Lecture-4

Buffer components
Preservatives and osmotic agents

4. 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**
3. **acetate**

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.

Even short, temporary pH changes can cause aggregation. Explain why?

- These conditions can occur, for example, during the freeze-drying process, when one of the buffer components is crystallizing and the other is not.
- In a phosphate buffer, Na_2HPO_4 crystallizes faster than NaH_2PO_4.

drop in pH during the freezing step.

➢ While other buffer components do not crystallize, but form amorphous systems and then pH changes are minimized.

5. Preservatives and Anti-oxidants

Antioxidants

- **Methionine, cysteine, tryptophane, tyrosine and histidine** are amino acids that are readily oxidized.
- Proteins rich in these amino acids are susceptible to oxidative degradation.

The solution !!!

1. **Replacement of oxygen by inert gases in the vials helps to reduce oxidative stress.**
2. **Addition of anti-oxidant such as ascorbic acid or sodium formaldehyde sulfoxylate can be considered.**

Preservatives

- Certain **proteins** are formulated in the container **designed for multiple injection** schemes.
- After administering the first dose, contamination with microorganism may occur and the preservatives are needed to minimize growth.
- Usually, these **preservatives** are present in concentrations that are **bacteriostatic rather than bactericide** in nature.
- Antimicrobial agents mentioned in the USP XXIV are the mercury-containing **pheylmercuric nitrate, thimerosal, p-hydroxybenzoic acids, phenol, benzyl alcohol and chlorobutanol.**

6. Osmotic Agents

- **For proteins, adjusting the tonicity-of parenteral products by using (Saline and mono- or disaccharide solutions).**

But

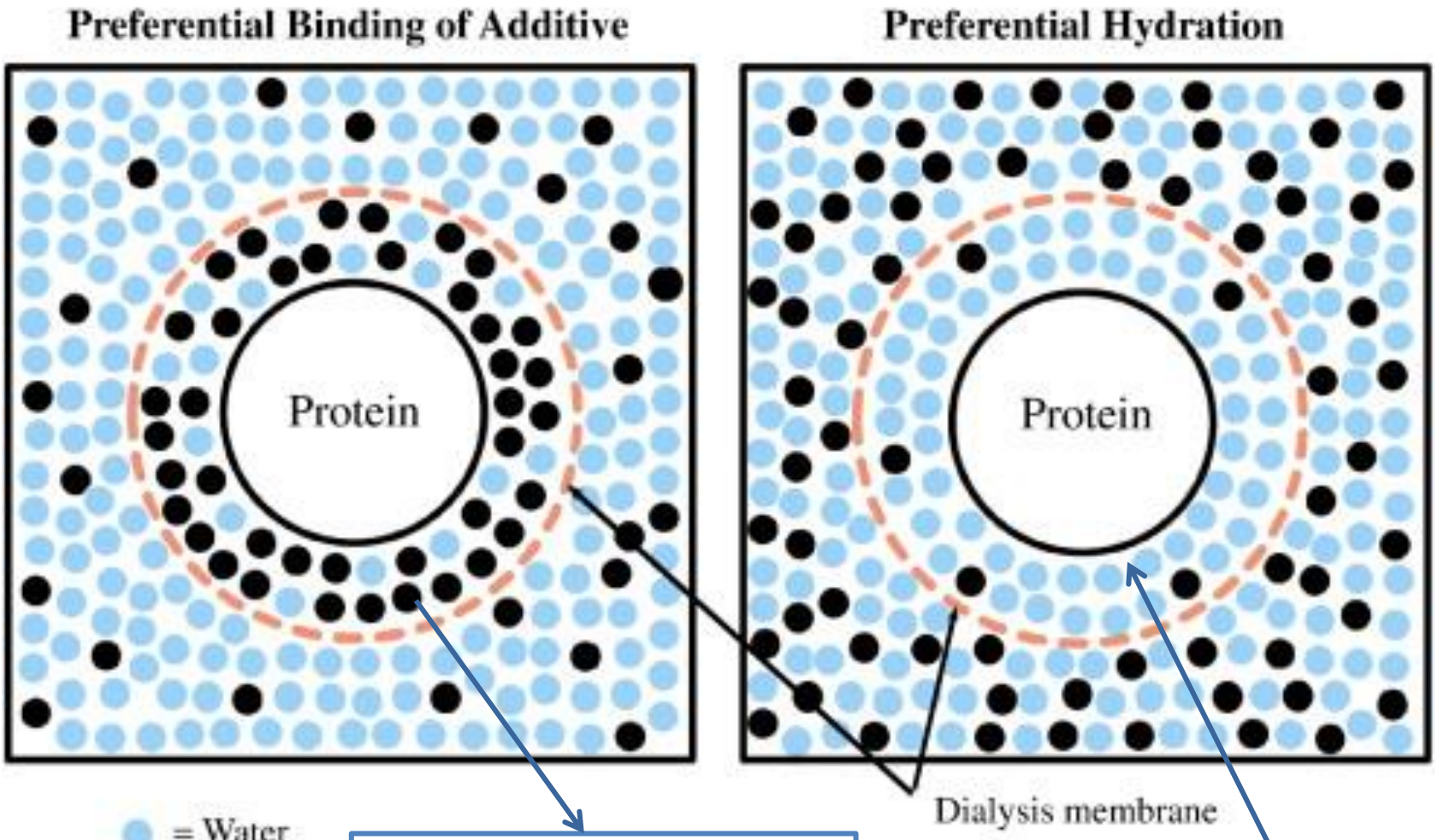
These excipients may not be inert; they may influence protein structural stability.

