LECTURE-2

Excipients Used in Parenteral Formulations of Biotech Product

- **In a protein formulation** (active substance), a number of **excipients selected** to serve different purposes.

This formulation design should be carried out with great care) to ensure therapeutic effectiveness and safe products.

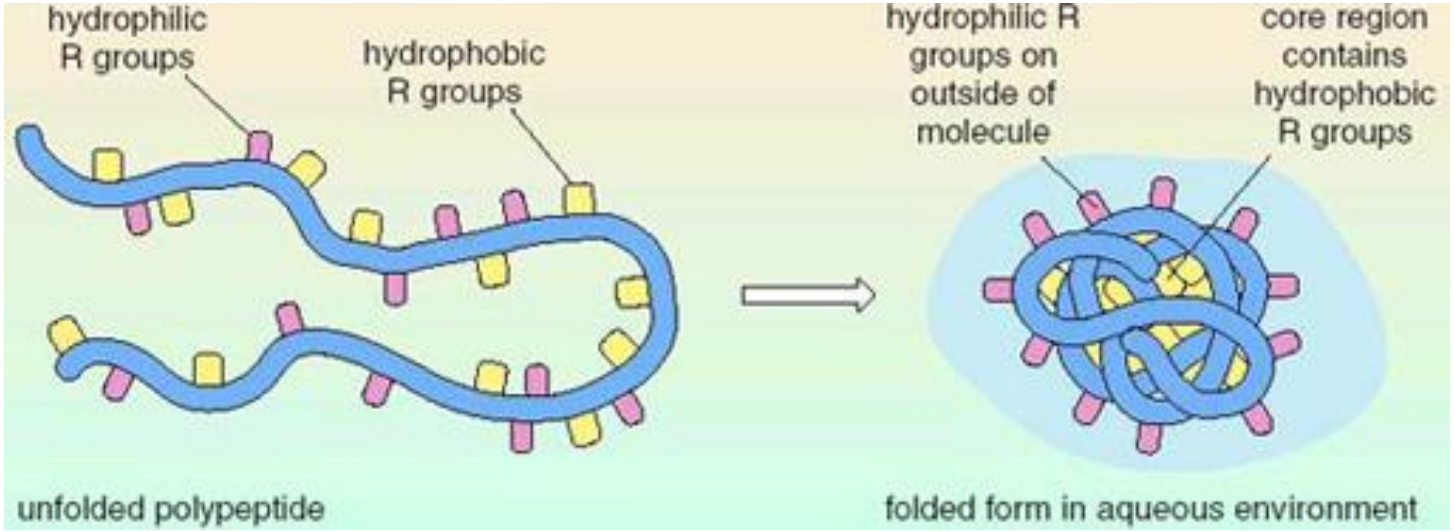
- **The nature of the protein** (e.g. lability-rapid change or destroyed-) and its **therapeutic use** (e.g. multiple injection systems) can **make these formulations quite complex in term of excipients profile and technology (freeze-drying, aseptic preparation**).

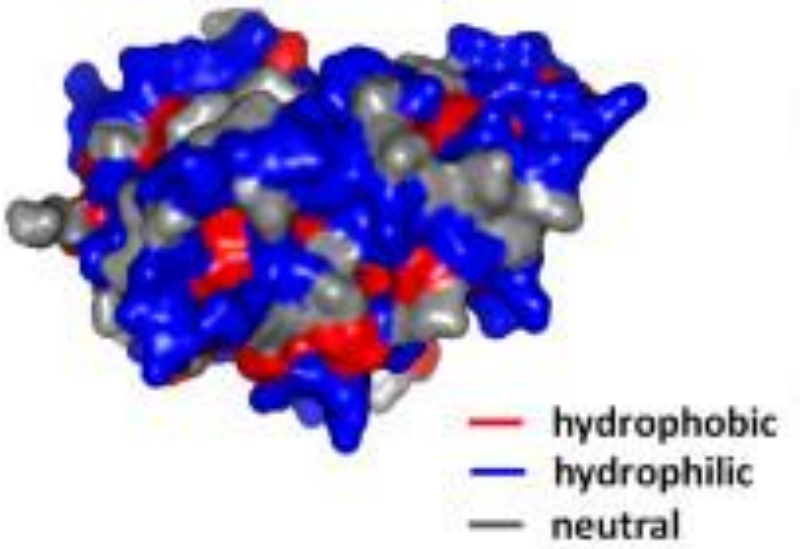
COMPONENTS FOUND IN PARENTERAL FORMULATIONS OF BIOTECH PRODUCTS

1. Active ingredient
2. Solubility enhancers
3. Anti-adsorption and anti-aggregation agents
4. Buffer components
5. Preservatives and anti-oxidants
6. Lyoprotectants/ cake formers
7. Osmotic agents
8. Carrier system

