Pharmaceutical Biotechnology

Lecture 3-

EXCIPIENTS USED IN PARENTERAL FORMULATIONS OF BIOTECH PRODUCTS

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EXCIPIENTS USED IN PARENTERAL FORMULATIONS OF BIOTECH PRODUCTS

- Active ingredient
- Solubility enhancers
- Anti-adsorption and anti-aggregation agents
- Buffer components
- Preservatives and antioxidants
- Lyoprotectants/cake formers
- Osmotic agents
- Carrier system (see later in this chapter)

Components found in parenteral formulations of biotech products. All of the above are not necessarily present in one particular protein formulation.

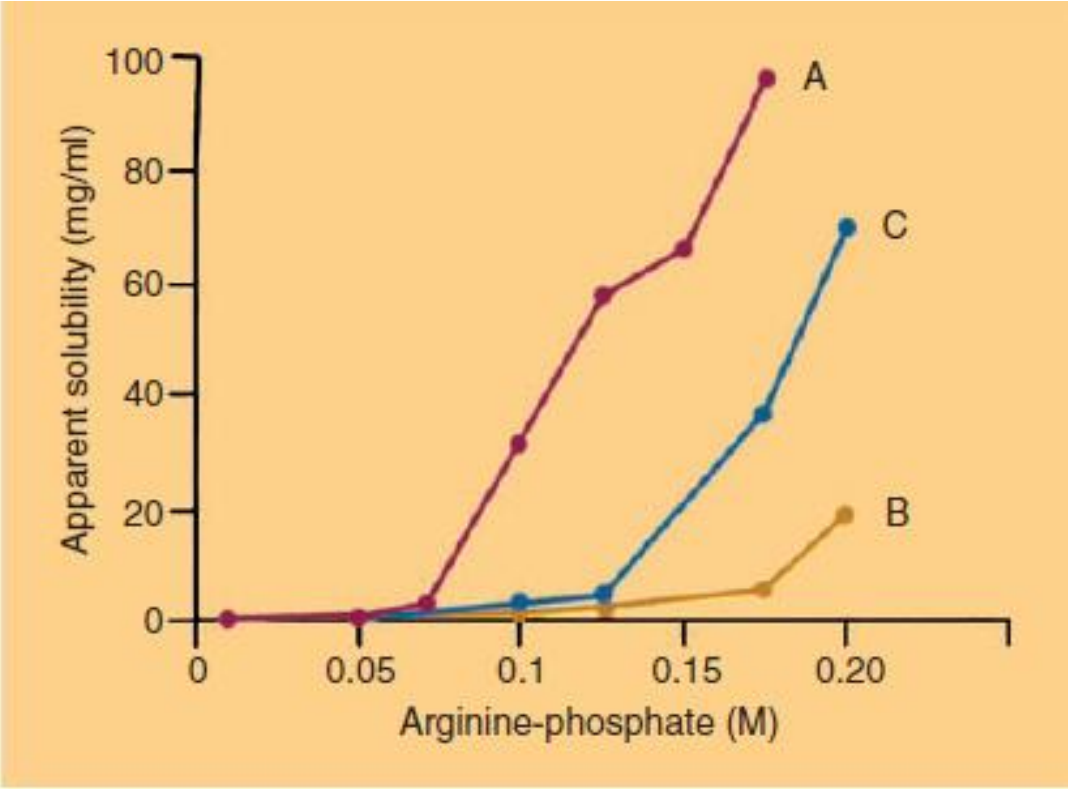
❑Solubility Enhancers

Proteins, in particular those that are non-glycosylated, may have a tendency to aggregate and precipitate.

Examples on solubility enhancers:

- ❖ proper pH and ionic strength conditions can enhance the solubility of proteins.
- ❖ Addition of amino acids such as lysine or arginine (used to solubilize tissue plasminogen activator, t-PA),
- ❖ or surfactants, such as sodium dodecylsulfate to stabilize non-glycosylated IL-2, can also help to increase the solubility

