
College of Pharmacy
Fifth Stage

# Pharmaceutical Biotechnology

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## Lectutre 4
## Excipients Used in Parenteral Formulations of Biopharmaceuticals

# Excipients Used in Parenteral Formulations of Biopharmaceuticals

# Excipients in Biopharmaceuticals

- **As other dosage form, biopharmaceuticals also contain a number of excipients that are selected to serve different purposes.**
- **Our concern is that the biopharmaceuticals are a complex dosage form that required special consideration in formulation as follow:**

1. **Most of these product are designed for parenteral administration.**
2. **The nature of protein which can be considered as unstable product due to multiple ways of instability that turns the protein inactive.**
3. **The special processing included in formulation of biotech product such as aseptic preparations.**
4. **In addition, if the dosage form is designed for multiple injection system, this will add additional complexity to the dosage form.**

- **Both the choice of the excipient and its concentration are important. For instance, low concentrations of polysorbates may stabilize the protein , while higher concentrations may cause denaturation.**

# Protein Instability

## Proteins are unstable

**Chemical instability** ⟷ **Physical instability**

- Deamidation
- Oxidation
- Proteolysis (hydrolysis)
- Disulfide shuffling
- Racemization
- Beta elimination
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- Conformational
  - Unfolding
  - Misfolding
- Colloidal
  - Aggregation
  - Precipitation
- Adsorption

# Example of Excipients in Marketed Biopharmaceuticals

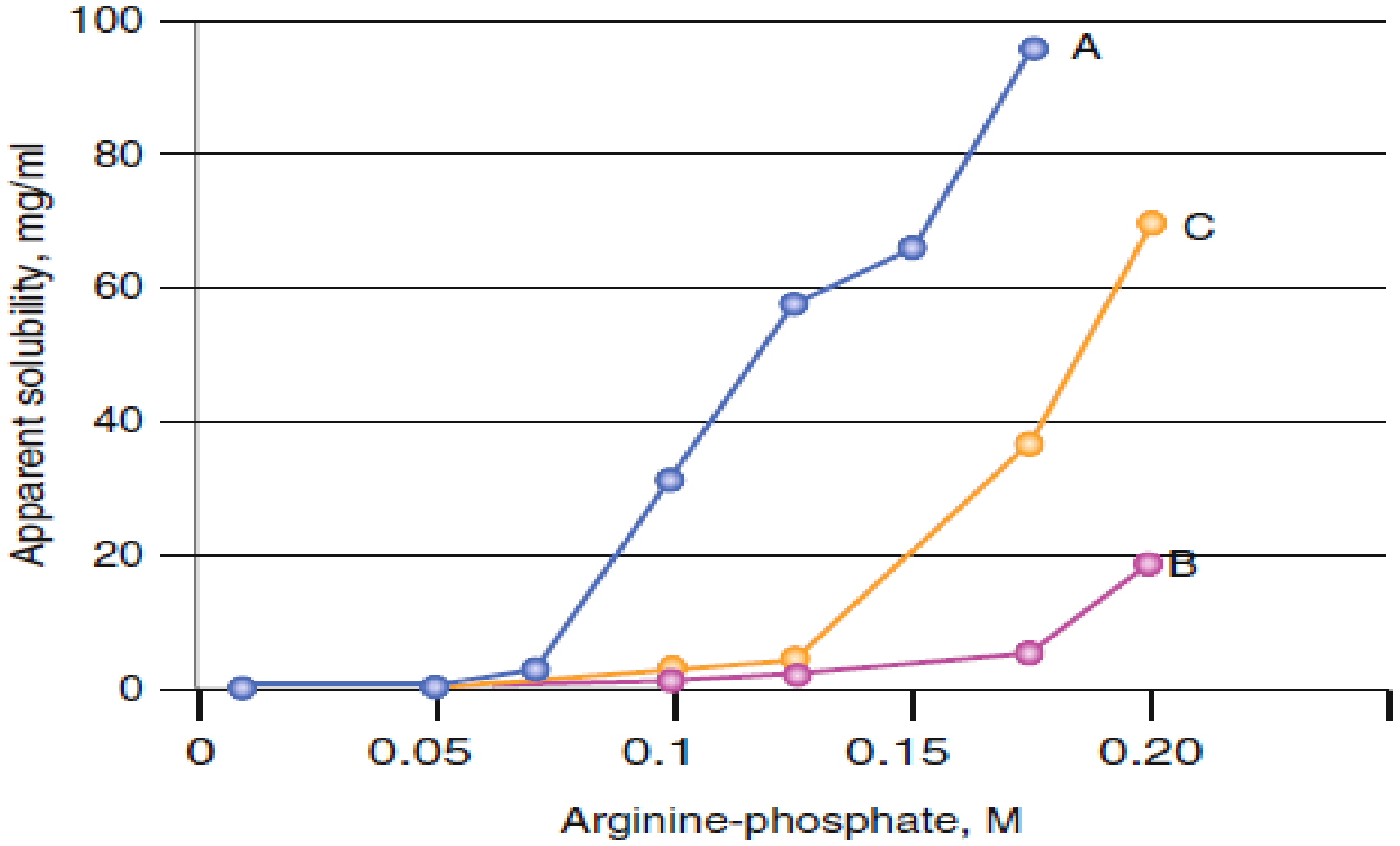
| Excipient class | Function | Examples |
|---|---|---|
| Buffers | pH control, tonicity | Histidine, phosphate, acetate, citrate, succinate |
| Salts | Tonicity, stabilization, viscosity reduction | Sodium chloride |
| Sugars[a], polyols | Tonicity, stabilization, cryoprotection, lyoprotection[b], bulking agent[b], reconstitution improvement[b] | Sucrose, trehalose, mannitol, sorbitol |
| Surfactants | Adsorption prevention, solubilization, stabilization, reconstitution improvement[b] | Polysorbate 20, polysorbate 80, poloxamer 188 |
| Amino acids | Stabilization, viscosity reduction, tonicity, pH control, bulking agent[b] | Arginine, glycine, histidine, lysine, proline |
| Anti-oxidants | Oxidation prevention | Methionine, sodium edetate |
| Preservatives[c] | Bacterial growth prevention | m-cresol, benzyl alcohol, phenol |