Note: All of the above are not necessarily present in one particular protein formulation

Structure of protein

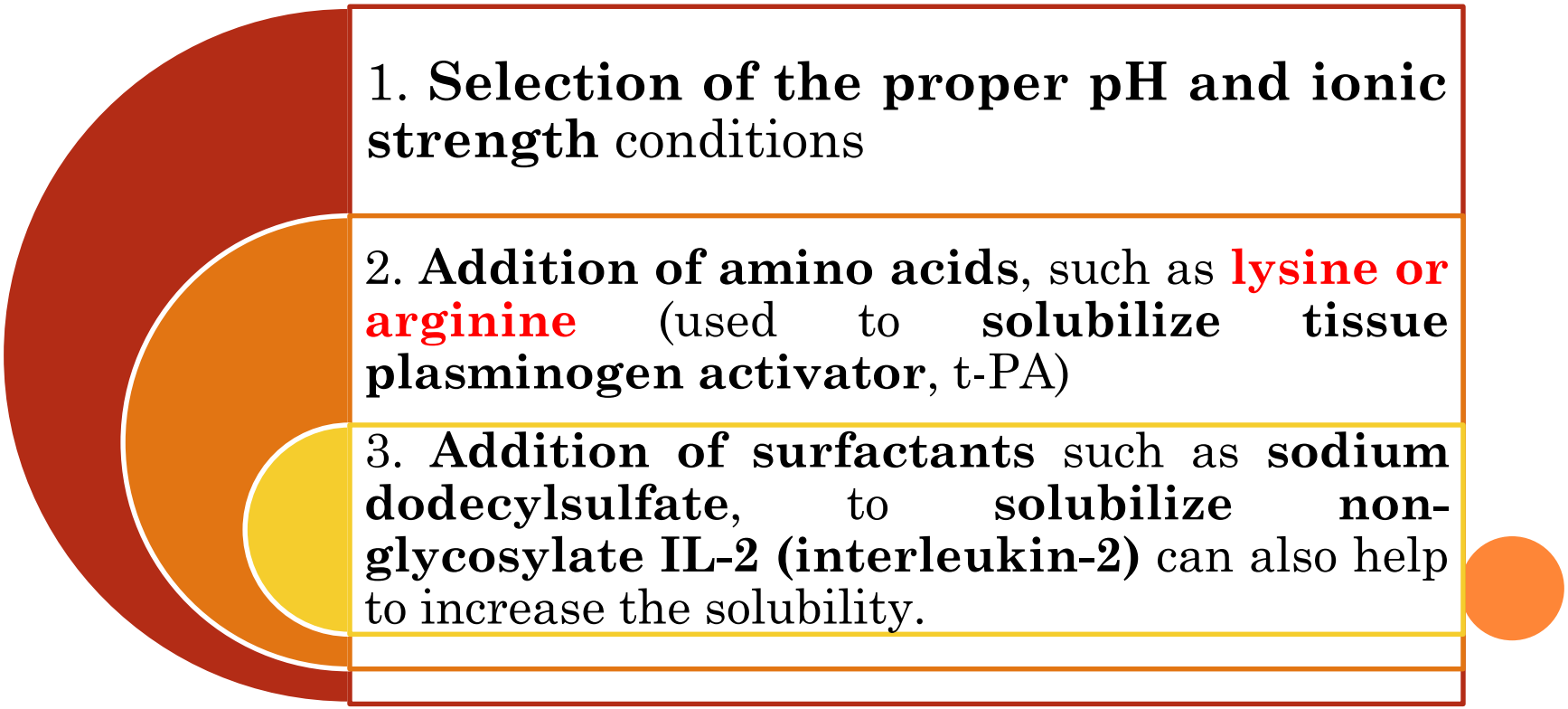




2. Solubility Enhancers

- **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:



