Shelf life of protein based pharmaceuticals

Lecture Seven

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- Proteins can be stored:

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

Aqueous solutions or dispersions:

- 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 (2-8^{o}C).
- **The abundant presence of water molecules promotes chemical and physical degradation processes**.

- What are the factors affecting the storage of aqueous protein **solutions** or **dispersions**?

1- Temperature (=,< or > 4°C)

2- Type of vehicle ant its proportions.

3- Frequency of use.

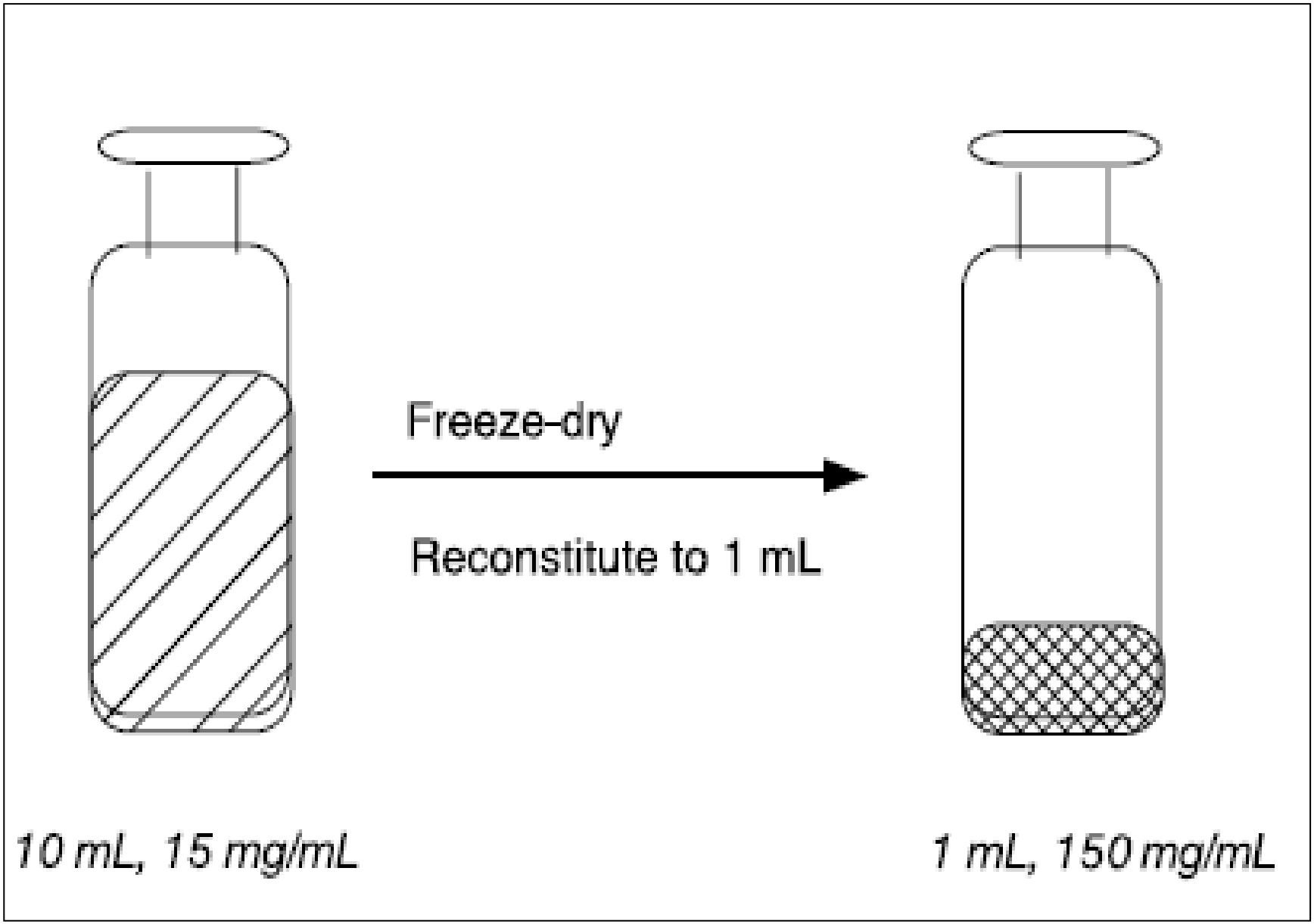
4- Type of container (glass or polypropylene).