E.g. sugars and polyhydric alcohol can stabilize the protein structure through the principle of preferential exclusion.

Enhance the interaction of the solvent (water structure promoters) **with the protein** and are themselves excluded from the protein surface layer; **the protein is preferentially hydrated**.

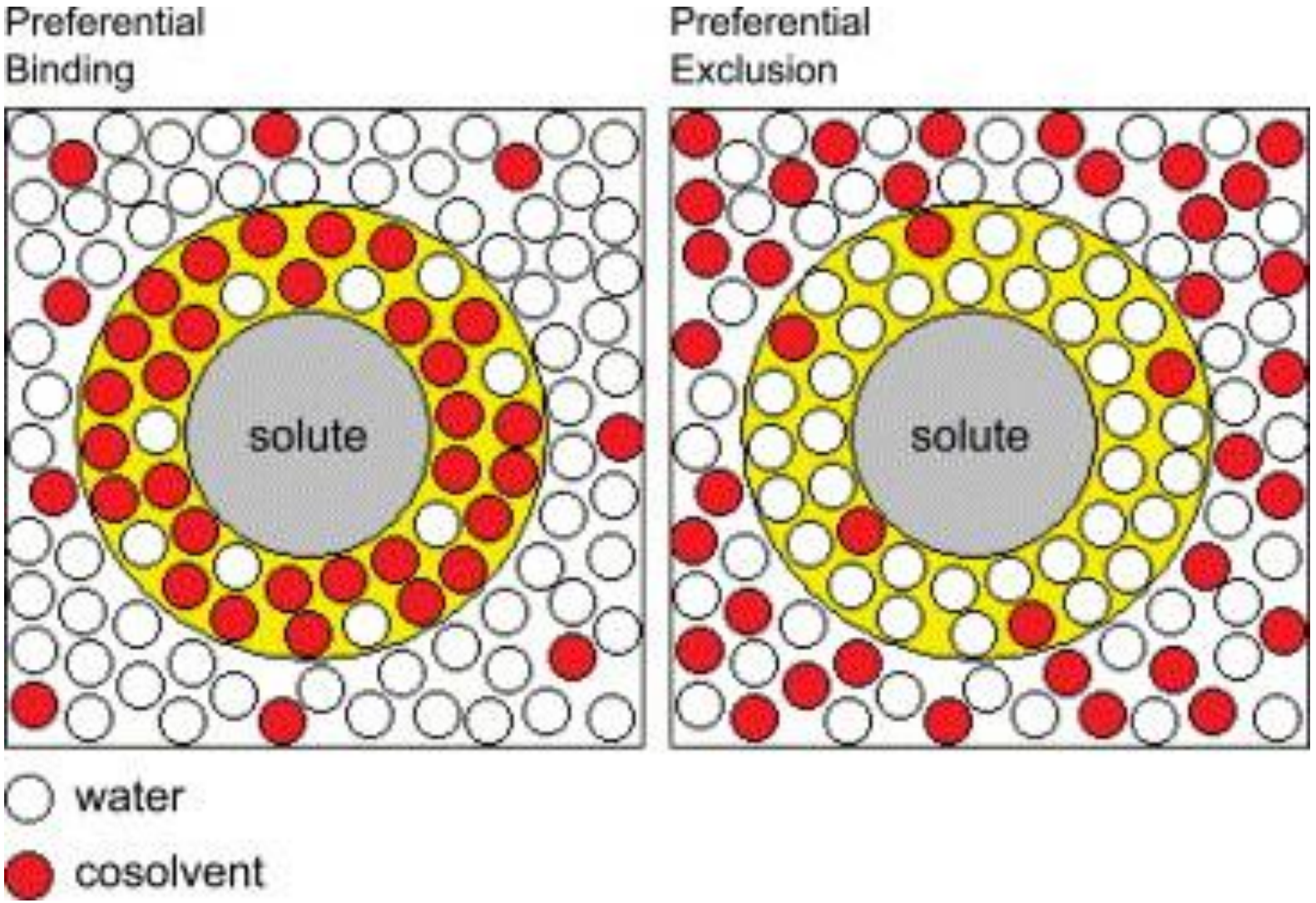
- **This phenomenon can be monitored through an increased thermal stability of the protein**.

