Shelf life of protein based pharmaceuticals

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- Proteins can be stored:

1) As an aqueous solution
2) In freeze-dried form
3) In dried form in a compacted state (tablets)

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Aqueous solutions:

- Stability of protein solutions strongly depends on factors such as pH, ionic strength, temperature and the presence of stabilizers.
- Stability is not more than (2 years) even when kept permanently under refrigerator conditions.
- **The abundant presence of water promotes chemical and physical degradation processes**.

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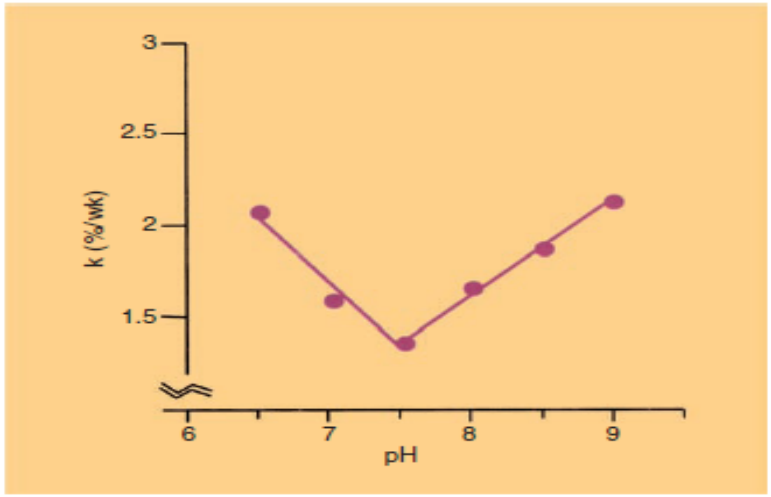

Figure 5 pH stability profile (at 25°C) of monomeric recombinant α_1-antitrypsin (rAAT)

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- Some mechanisms behind chemical and physical degradation processes during purification and storage like:

1) Oxidation: occur in cysteine (-SH), methionine, tryptophan and tyrosine residues. It can affect the size and hydrophobicity of proteins.

2) Hydrolysis: occur in peptide bonds, it can affect the size.

3) Deamidation: on amides of asparagine and glutamine residues, it can affect the charge

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4) Acylation: On amino groups, it can affect the charge.

5) Carboxylation (esterification): occur on glutamate and aspartate residues. It can affect the charge.

6) Upon heating, denaturation and then aggregation

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Freeze drying of proteins:

- Freeze drying may provide the requested stability, in which water is removed through sublimation and not by evaporation.
- The stages of freeze drying are:

1) Freezing step.
2) The primary drying step.
3) The secondary drying step.

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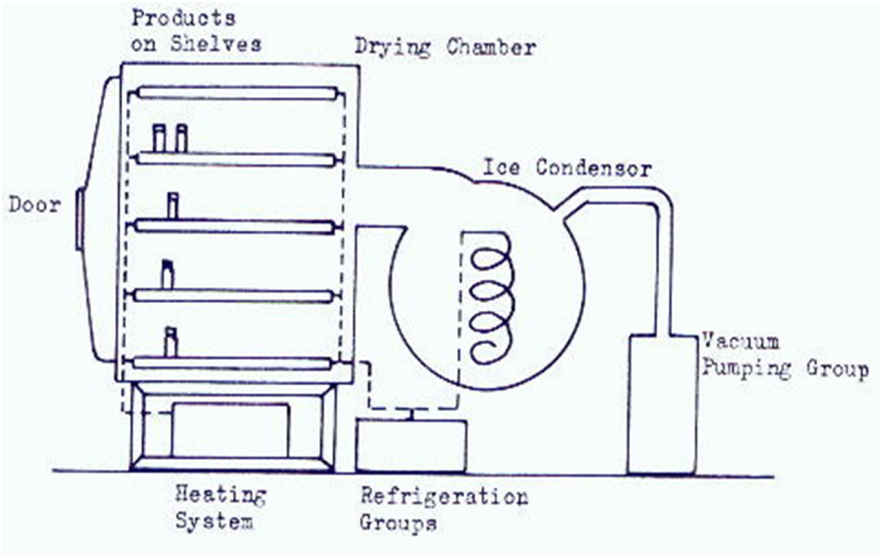