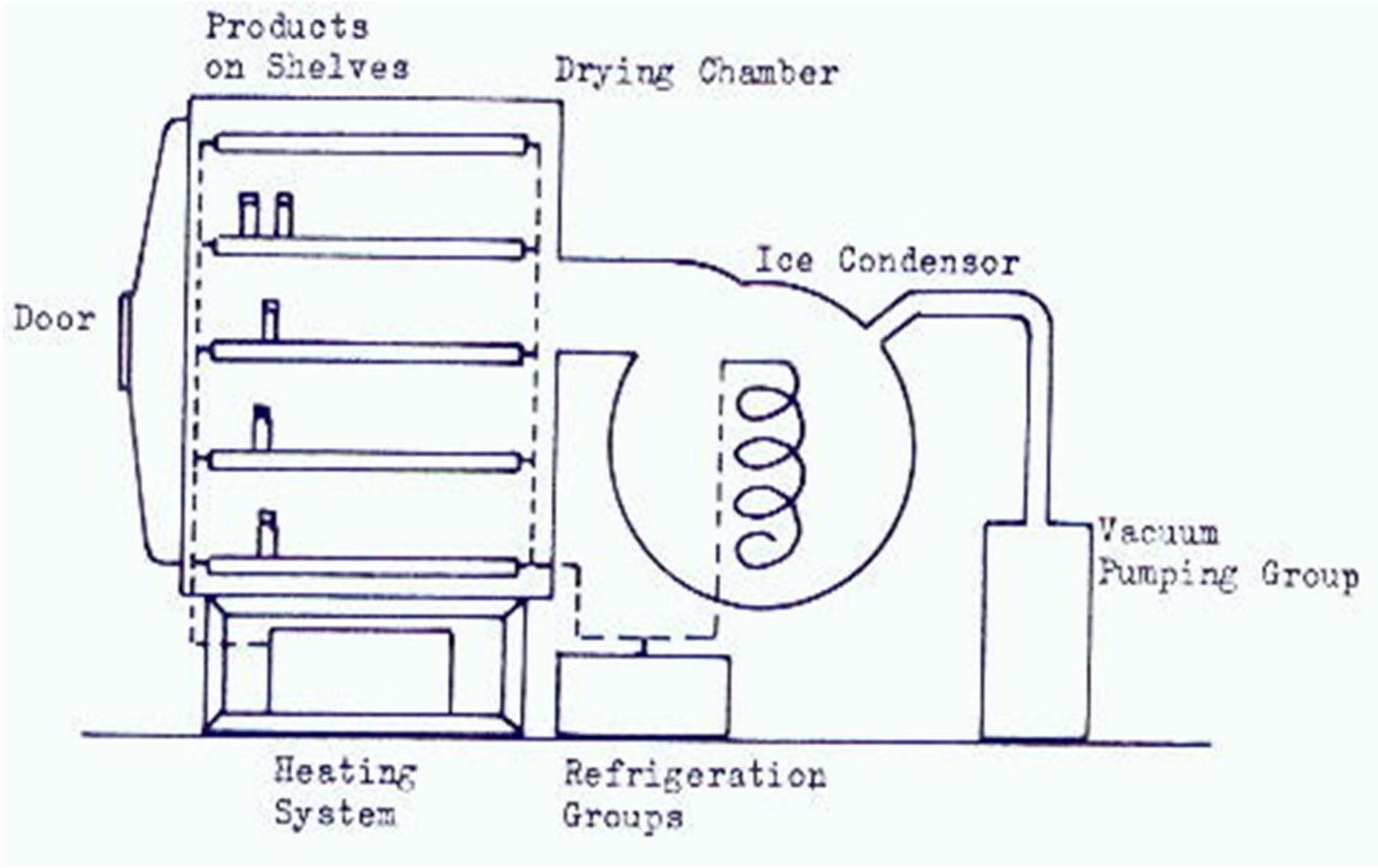
5- pH

6- Protein concentration (The need for filler).

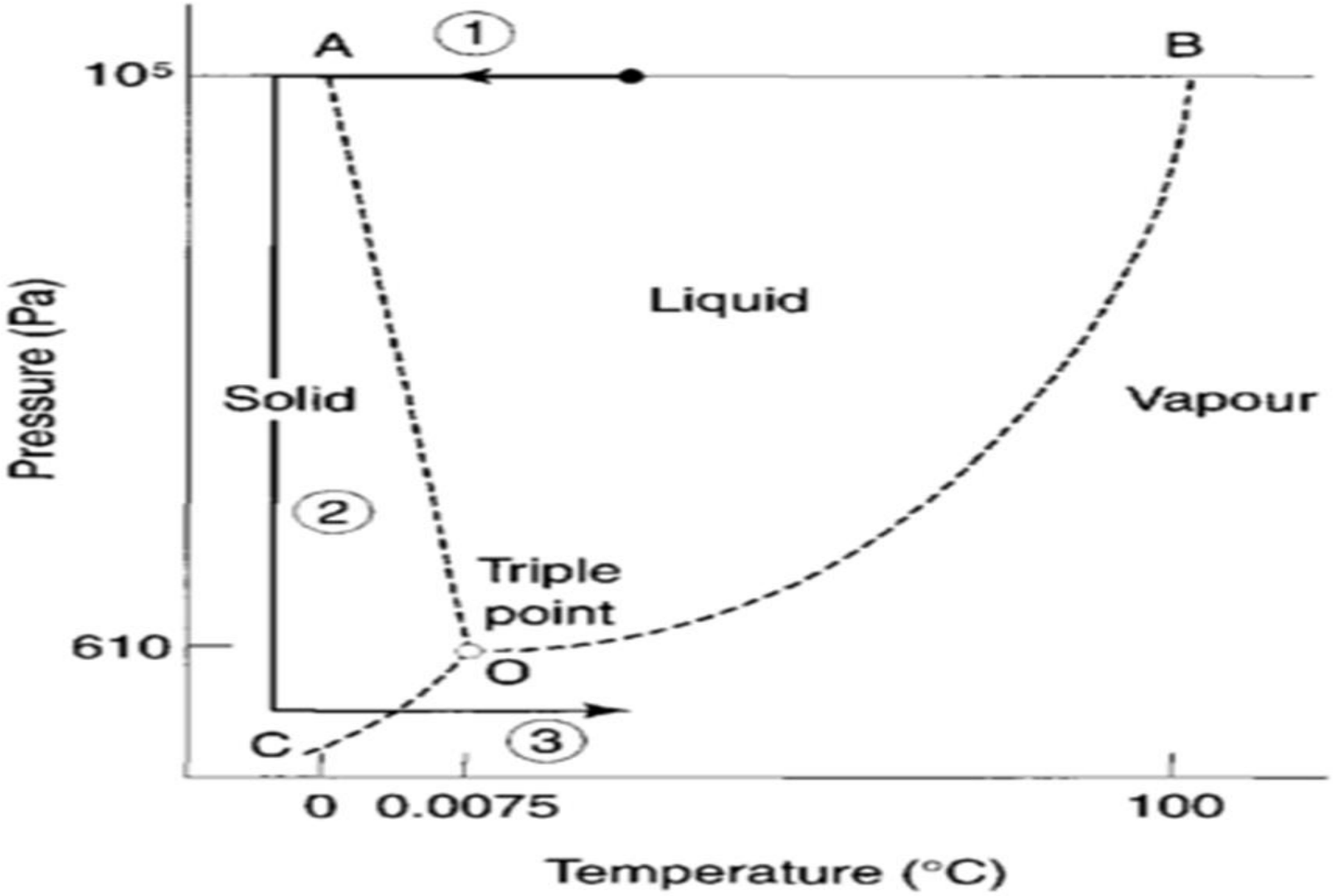
Freeze drying of proteins:

- Freeze drying may provide the requested stability, in which water is removed through sublimation and not by evaporation.
- Lyophilized products, sometimes, can also provide **the flexibility of dose concentration and injection volume.**

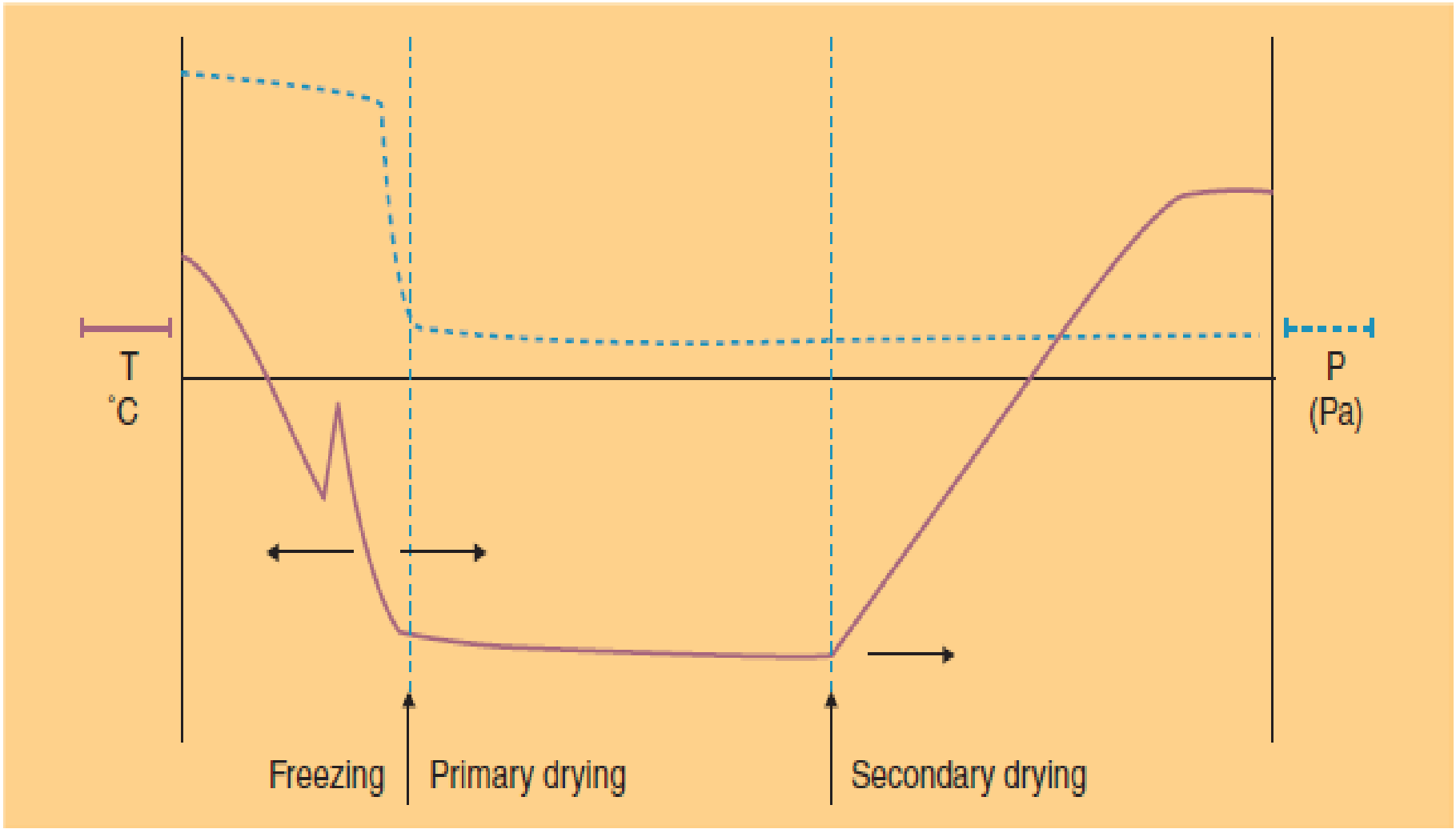

Freeze-dry
Reconstitute to 1 mL
10 mL, 15 mg/mL
1 mL, 150 mg/mL



Freeze dryer


A
1
B
10^5
Liquid
Solid
Vapour
2
Triple
point
610
O
C
3
0
0.0075
100
Temperature (°C)
Pressure (Pa)

Stages of freeze drying process



Excipients involved in freeze drying process

The freeze- drying of a protein solution without the proper excipients may result in (irreversible damage to the protein).

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

Lyo-protectant/cake formers:

- Sugars like glucose, fructose, lactose, maltose, mannitol, sorbitol, sucrose, terhalose and inositol.
- These excipients may be not considered as inert excipiens?? They may influence protein structural stability (conformation) through the principle of **preferential exclusion.**

- Their mechanisms are not fully understood. Factors that might play a role are:

1) They replace water as stabilizing agent (water replacement theory).

2) They modify the T_g of the cake/frozen system.

3) They will absorb moisture from the stoppers.

4) They slow down the secondary drying process and minimize the chances for over-drying of the protein. Over-drying might occur when residual water levels after secondary drying become too low.

Carrier systems (bulking agents):

- Are typical excipients used in a freeze-dried protein formulation for elegance shape and blowout prevention like mannitol and glycine.
- 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.

Notes on stages of process:

1) **In freezing step**, ice crystal formation does not start right at the thermodynamic freezing point, but super-cooling occurs.

That means, the crystallization often only occurs when temperatures of (– 15°C) or lower have been reached.