Dialysis Equilibrium



PTN surrounded by additives (saline i.e. salt) tend to remove water from PTN molecule and around it and tend to ppt.

PTN surrounded by water so keep high solubility by addition of sugar or glycerol (hydrophilic) by keeping repulsive force (Preferential Exclusion).


Preferential
Binding
Preferential
Exclusion
solute
solute
water
cosolvent

SHELF LIFE OF PROTEIN BASED PHARMACEUTICALS

Protein can be stored:

(1) as an aqueous solution

(2) in freeze-dried form

(3) in dried form in a compacted state (tablet).

The **stability of protein solutions** strongly **depends on** factors such as **pH, ionic strength, temperature, and the presence of stabilizers**.

E.g.: Figure 2 shows the pH dependence of α_1-antitrypsin and clearly demonstrates the critical importance of pH on the shelf-life of proteins.

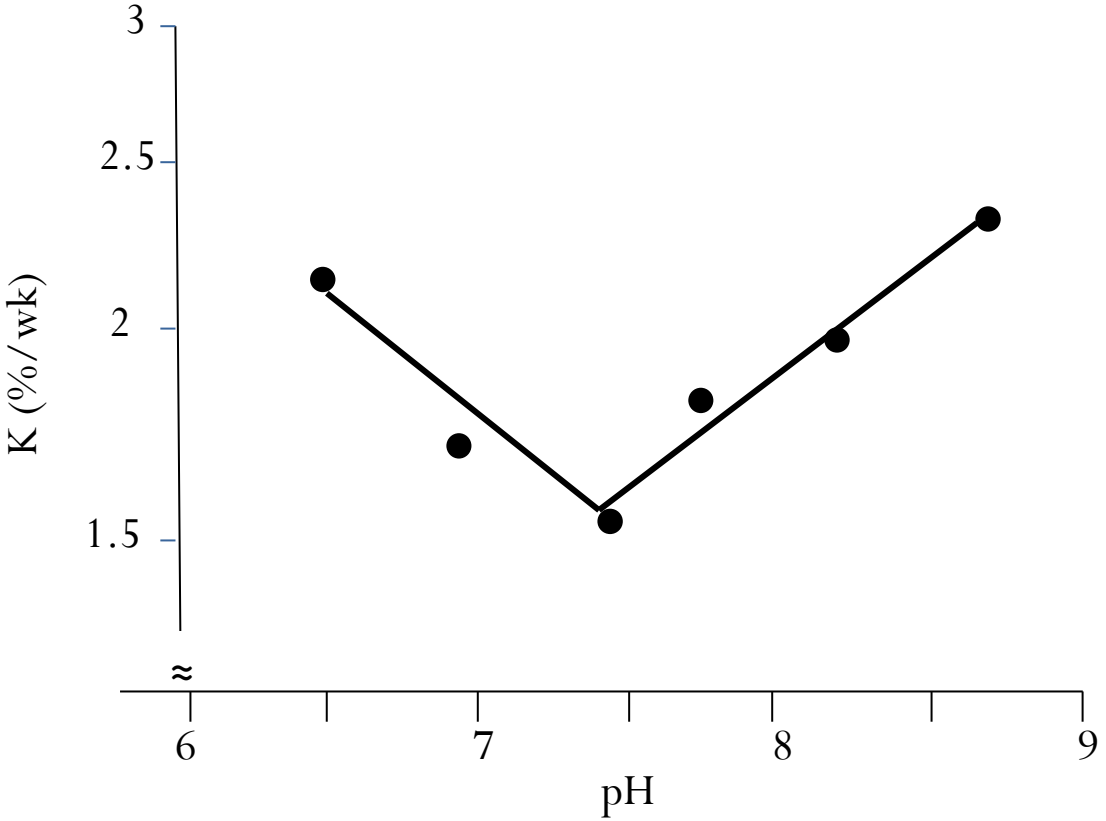


Figure 2 pH stability profile (at 25°C) of monomeric recombinant α_1-antitrypsin (rAAT) by size exclusion-HPLC assay. K = degradation rate constant. Monomeric rAAT decreased rapidly in concentration both under acidic and basic conditions. Optimal stability occurred at pH 7.5 (in which PTN –ve charge PI 5.3).

Freeze-Drying of Proteins

- 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.

Importance of Freeze Drying

- Freeze-drying may **provide the desired stability** by extending shelf life. During freeze-drying **water is removed via sublimation and not by evaporation**.