Freeze dryer

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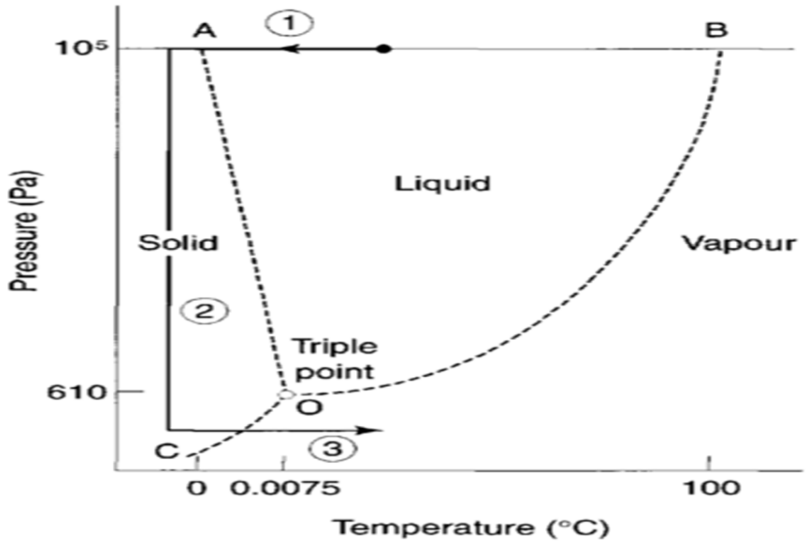

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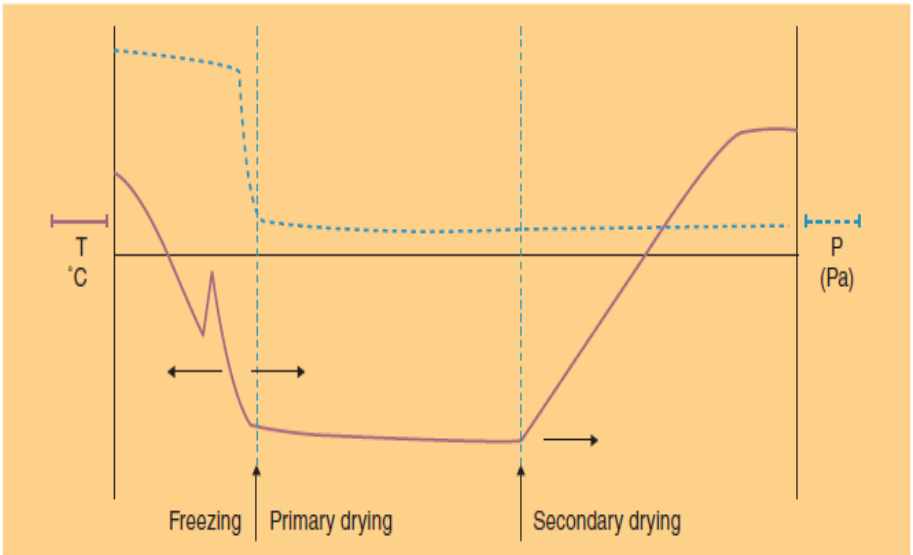

Stages of freeze drying process

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Notes:

1) The freeze- drying of a protein solution without the proper excipients causes, as a rule, irreversible damage to the protein.

- As excipients, we have bulking agent, collapse temperature modifier and lyo-protectants.
- Collapse temperature modifiers like dextran, albumin and gelatin, can increases collapse temperature???

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2) In freezing step, ice crystal formation does not start right at the thermodynamic freezing point, but super-cooling occurs. That means that crystallization often only occurs when temperatures of (– 15°C) or lower have been reached.

During the crystallization step the temperature may temporarily rise in the vial, because of the generation of crystallization heat.