# Solubility Enhancement

- Proteins, in particular those that are **non-glycosylated**, may have a **tendency to aggregate** and **precipitate**.
- Approaches that can be used to **enhance solubility** include:

1. **Selection of the proper pH** conditions.
2. **Addition of amino acids** such as **lysine or arginine** (used to solubilize tissue plasminogen activator, t-PA).
3. **Surfactants** such as **sodium dodecyl sulfate** to solubilize non-glycosylated IL-2 can also help to increase the solubility.

# SOLUBILITY ENHANCEMENT




Effect of arginine on type I and type II alteplase at pH 7.2 and 25 °C. **A** type I alteplase, **B** type II alteplase, **C** 50:50 mixture of type I and type II alteplase.

# Solubility Enhancement

- The **mechanism of action** of these solubility enhancers **depends on the type of enhancer** and the **protein involved**.
- This figure clearly indicates the dramatic effect of arginine concentration on the apparent solubility of t-PA.
- It is believed that **arginine** will **increase the hydrogen bonding** ability of the **protein**.
- In the above examples, **aggregation** is **physical in nature**, i.e., based on **hydrophobic and/or electrostatic** interactions between molecules.

# Solubility Enhancement

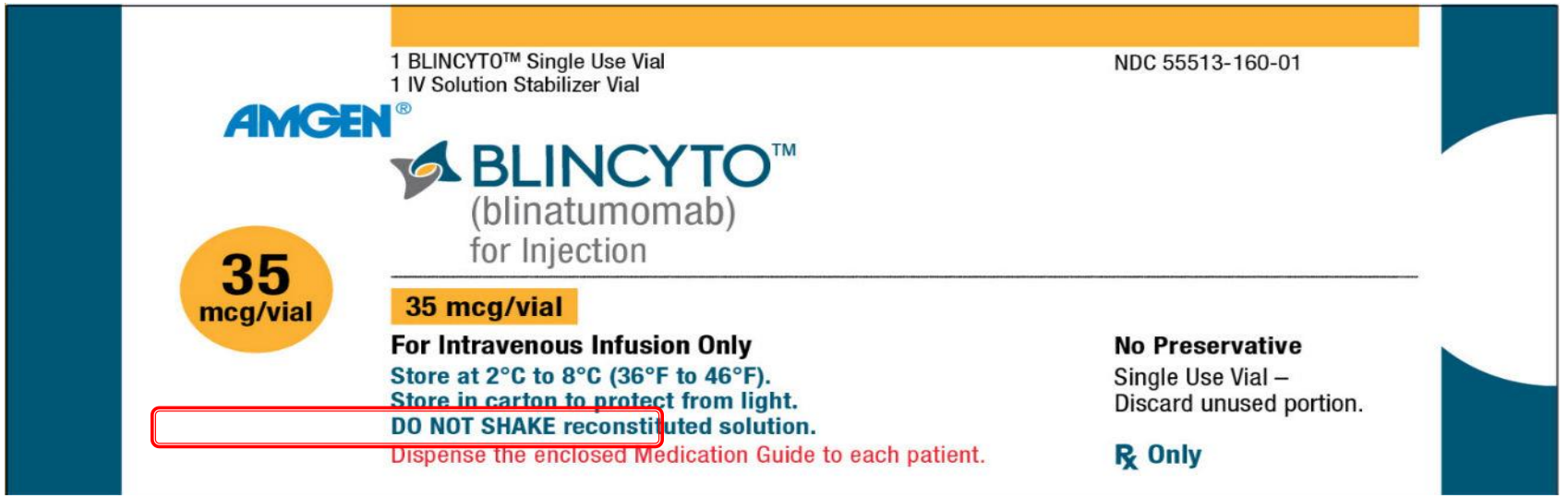
- However, **aggregation** based on the formation of **covalent bridges between** molecules through **disulfide bonds** and **ester** or **amide** linkages has been described as well.
- In those cases, proper conditions should be found to avoid these **chemical reactions** such as **controlling pH** of the solution and adding **anti-adsorption** agents.