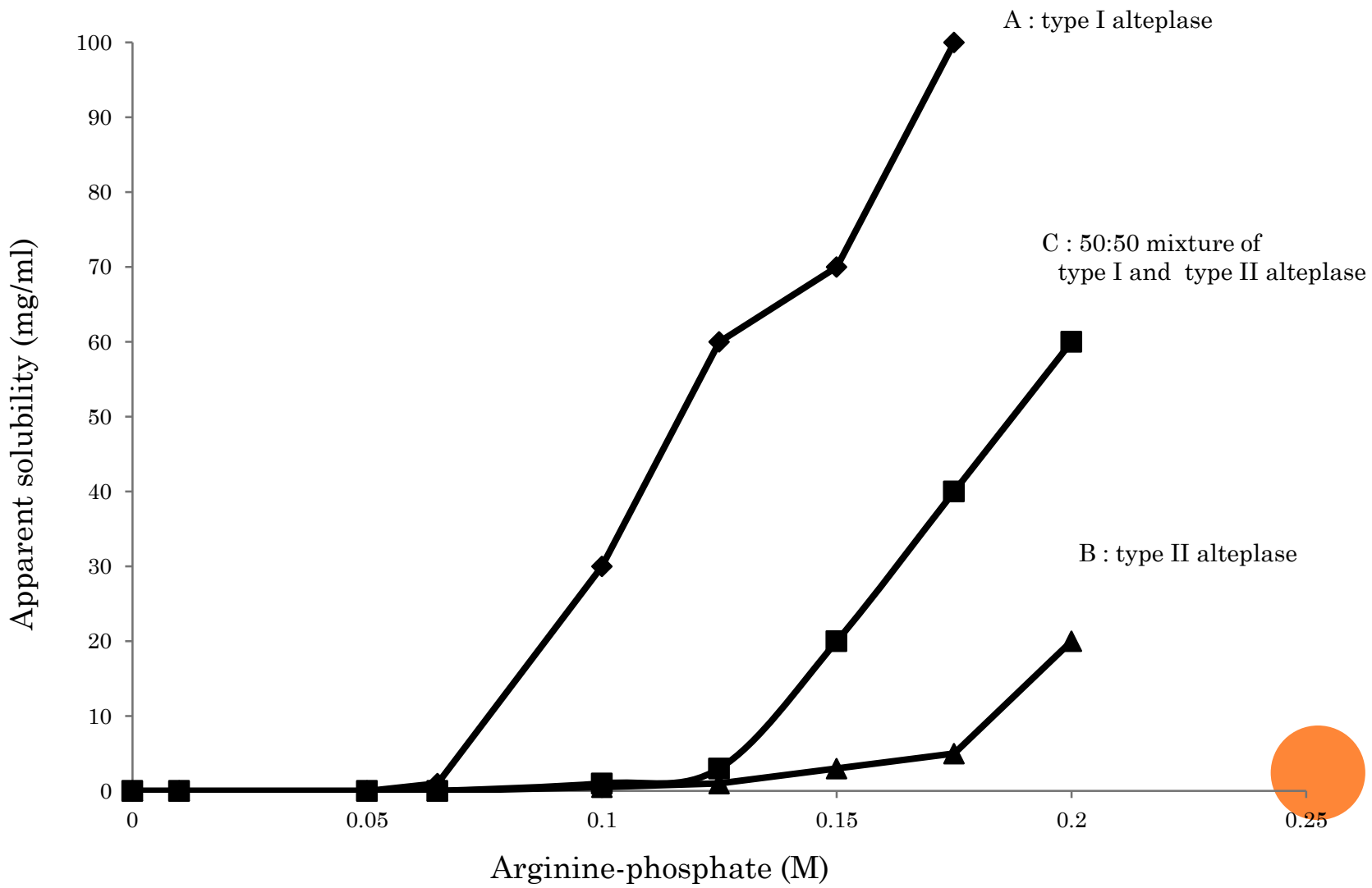
The mechanism of action of these solubility enhancers

depends on

Type of enhancer and protein involved and is not always fully understood.

- In the previous examples (t-PA and **IL-2**) aggregation is physical in nature, i.e. based on hydrophobic and/ or electrostatic interactions between molecules by Formation of covalent bridges between molecules through disulfide bonds, and ester or amide linkages
- In these cases proper conditions should be found to avoid these chemical reactions (the figure above clearly indicates the dramatic effect of this basic amino acid on the apparent solubility of t-PA).

Figure 1: Shows the effect of arginine concentration on the solubility of t-PA (alteplase) at pH 7.2 and 25°C.