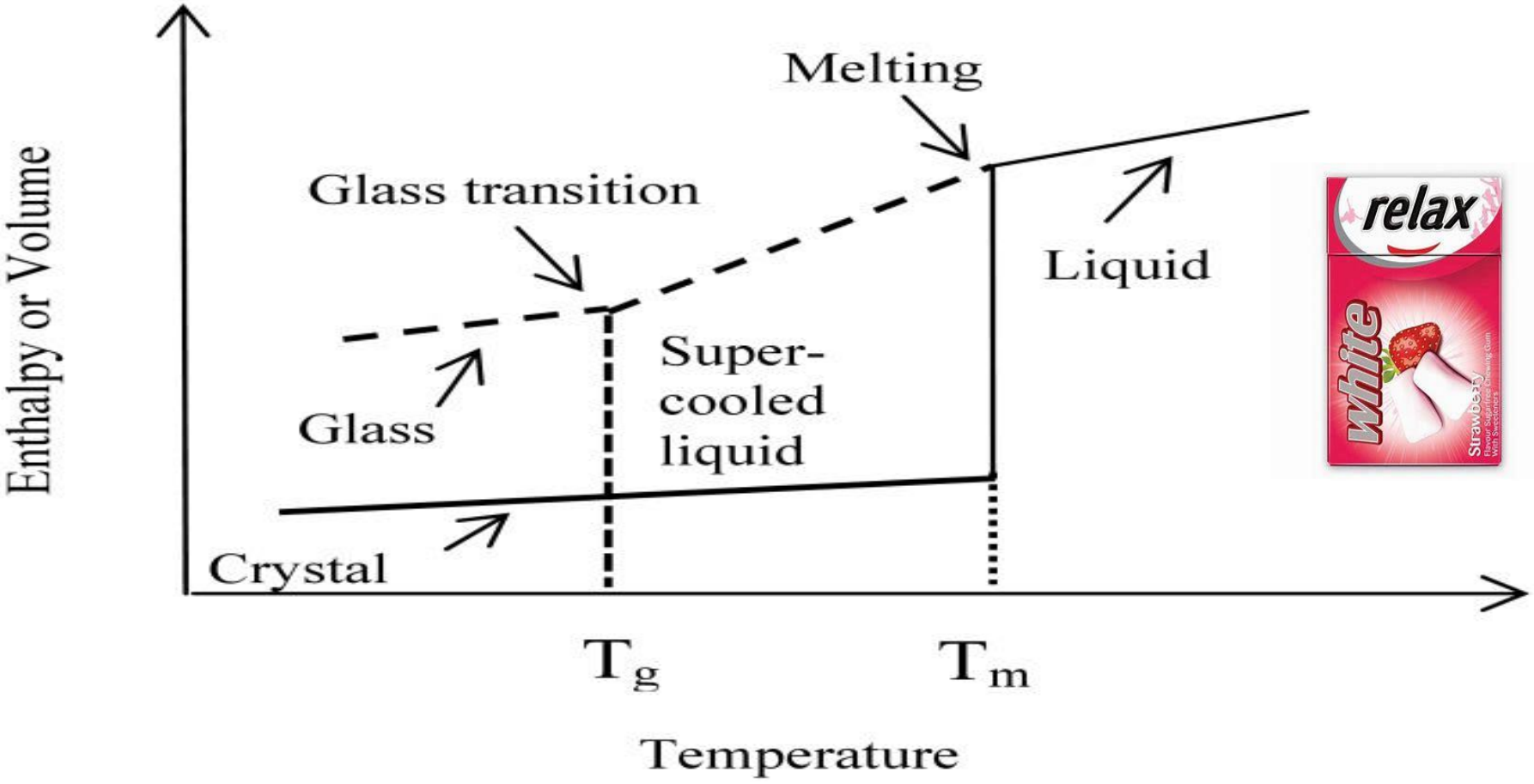
During the crystallization step the temperature may temporarily rise in the vial, because of the generation of crystallization heat.

- During cooling stage, aggregation 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**.

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).

*Melting is a transition which occurs in crystalline polymers. ... But even crystalline polymers will have some amorphous portions. These usually makes up 40-70% of the polymer sample. This is why the same sample of a polymer can have both a glass transition temperature and a melting temperature.



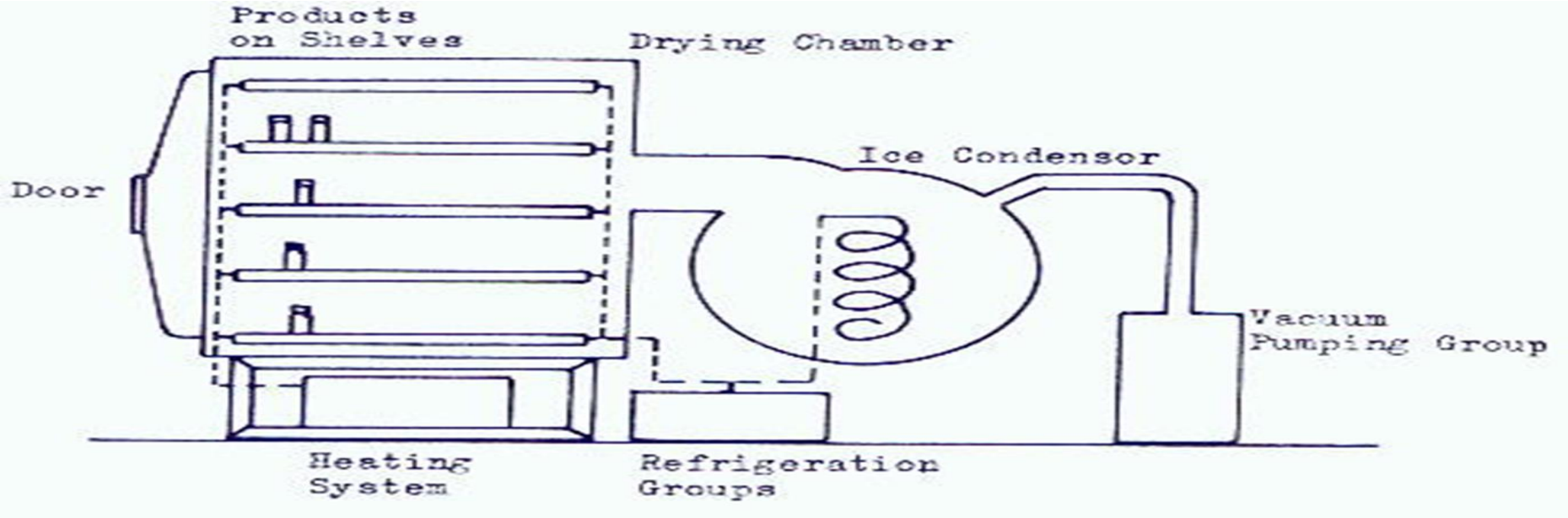
Glass transition temperature can be observed in amorphous and semi-crystalline polymer compounds. Melting temperature can be observed in crystalline compounds.