# Anti-adsorption and Anti-aggregation Agents:

- Most **proteins** are **prone to adsorb** to interfaces.
- **Anti-adsorption** agents are added to **reduce adsorption** of the active protein to **interfaces**.
- **These interfaces can be water–air, water–container wall**, or **interfaces formed** between the **aqueous phase** and **utensils** used to administer the drug (e.g., catheter, needle).
- **For solid surfaces (such as protein - container wall):**
- Adsorbed, **partially unfolded protein** molecules not only present a **loss of API (**Active Pharmaceutical Ingredient**)** but also may **form aggregates**, leave the surface, return to the aqueous phase, and form **larger aggregates**, and **precipitate**.

# Anti-adsorption and Anti-aggregation Agents

- A similar situation may occur at **gas-liquid** interfaces.
- For some proteins the reconstitution protocol instructs to **swirl** the vial **instead of shaking** it to avoid protein exposure to large liquid-air interfaces.



# Anti-adsorption and Anti-aggregation Agents

- Techniques used to decrease (or prevent) adsorption and suppress aggregation.

1) **For interface-induced aggregation:**

a) **Use of surfactant:** surfactants will **adsorb at the interfaces** → make the interface more hydrophilic → Protein accumulation at the interface is suppressed and thereby aggregate formation.

- The most commonly used surfactants for parenteral use are **poly-sorbate 20, 80,** and **Poloxamer 188.**

b) **Use of human serum albumin:** also **prevent adsorption.**

- Albumin is **commonly avoided** nowadays due to probability of **transferring infections**.

# Anti-adsorption and Anti-aggregation Agents

2) **For the aggregates that occur in the bulk:** these are formed because of **colloidal** and/or **conformational instability**.

a) **Adding sugars: Glucose** may perfectly act as a conformational stabilizer.

b) **Selecting a proper pH and buffer medium for maximum stability**.

- Variation in pH of the medium can cause protein instability and aggregation.

# Buffer Components

Buffer selection is an important part of the formulation process, because of the pH dependence of protein **solubility** and **physical and chemical stability**.

 Buffer systems regularly encountered in biotech formulations are:

1. Phosphate
2. Citrate and
3. Acetate

# Buffer Components

## The isoelectric point (pI)

- pH of a solution at which the **net primary charge** of a protein becomes **zero**.
- At a solution **pH that is above the pI** the surface of the protein is predominantly **negatively charged** and like charged molecules will exhibit **repulsive forces**.
- At a solution **pH that is below the pI**, the surface of the protein is predominantly **positively charged** and **repulsion** between proteins occurs.
- At the **pI the negative and positive charges cancel**, **repulsive electrostatic** forces are **reduced** and the **attraction forces predominate**. The attraction forces will cause **aggregation and precipitation**.
- The pI of most proteins is in the pH range of 4-6.

# Buffer Components

**pI:** is the pH at a particular molecule carries **no net electrical charges** (overall charge).

- Thus molecule is affected by **pH** of its surrounding environment and can **become more positively or negatively** charged due to the gain or loss, respectively, of ($H^+$).
- Such molecules have **minimum solubility** in water or salt solutions at the **pH that corresponds to their pI** and often **precipitate** out of solution.