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- During cooling stage, concentration of the protein and excipients occurs because of the growing ice crystal mass at the expense of the aqueous water phase.
- The crystal formation can cause ppt. of one or more of the excipients (like buffer components) which may consequently result in pH shifts or ionic strength changes.
- It may also induce protein denaturation.

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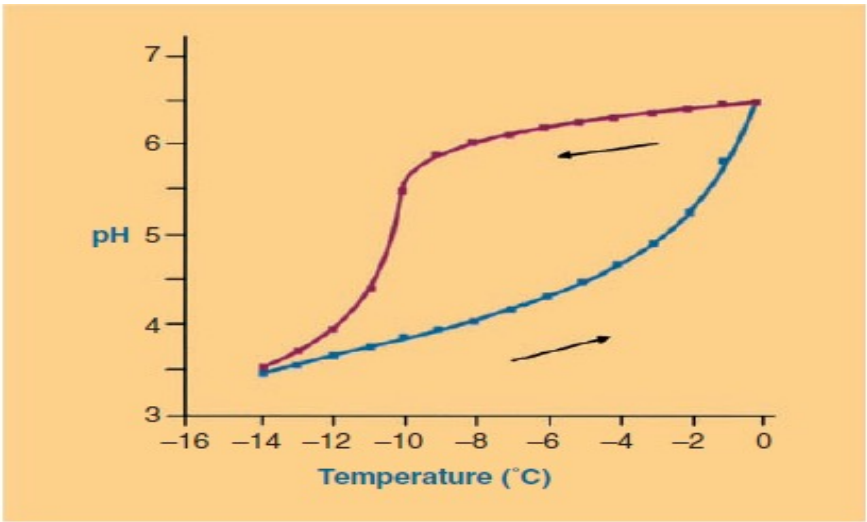

Figure 7 Thawing/cooling (*blue indicates thawing; red indicates cooling*). The effect of freezing on the pH of a citric acid-disodium phosphate buffer system. *Source*: Adapted from

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3) If the system does not (fully) crystallize but forms an amorphous mass upon cooling, the temperature in the freezing stage should drop below T_g.

In amorphous systems the viscosity changes dramatically in the temperatures range around the T_g (a rubbery state exists above T_g , while a glass state below T_g).

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4) In the primary drying stage, sublimation of the water mass in the vial is initiated by lowering the pressure.

The water vapor is collected on a condenser, with a (substantially) lower temperature than the shelf with the vial.

Temperature drops are avoided by the supply of heat from the shelf to the vials, so the shelf is heated during this stage.

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5) Heat is transferred to the vials through,

i) Direct shelf-vial contact (conductance).

ii) Radiation

iii) Gas conduction

6) During the primary drying stage one transfers heat from the shelf through the vial bottom and the frozen mass to the interface frozen mass/dry powder, to keep the sublimation process going.

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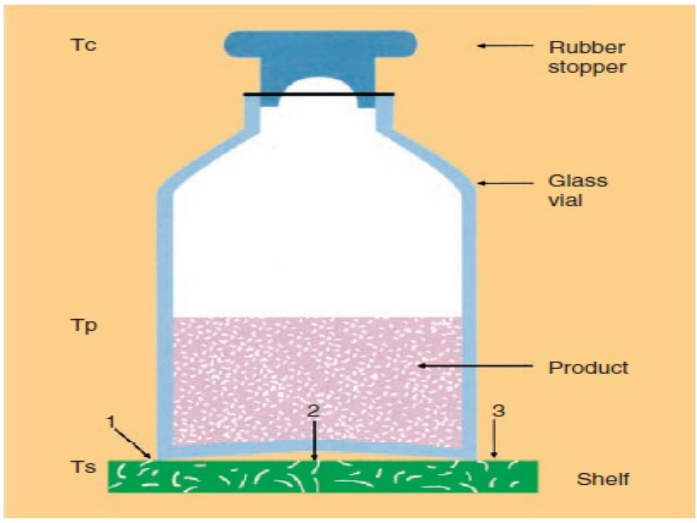

Figure 8 Heat transfer mechanisms during the freeze-drying process: (1) direct conduction via shelf and glass at points of actual contact, (2) gas conduction: contribution heat transfer via conduction through gas between shelf and vial bottom, and (3) radiation heat transfer. *Abbreviations*: Ts, shelf temperature; Tp, temperature sublimating product; Tc, temperature condensor. Ts > Tp > Tc.