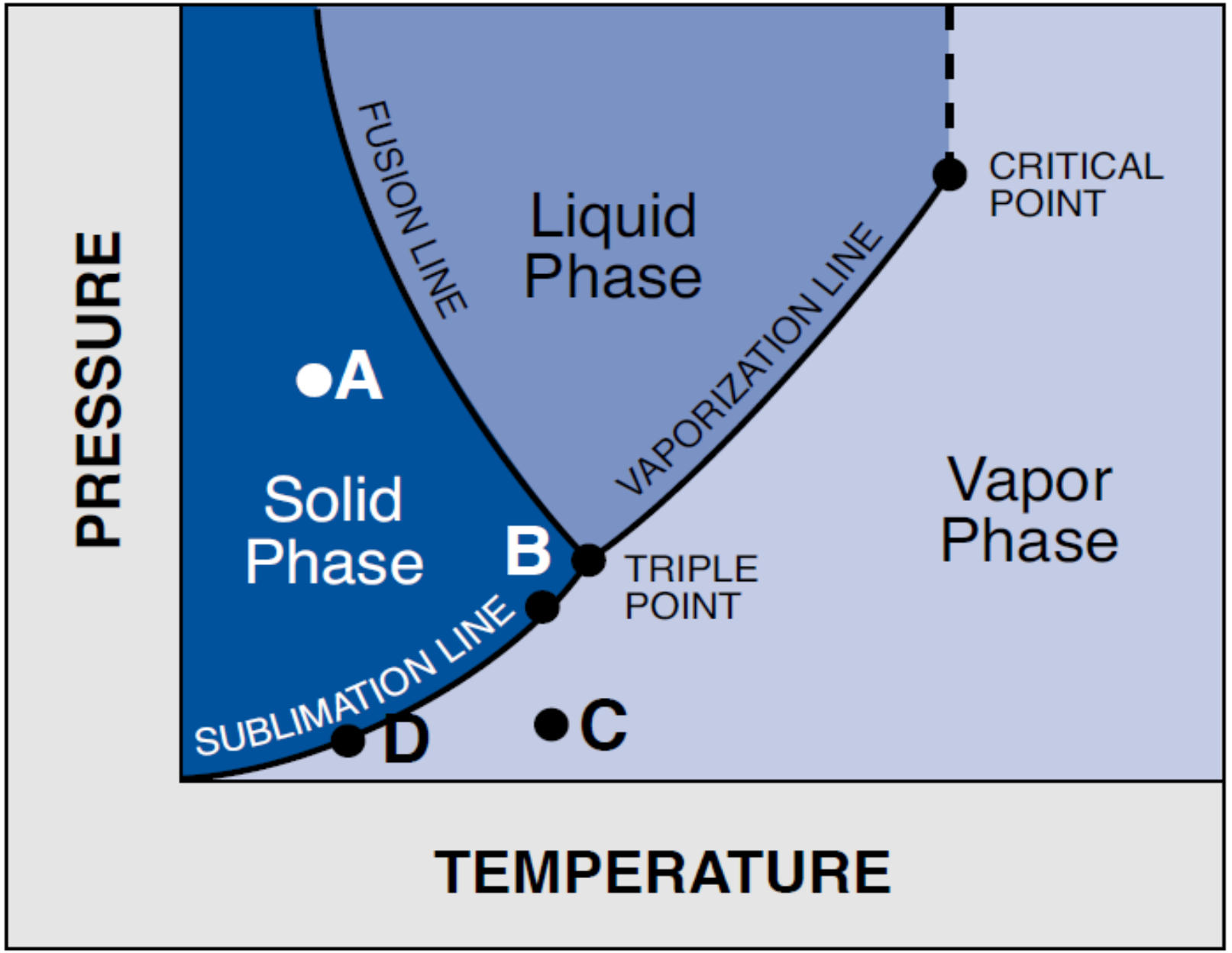
it works by freezing the material, **then reducing the pressure and adding heat** to allow the frozen water in the material to sublimate.

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

  (1) freezing step

  (2) primary drying step

  (3) secondary drying step.



Sublimation: convert from solid to gas with no liquid state

Table 1. Three stages in the freeze drying process of protein formulations.

1. ***Freezing***

The temperature of the product is reduced from ambient temperature to a temperature below the eutectic temperature (Te), or below the glass transition temperature (Tg) of the system. A Tg is encountered if amorphous phases are present.

2. ***Primary drying***

Crystallized and water not bound to protein/excipients is removed by sublimation. The temperature is below the Te or Tg; the temperature is for example -40°C and reduced pressures are used.

3. ***Secondary drying***

Removal of water interacting with the protein and excipients. The temperature in the chamber is kept below Tg and rises gradually, e.g., from -40°C to 20°C.

- The **freeze-drying of a protein solution without the proper excipients causes**, as a rule, **irreversible damage to the protein**.
- Table 4.3 lists excipients typically encountered in successfully freeze-drying protein products.

Table 4.3. typical excipients in a freeze-dried protein formulation

1. **Bulking agents: mannitol/ glycine**

➢ Reason: elegance/ blowout prevention

❖ Blowout is the loss of material taken away by the water vapor that leaves the vial. It occurs when little solid material is present in the vial.

2. **Collapse temperature modifier: dextran, albumin/ gelatine**

➢ Reason: increase collapse temperature.

3. **Lyoprotectant: sugars, albumin**

➢ Reason: protection of the physical structure of the protein.