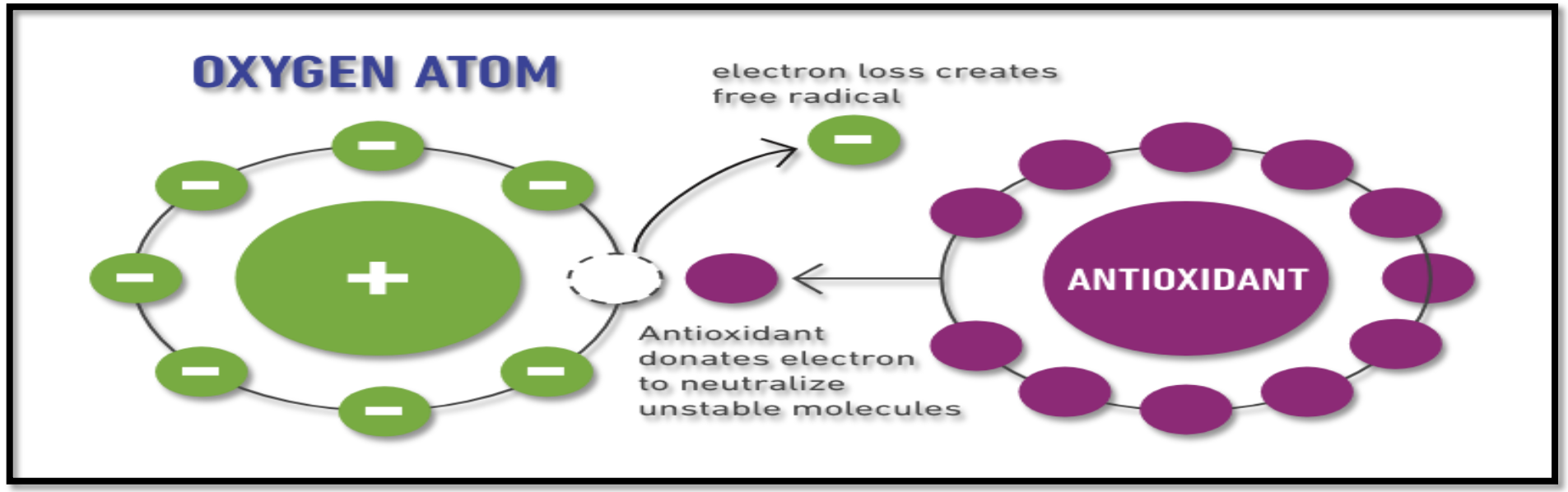
# Preservatives and Antioxidants

- **Antioxidant (Protection against oxidation):**
- Methionine, cysteine, tryptophan, tyrosine, and histidine are amino acids that are readily oxidized.
- **Proteins** rich in these **amino acids** are liable to **oxidative degradation**.
- These changes can be **decreased** (or **prevented**) by:

1. **Replacement of oxygen** by inert gases (e.g. **argon**) in the vials helps to **reduce oxidative stress**.
2. **Decrease the headspace in the vial** (such as in pre-filled syringes) will **decrease** the amount of **oxygen** available and **decrease** the **oxidative stress**.
3. **Addition of antioxidants** such as **methionine** that competes with the methionine residues for oxidation.

# Preservatives and Antioxidants

- **Note**: **some antioxidant** can act as an **oxidant** in certain condition that need to be consider.
- Ascorbic acid, for example, can act as an **oxidant** in the presence of a number of **heavy metals**.
- So, if **ascorbic acid** had to be used for any reason we need to add **chelating agents** such as **EDTA** to **reduce** the effect of **heavy metal**.



# Preservatives and Antioxidants

- **Preservation:**
- Certain proteins are formulated in containers designed for **multiple injection schemes**.
- **After** administering the **first dose**, **contamination with microorganisms may occur**.
- **Preservatives** must be added to **minimize growth**.
- **Common** antimicrobial agents include phenol, meta-cresol, benzyl alcohol, and chlorobutanol.
- Usually, these **preservatives** are present in **concentrations that are bacteriostatic rather than bactericidal in nature**.
- **They can interact with the protein, which may compromise both the activity of the protein and the effectivity of the preservative**.

# Cryoprotectant

- **Proteins** in solution often do not meet the preferred **stability requirements** for industrially pharmaceutical products (**>2 years**), even when kept permanently under **refrigerator conditions** (cold chain).
- The abundant presence of **water** promotes **chemical and physical degradation** processes.
- **Due to heat instability** of biopharmaceuticals → **freeze drying has become** the gold standard drying process for dosages that need to be in dry state for **maximum stability**.
- Freeze drying includes **removing of water by sublimation** (from **solid phase** into **vapor phase** without passing in **liquid phase**).

# Freeze Drying

- Three stages can be discerned in the **freeze drying process**:

**(1) A freezing step**

The temperature of the product is reduced.