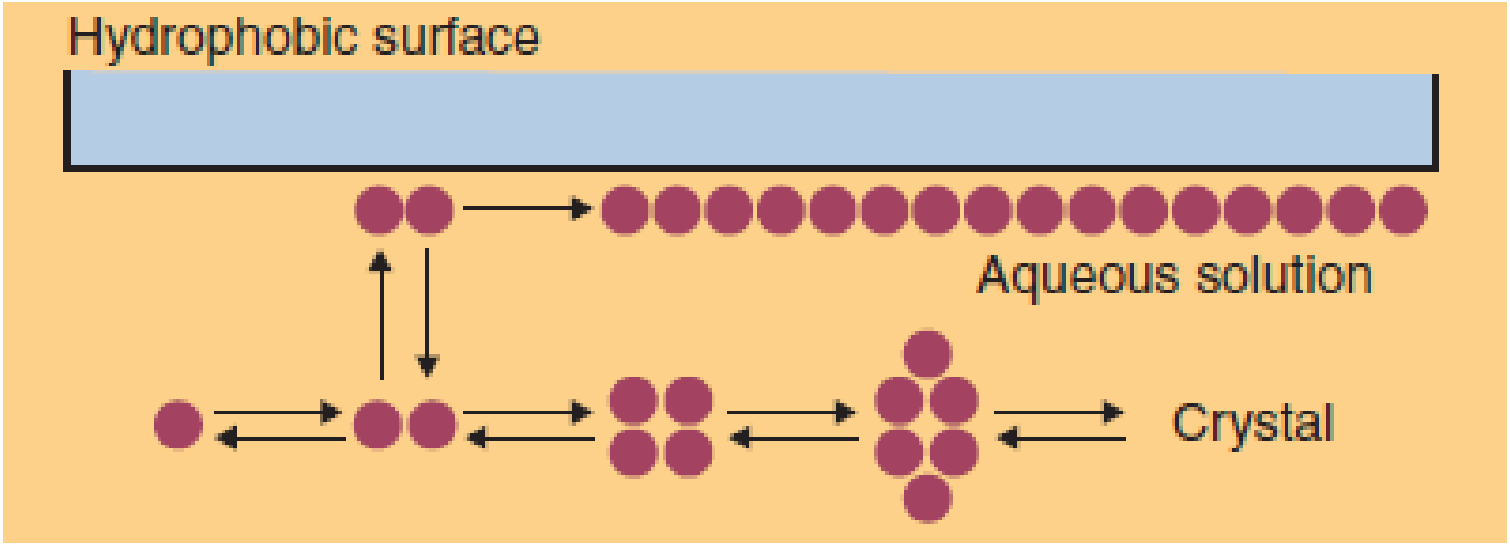
Solubility Enhancers



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. Source: From Nguyen and Ward, 1993.

❑ Anti-Adsorption and Anti-Aggregation Agents

- **Anti-adsorption agents are added to reduce adsorption of the active protein to interfaces.**
- **Some proteins tend to expose hydrophobic sites, normally present inthe core of the native protein structure 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 unfold 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 is presented in the following figure.**



Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein films. Each circle represents a monomeric insulin molecule.

Native insulin in solution is in an equilibrium state between monomeric, dimeric tetrameric, and hexameric forms . The relative abundance of the different aggregation states depend on the pH, insulin concentration, ionic strength, and specific excipients (e.g., Zn2þ and phenol). It has been suggested that the dimeric form of insulin adsorbs to hydrophobic interfaces and subsequently forms larger aggregates at the interface. This explains why anti adhesion agents can also act as anti-aggregation agents, Insulin is one of the many proteins that can form fibrillar precipitates (long rod-shaped structures with diameters in the 0.1 mm range).

- **Low concentrations of phospholipids** and **surfactants** have been shown to exert a fibrillation-inhibitory effect The **selection of the proper pH** can also help to prevent this unwanted phenomenon.

❑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.

- **Buffers used in biotech formulation are:**

✓ phosphate
✓ citrate
✓ acetate

- **temporary pH changes can cause aggregation. These conditions can occur, for example during the freezing step in a 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. This causes a noticed drop in pH during the freezing step. Other buffer components do not crystallize, but form amorphous systems and then pH changes are minimized.**

❑Preservatives and Antioxidants

- Methionine, cysteine, tryptophan, tyrosine, and histidine are amino acids that are readily oxidized.

- **Proteins rich in these amino acids are susceptible to oxidative degradation.**

- **Replacement of oxygen by inert gases in the vials helps to reduce oxidative stress.**

- **Moreover, the addition of antioxidants such as ascorbic acid or acetylcysteine can be considered.**

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❑ Preservatives and Antioxidants

- Certain proteins are formulated in containers designed for multiple injection schemes.
- After administering the first dose, contamination with microorganisms may occur and preservatives are needed to minimize growth.
- Usually, these preservatives are present in concentrations that are bacteriostatic rather than bactericidal in nature.
- Antimicrobial agents are the mercury-containing phenylmercuric nitrate and thimerosal and p-hydroxybenzoic acids, phenol, benzyl alcohol and chlorobutanol.
- The use of mercury containing preservatives is presently under discussion (FDA)

❑Osmotic Agents:

- For proteins the regular rules apply for adjusting the tonicity of parenteral products. Saline and mono- or disaccharide solutions are commonly used.