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7) Temperature of the vial content should never reach or exceed T_e or T_g range. Typically a safety margin of 2-5 ˚C is used, otherwise the cake will collapse. Collapse causes a strong reduction in sublimation rate and poor cake formation.

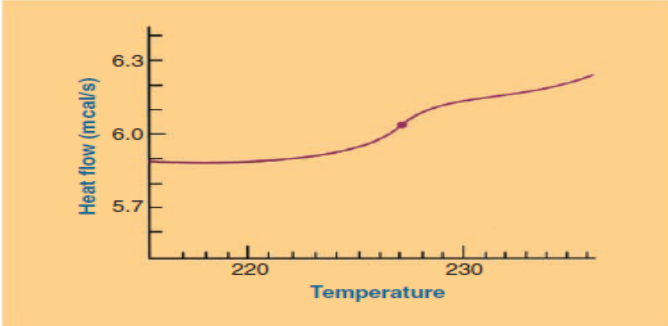

Figure 9 9 DSC heating trace for a frozen solution of sucrose and sodium chloride, showing the glass transition temperature of the freeze concentrate at 227 K. For pure freeze-concentrated sucrose, T_g=241 K (1 Cal=4.2 J). *Source*:

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8) When all frozen or amorphous water that is non protein and non-excipient bound is removed, the secondary drying step starts. The end of the primary drying stage is reached when product temperature and shelf temperature become equal, or when the partial water pressure drops.

9) In this stage, the temperature is slowly increased to remove (bound) water, the chamber pressure is still reduced.

The temperature should stay all the time below the collapse/eutectic temperature, which continues to rise when residual water contents drop.

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10) Typically, the secondary drying step ends when the product has been kept at 20°C for some time.

The residual water content is a critical, end point indicating parameter. Values as low as 1% residual water in the cake have been recommended.

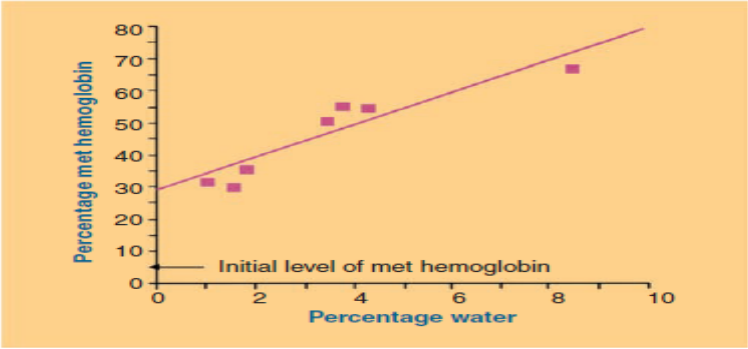

Figure 10 The effect of residual moisture on the stability of freeze-dried hemoglobin (–6%) formulated with 0.2 M sucrose; decomposition to met hemoglobin during storage at 23°C for 4 years. *Source*: From Pikal, 1990a; data reported by Pritoupil

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As summary :

- **Freezing**
  The temperature of the product is reduced from ambient temperature to a temperature below the eutectic temperature (T_e), or below the glass transition temperature (T_g) of the system. A T_g is encountered if amorphous phases are present.
- **Primary drying**
  Crystallized and water not bound to protein/excipient is removed by sublimation. The temperature is below the T_e or T_g (e.g., –40°C) and reduced pressures are used.
- **Secondary drying**
  Removal of water interacting with the protein and excipients. The temperature in the chamber is kept below T_g and rises gradually, e.g., from –40°C to 20°C.

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Other approaches to stabilize proteins

- Compacted forms of proteins are being used for certain veterinary applications, such as a sustained release formulations of growth hormones.
- The pellets should contain as few additives as possible.
- They can be applied sub-dermally or intramuscularly when the compact pellets are introduced by compressed air powered rifles into the animals

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