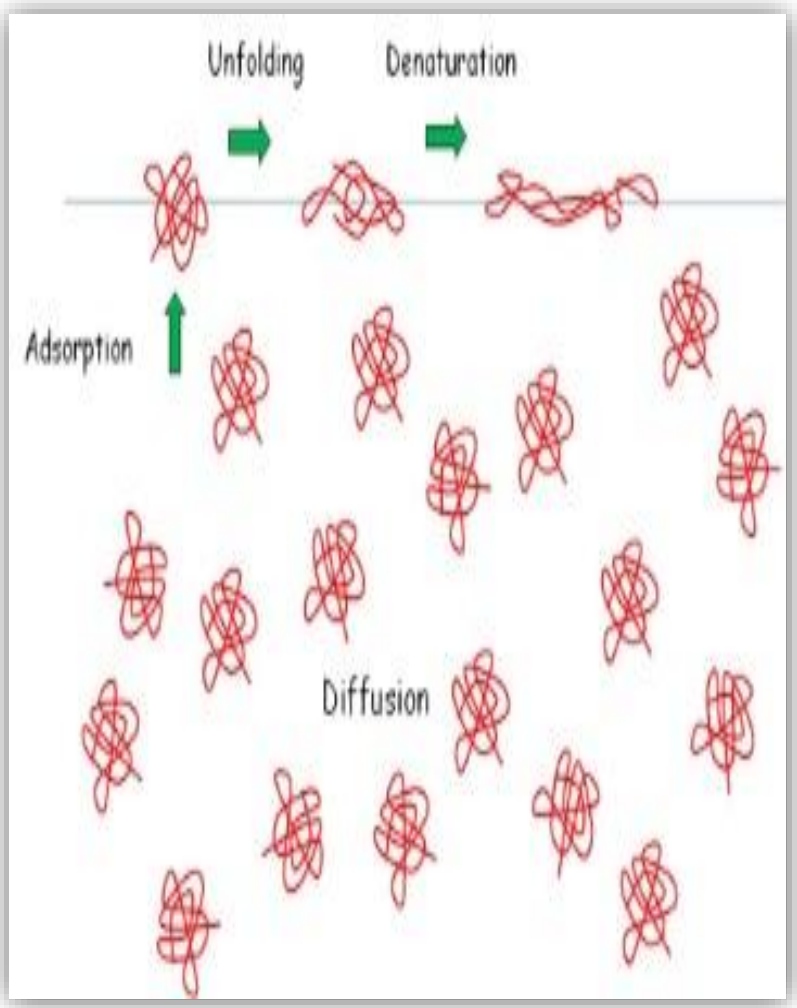
3. Anti-adsorption and anti-aggregation agents

- **Anti-adsorption agents (added to reduce adsorption of the active protein to interfaces**).

- Some **proteins** normally have **hydrophobic sites in the core** structure. They tend to **expose hydrophobic sites** when an interface is present.