❖ Mechanism of action of lyoprotectants is not fully understood. Factors that might play a role are:

Notes

- Cryoprotectants lower the melting point of water on dissolving in it and hence protect the cells. **Ethylene glycol, dimethyl sulfoxide (DMSO), glycerol**

- A protectant is substance that is added to a formulation in order to protect the active ingredients. Note that **lyoprotectants protect during the drying stages whereas cryoprotectants protect during the freezing stages**. A lyoprotectant can also be used as the bulking agent.

Mechanisms of action of lyoprotectants

1. **Lyoprotectants** **replace water as stabilizing agent** (water replacement theory),
2. Lyoprotectants increase the Tg of the cake/ frozen system
3. Lyoprotectants will absorb moisture from the stoppers
4. Lyoprotectants slow down the secondary drying process and minimize the chances for overdrying of the protein. Overdrying might occur when residual water levels after secondary drying become too low.

Freeze Drying

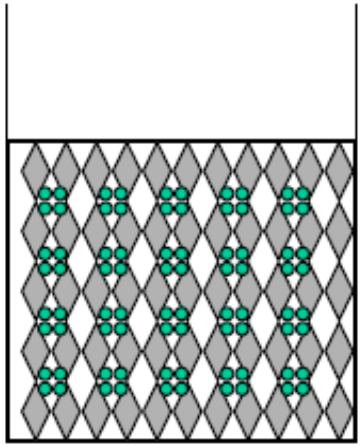
Solution

Temperature
Time
Pressure

Powder

Freezing step

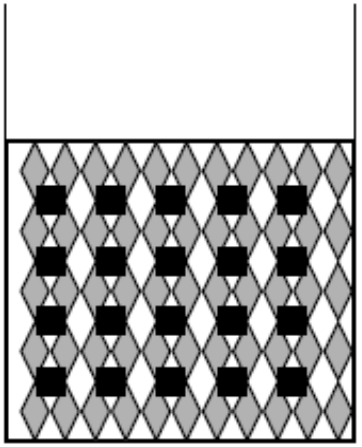
Crystalline Solutes



After Freezing
(Freeze Concentrate)

Some solutes crystallize with ice during freezing

- **Crystalline solutes**



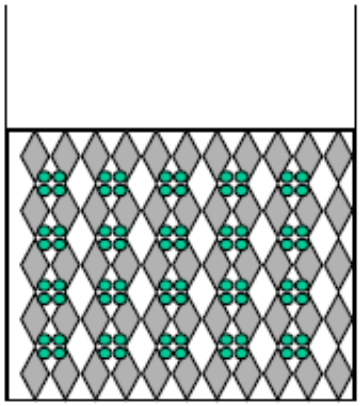
Eutectic Mixture

The temperature where solute and ice both exist in a rigid crystalline state is the "eutectic temperature".

For example, NaCl forms a eutectic mixture containing 23.3%NaCl and melts at -21.13°C.

Freezing step

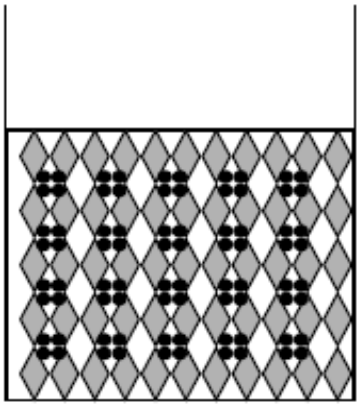
Amorphous Solutes



After Freezing
(Freeze Concentrate)

Most solutes don't crystallize and form a random (amorphous) viscous glassy phase