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

- For example, sugars and polyhydric alcohols can stabilize the protein structure through the principle of "**preferential exclusion**"

- These additives enhance the interaction of the solvent with the protein and are themselves excluded from the protein surface layer; the protein is preferentially hydrated.

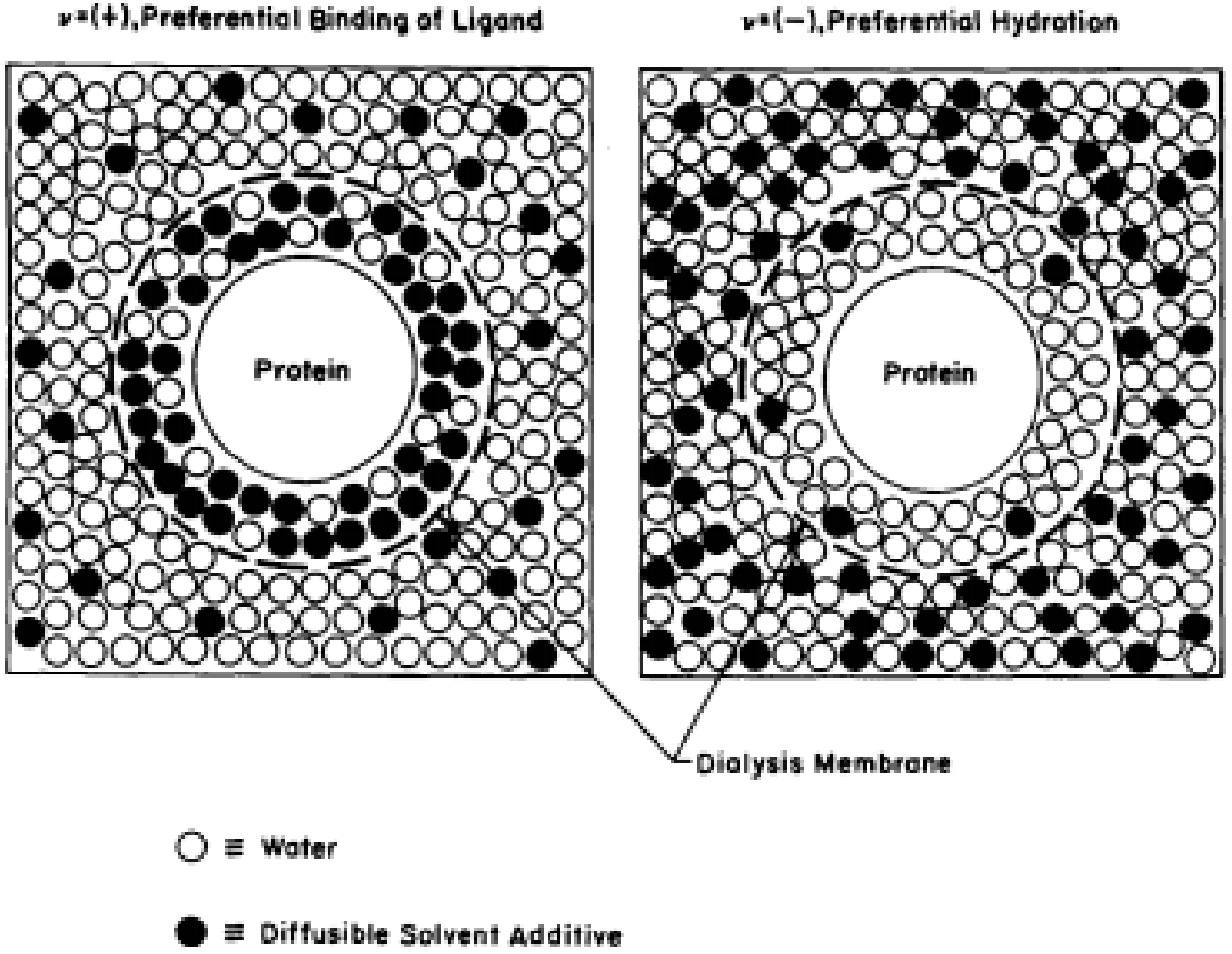

Fig. 1-5: Schematic representation of preferential binding and preferential hydration. Adapted from Timasheff (1992a).

Thanks!