**(2) The primary drying step**

Crystallized and water not bound to protein/excipients is removed by sublimation. The **temperature** is **- 40 $^{o}$C** and **reduced pressures** are used.

**(3) The secondary drying step**

Removal of water interacting with the protein and excipients. The **temperature** is rises gradually, e.g., from **- 40 $^{o}$C to 20 $^{o}$C**.

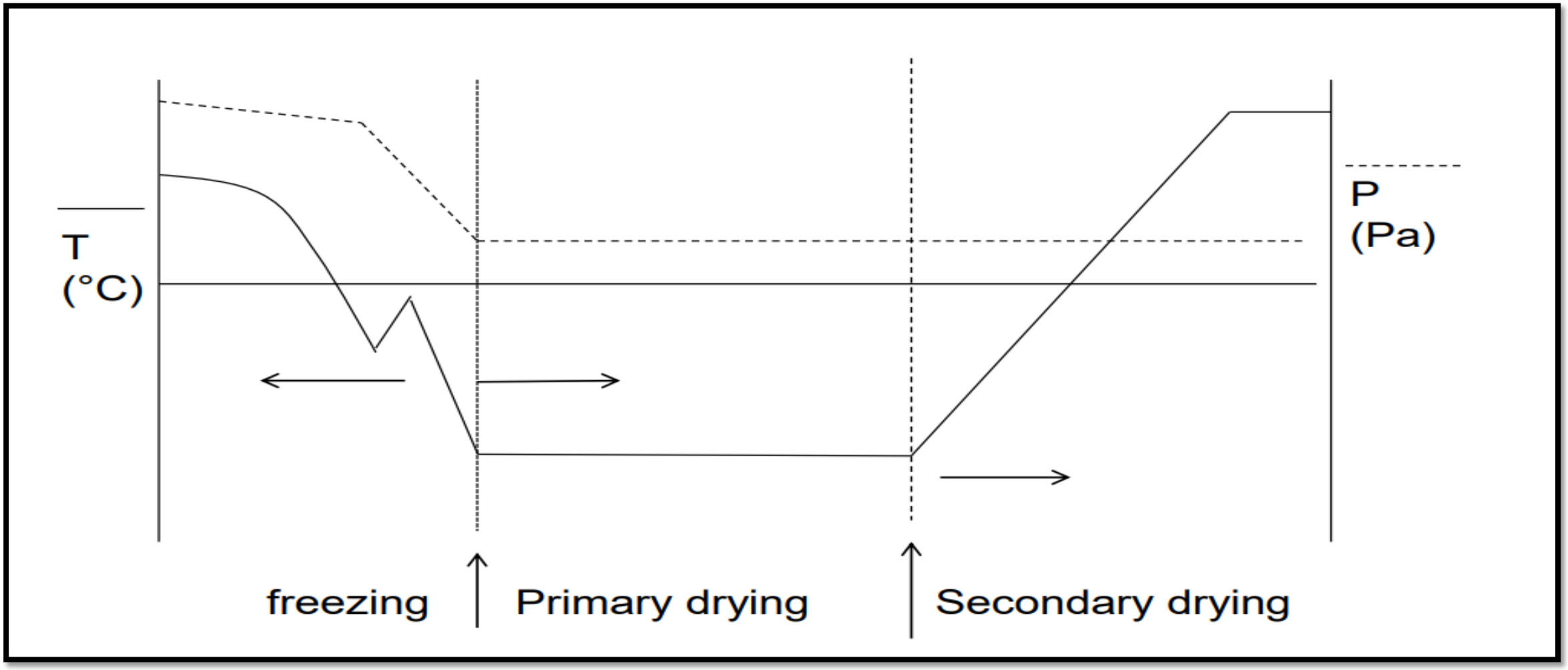
# Freeze Drying

Solution

Temperature
Time
Pressure

Powder

# Freeze Drying




**Example of freeze-drying protocol for systems with crystallizing water. Abbreviations: T, temperature; P, pressure.**

# Cryoprotectant

- During **freezing stage**, **ice crystal may form** and grow causing **structural changes** and **protein instability**.
- **Cryoprotectants are excipients** that **protect** a protein **during freezing or in the frozen state** (mainly sugars: sucrose, trehalose and sugar alcohols: mannitol, sorbitol).
- These work by **increasing the solute concentration** and **lower the melting point** (keep water as liquid as possible).
- This will **prevent rapid ice formation** which is the cause of **structural changes**.
- **So cryoprotectants keep the structure integrity during freezing stage**.


Thank You