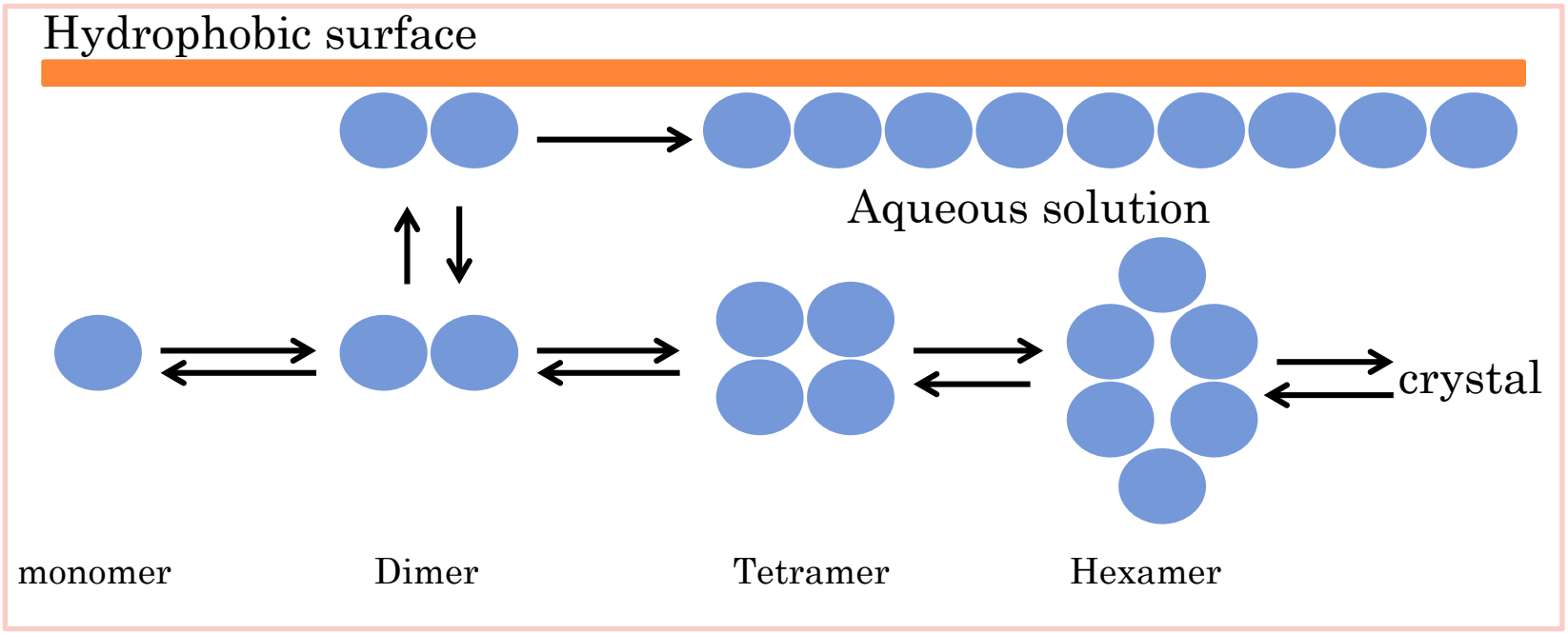
- These **interfaces** can be **water/air, water/container wall** or **interfaces formed between the aqueous phase and utensils** used to administer the drug (e.g. **catheter, needle).**

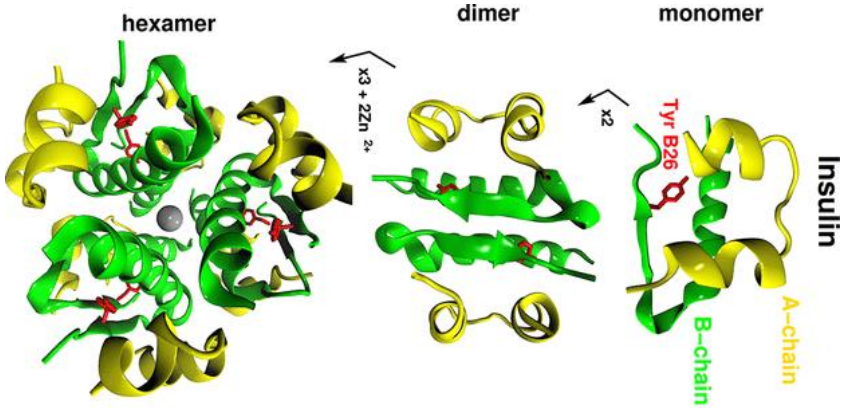
- These **adsorbed**, **partially unfolded protein molecules form aggregates**, leave the surface, return to the aqueous phase, **form larger aggregates and precipitate**.



Example:

- The proposed mechanism for aggregation of insulin in aqueous media through contact with a hydrophobic surface (or water-air interface).
- Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein film





- **Native insulin in solution** is in an **equilibrium state** between monomeric, dimeric, tetrameric and hexameric form.
- The relative **abundance** of the different aggregation states **depends on the pH, insulin concentration, ionic strength and specific excipients (Zn^{2+} and phenol)**.
- **Suggestion:** dimeric form of insulin adsorbs to hydrophobic interfaces and subsequently forms larger aggregates at the interface.
- **This adsorption explains why anti-adhesion agents can also act as anti-aggregation agents.**
- Ex: Albumin (strong tendency to adsorb to surfaces) and is therefore added in relatively high concentration (e.g. 1%) as an anti-adhesion agent to protein formulations.
- **MECHANISM:** albumin competes with the therapeutic protein for binding sites and prevents adhesion of the therapeutically active agent by combination of its binding tendency and abundant presence.

- **Insulin** is one of the many proteins that can form **fibrillar precipitates** (long rod-shaped structures with diameters in the 0.1 μm range).