- Amorphous solute

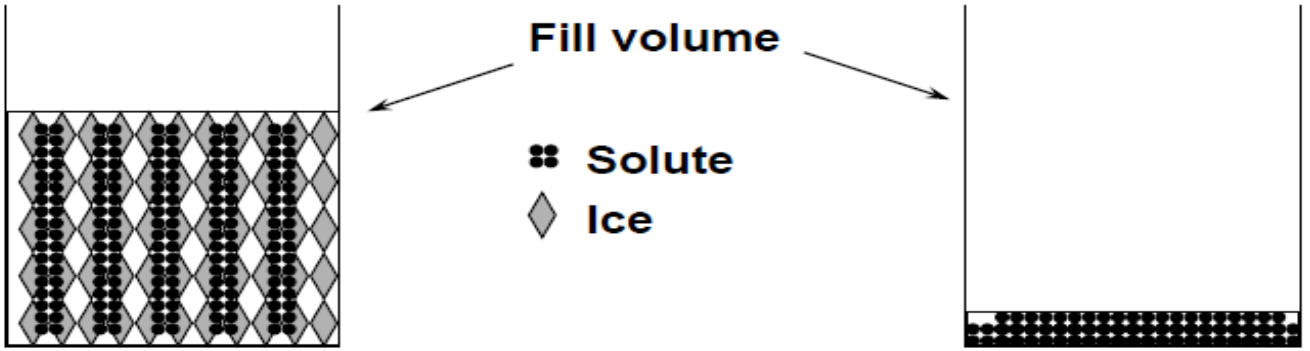


Glassy Mixture

In these systems the viscosity of solute phase increases until the solute is completely immobile and behaves like a glass.

The temperature where the solute behavior changes from solution to a rigid glass is the "glass transition" temperature.

Product Collapse - during freeze drying product temperature exceeds the collapse temperature and the material "collapse" as ice is sublimed.

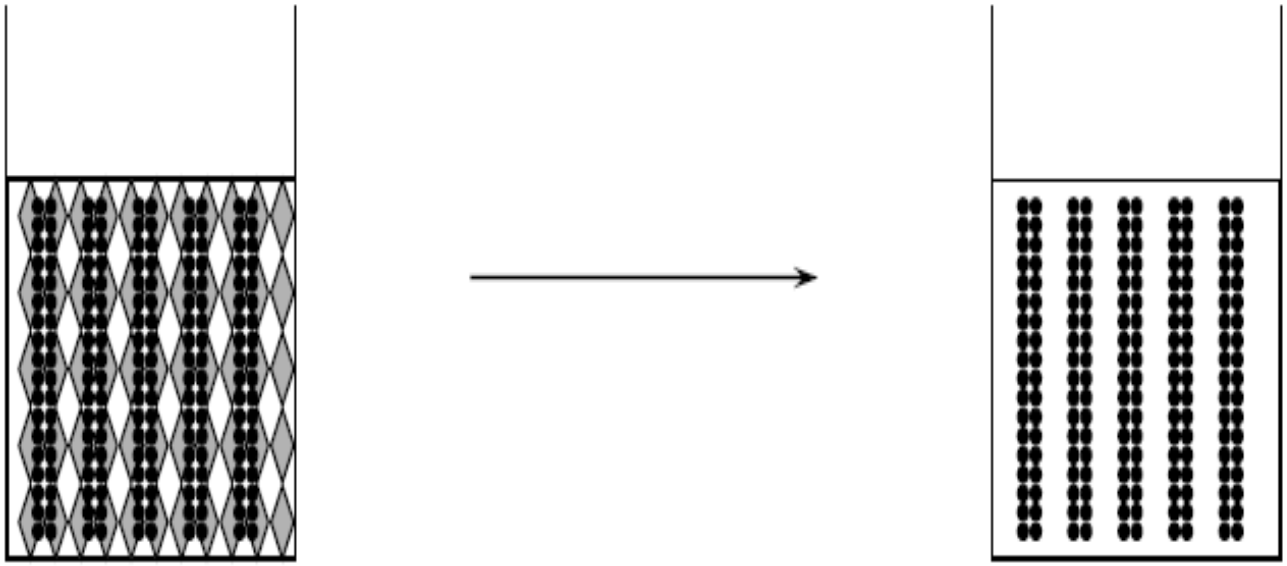


- After ice sublimed a dried residue of solute is produced.

Collapse lead to loss of material structure

Collapse glycerin/ sucrose formulations

Properly dried material produces a well formed cake with no apparent shrinkage.

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Home
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