But the main difference between glass transition temperature and melting temperature is that glass transition temperature describes the transition of a glass state into a rubbery state whereas melting temperature describes the transition of a solid phase into a liquid phase.

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

The collected water vapors in (ice condenser) with (substantially) lower temperature.

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



Heat is transferred to the vials through,

i) Direct shelf-vial contact (conductance).

ii) Radiation

iii) Gas conduction

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

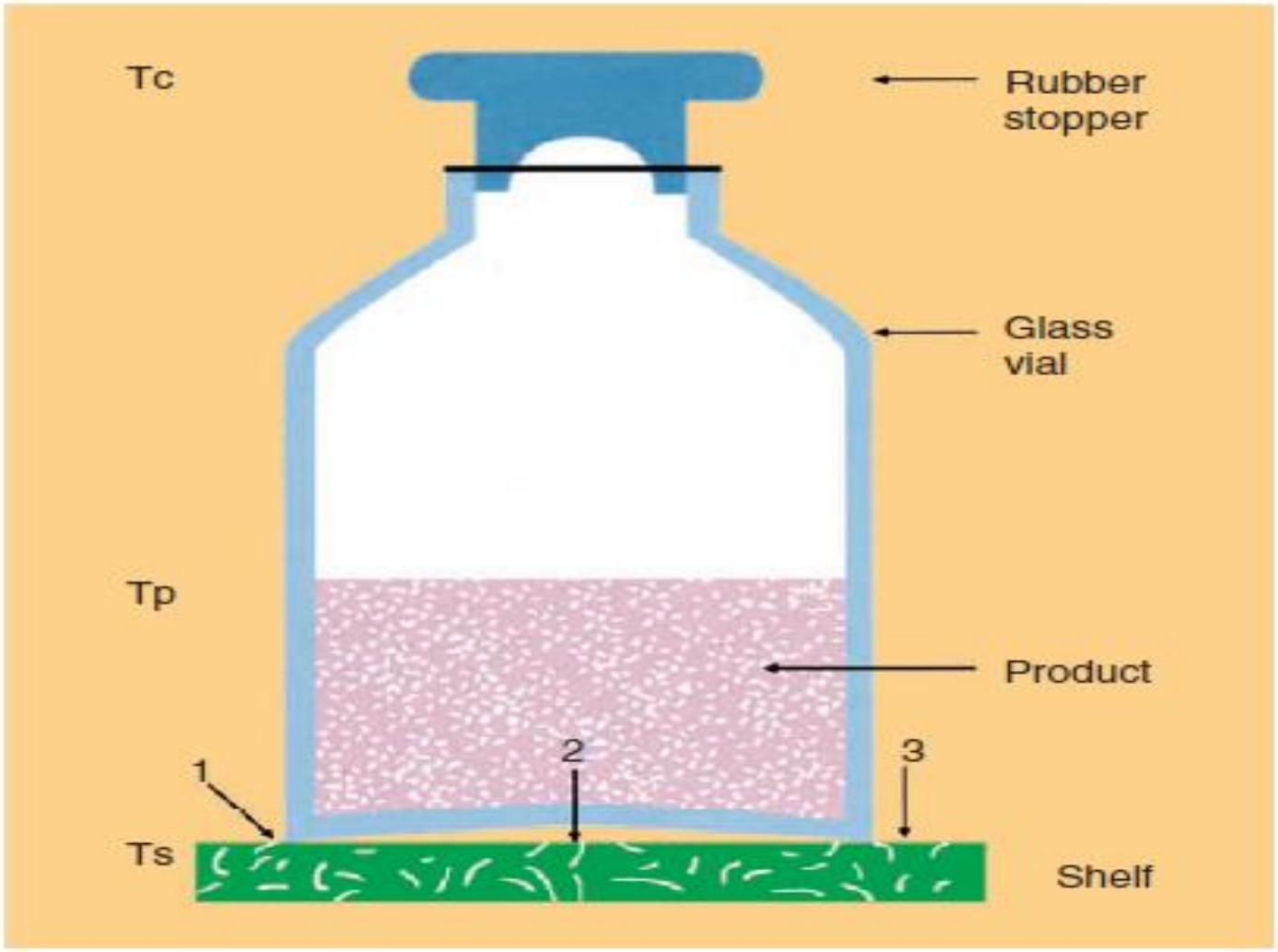


Figure 8 Heat transfer mechanisms during the freeze-drying process: (1) direct conduction via shelf and glass at points of actual contact, (2) gas conduction: contribution heat transfer via conduction through gas between shelf and vial bottom, and (3) radiation heat transfer. *Abbreviations*: Ts, shelf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

Temperature of the vial content should never reach or exceed T_e (of water) or T_g range (of protein). 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.

3) 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.

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

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.

