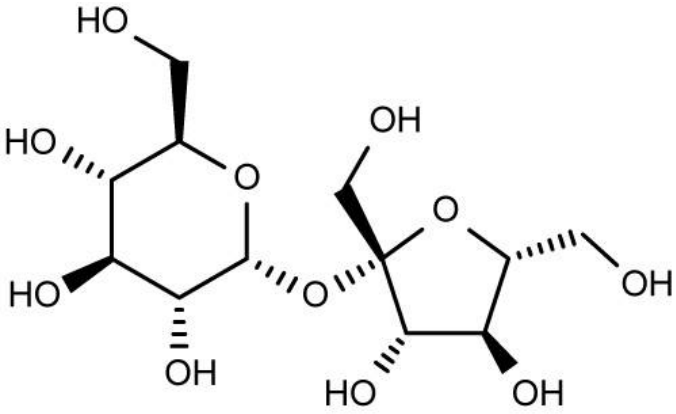
In absence of proper excipients, irreversible damage to the protein

Lyoprotectant/Cryoprotectant

- **PEG:**
  - Coats the protein
  - Not a very good stabilizer



- **Sucrose:**
  - Freezes the water molecules around the protein **(preferential exclusion)**
  - Also preservative above 60%



Lyoprotectants prevent over drying of proteins during freeze drying

You are now able to:

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- ✓ Numerate strategies to improve protein formulations
- ✓ Understand the difficulty of scaling up pharmaceutical protein industry