This can be prevented by:

1. **Low concentrations of phospholipids and surfactants** (as a fibrillation-inhibitory effect).
2. The **selection of the proper pH** to prevent this unwanted phenomenon.

- Apart from albumin, **surfactants** can also **prevent adhesion to interfaces and precipitation**.

Readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups phase.

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

- **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 is:

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

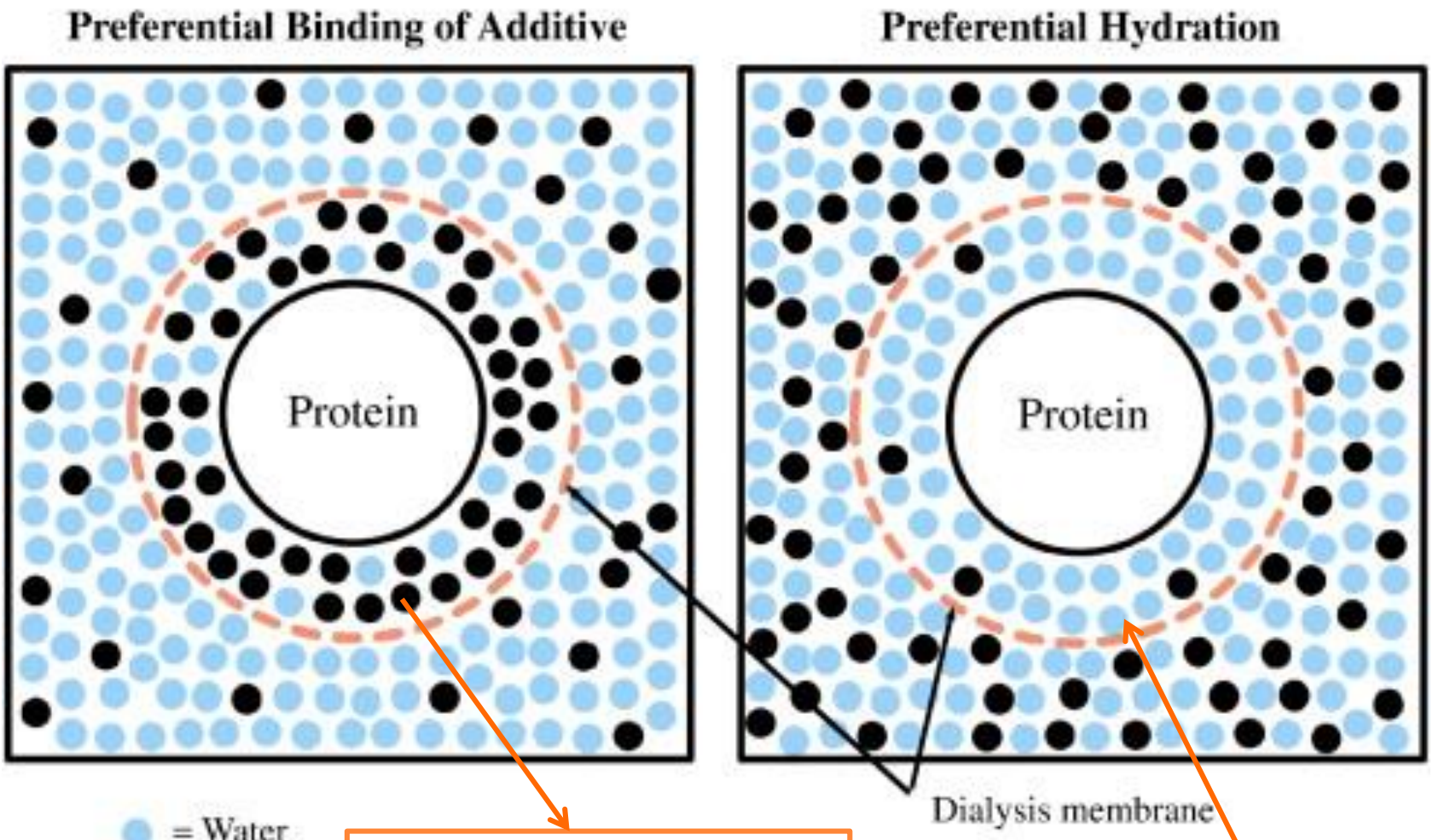
These excipients may not be inert; they may influence protein structural stability.

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).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