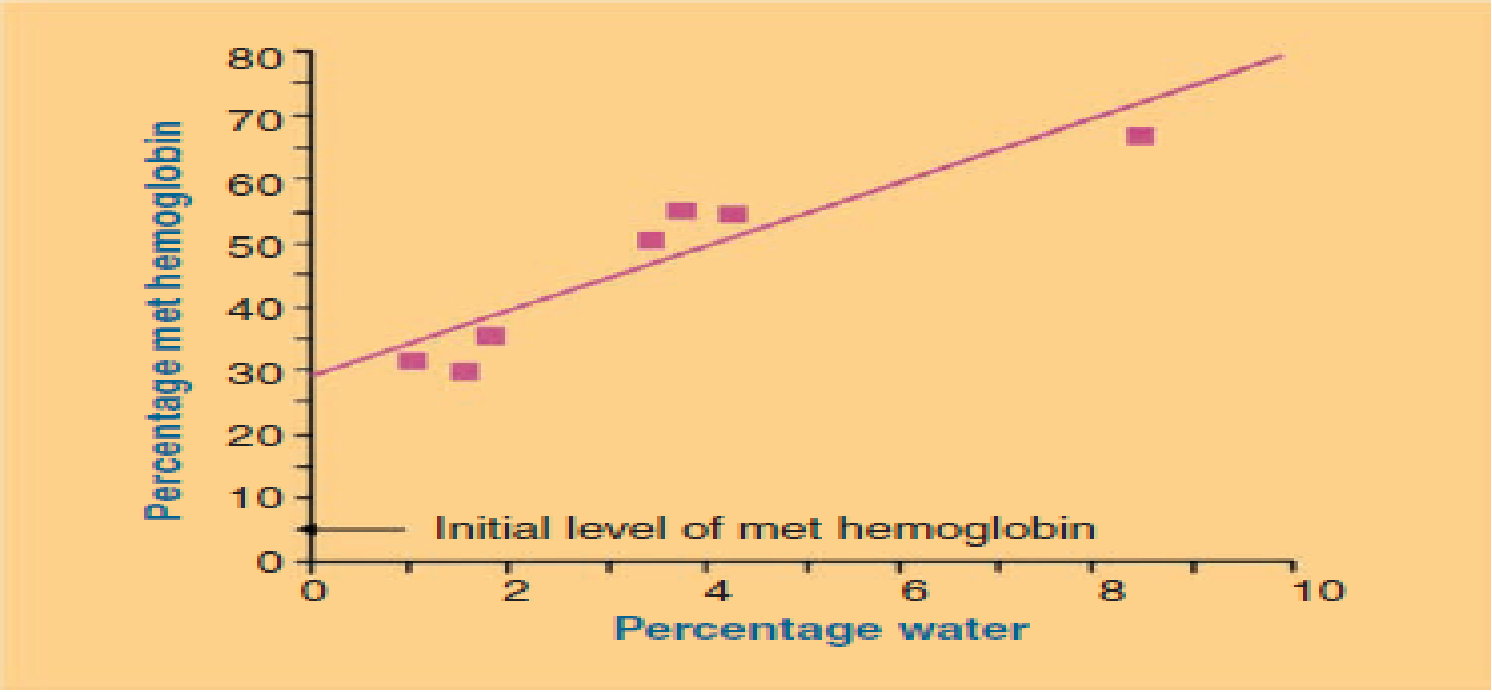


Figure 10 The effect of residual moisture on the stability of freeze-dried hemoglobin (–6%) formulated with 0.2 M sucrose; decomposition to met hemoglobin during storage at 23°C for 4 years. *Source*: From Pikal, 1990a; data reported by Pritoupil

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.

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 (mostly).

Delivery of proteins
(Routes of administration)

Routes of administration:

(1) Parenteral systemic delivery (Injectable):

by IV., SC., IP. and IM

(2) Non-parenteral systemic delivery:

All methods of administering a therapeutic substance or medication to a patient that do not involve puncture of the skin or any active delivery through the skin through the use of a device.

Oral, nasal, buccal, ocular, rectal, transdermal and pulmonary routes

The parenteral route of administration:

For the systemic delivery of therapeutic peptides and proteins, it is currently believed to be the most efficient route and also the delivery method of choice to achieve therapeutic activity.

Product trade name
Gonal F (rhFSH)
Puregon (rhFSH)
Follistim (rhFSH)
Luveris (rhLH)
Ovitrelle (rhCG)

Parenteral administration is here defined as administration via those routes where a needle is used, including IV., IM., SC. and IP. Injections.

These routes with different residence times and dispositions, so there is a significant effect on the therapeutic performance of the drug.

Among them, IV. Adm. is currently the method of choice for systemic delivery

- These changes are related to:

1) The prolonged residence time at the IM or SC site of injection compared to IV administration and enhanced exposure to degradation reactions (peptidases), in addition to protein-tissue bounding.
2) Differences in disposition

- **Regarding point 1:**

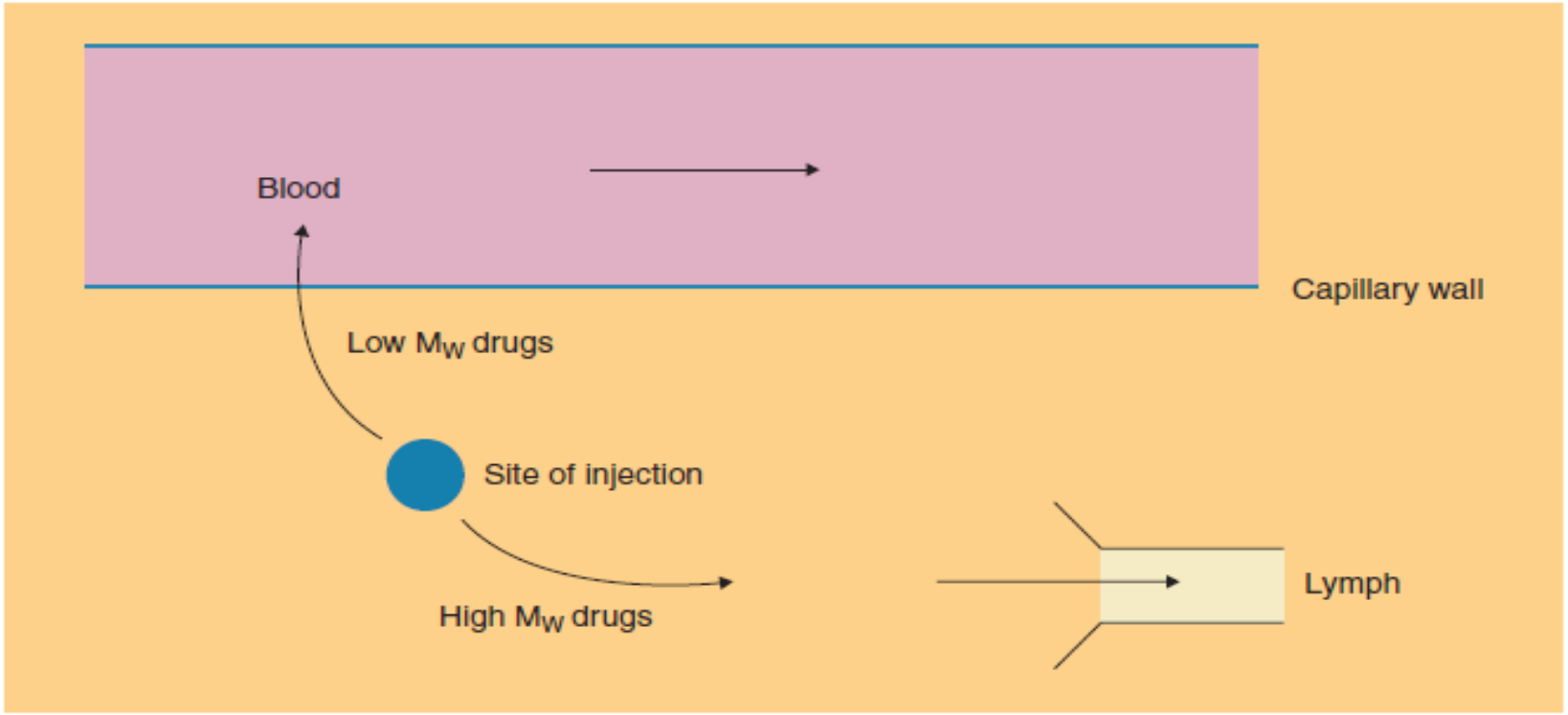
As factors involved:

1) Diabetics can become insulin resistant through high tissue peptidase activity.
2) Exercise level of the muscle at the injection site
3) Message
4) Heat
5) The state of the tissue

- **Regarding point 2:**

As factor involved is (Molecular weight of protein)

- Upon administration, the protein may be transported to the blood through the lymphatics or may enter the blood circulation through the capillary wall at the site of injection.



Routes of uptake of SC or IM injected drugs

- The fraction of the administered dose taken by this lymphatic route is molecular weight dependent.
- Lymphatic transport takes time (hours) and uptake in the blood circulation is highly dependent on the injection site.
- On its way to the blood, the lymph passes through draining lymph nodes and contact is possible between lymph contents and cells of the immune system such as macrophages, B- and T- lymphocytes residing in the lymph nodes.

The oral route of administration:

- It is preferred ? because it is patient friendly and no intervention by a healthcare professional is necessary to administer the drug.
- Oral bioavailability is usually very low?

Because of:

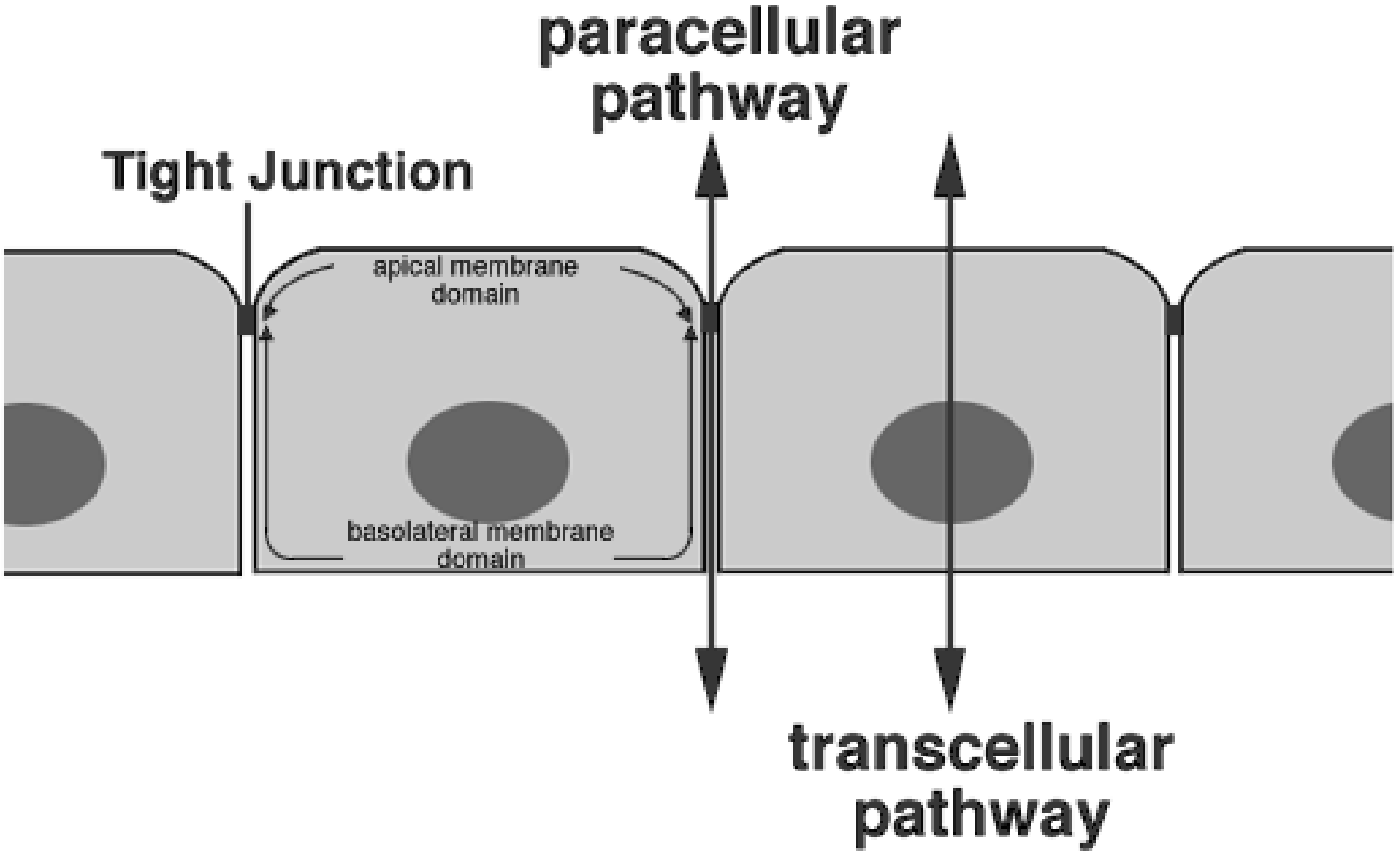
(1) Protein degradation in the GIT.

(2) Poor permeation within GIT in case of a passive transport process.

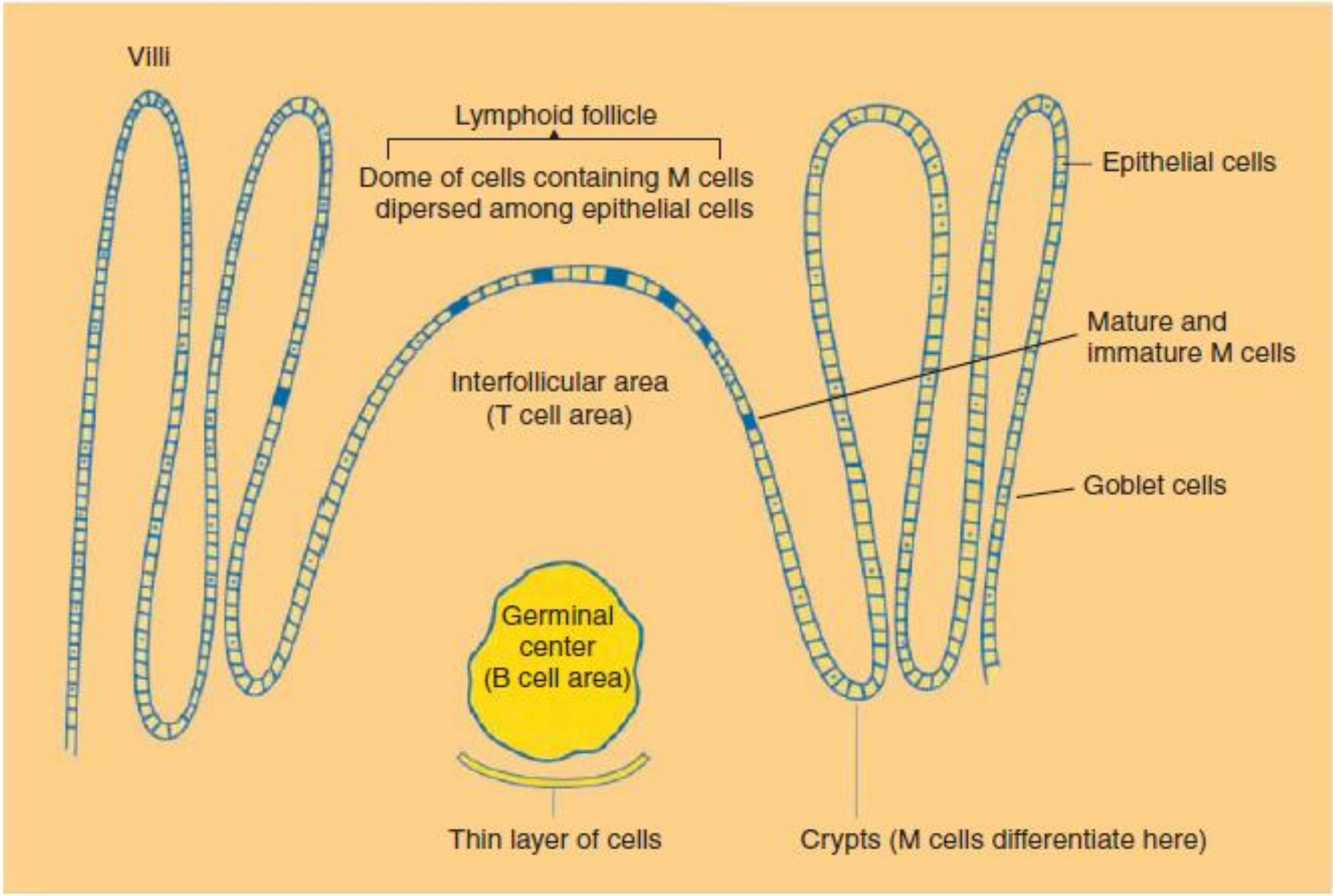
- For degradation, occurred due to presence of proteolytic enzymes (proteases) for breaking down protein in our food to amino acids, or di-tri-peptides to be absorbed.
- In the stomach, pepsins , a family of aspartic proteases are secreted, active between pH(3-5) and lose activity at higher pH values.
- Pepsins are endo-peptidases capable of cleaving peptide bonds distant from the ends of the peptide chain, selectively cleave peptide bonds between two hydrophobic amino acids.

- Other endo-peptidases are active in the GIT at neutral pH values, e.g., **trypsin, chymotrypsin, and elastase** (with different peptide bond cleavage properties that more or less complement each other).
- In addition, exo-peptidases, proteases degrading peptide chains from their ends. Examples, **carboxy-peptidase A and B**.
- In the GI lumen, the proteins are cut into fragments that effectively further break down to amino acids, di- and tri- peptides by brush border and cytoplasmic proteases of the enterocytes.

- For permeation, high molecular weight molecules do not readily penetrate the intact and mature epithelial barrier depending on diffusion principle.
- Where diffusion coefficient decreases with increasing molecular size.
- A typical transport mechanism is paracellular transport in which there are tight junctions between each of the cells in the epithelium that prevent water and aqueous soluble compounds from moving past these cells.


paracellular
pathway
Tight Junction
apical membrane
domain
basolateral membrane
domain
transcellular
pathway

- For oral immunization (vaccination), only a small fraction of the antigen (protein) has to reach its target site to elicit an immune response.
- The target cells are lymphocytes and antigen presenting accessory cells located in Payer's patches



The intestinal Payer's patches

Notes :

1) These regions characterized with:

a) Little lysosomal degradation capacity

b) Mucous producing goblet cell density is reduced (less mucus production).

2) As attempts to improve the antigens delivery here, microspheres, liposomes or modified live vectors (like attenuated bacteria and viruses) are used.