Pharmaceutical Biotechnology
Lec. 10

Chapter 4
Formulation Of Biotech Products, Including Biopharmaceutical Considerations

Dr. Marwan Abu-Halaweh
Office 908
Email: mhalaweh@philadelphia.edu.jo
Subjects: Pharmaceutical Biotechnology
Philadelphia University

Microbiological Consideration

- **1- Sterility:**
- Proteins are administrated parenterally and have to be sterile.
- Proteins are sensitive to heat so they cannot be autoclaved, gas sterilization or by ionizing radiation. Which mean that sterilization of the end product is not possible.
- Therefore, Protein pharmaceutical have to be assembled under a septic conditions.

Microbiological Consideration

1- Sterility:

- Equipment and excipients are treated separately and autoclaved, or sterilized by dry heat more than 160 C°.
- Chemical treatment or gamma radiation to minimize the bioburden.
- Filtration techniques are used for the removal of microbial contaminants (filtration through 0.2 or 0.22 μm membrane).
- Product assembly done in class I00 (maximum I00 particles > 0.5 μm per cubic foot) room, with laminar air flow filtered through HEPA (high Efficiency Particulate Air) filter.

Microbiological Consideration

2- Viral decontamination:

- Recombinant DNA products are grown in microorganisms; these organisms should be tested for viral contamination.
- Excipients with a certain risk factor, such as blood derived human serum albumin, should be carefully tested before use.

Microbiological Consideration

3- Pyrogens removal:

- Pyrogens are compounds that induce fever
- Exogenous pyrogens: not produced by the body, come from outside.
- - Sources: Bacterial, viral or fungal.
- Bacterial endotoxin are mainly lipopolysaccharides (LPS) Gram negative.
- Ion exchange chromatography procedures can effectively reduce endotoxin level in solution. By utilizing its –ve charge.
- Pharmaceutical products and excipients used in protein formulation should be endotoxin free.

Microbiological Consideration

3- Pyrogens removal:

- should be endotoxin free which can be done by reverse osmosis.
- Clean of the containers could be done by using activated charcoal or other material with large surface offering hydrophobic interaction All solutions and material used throughout the production step.

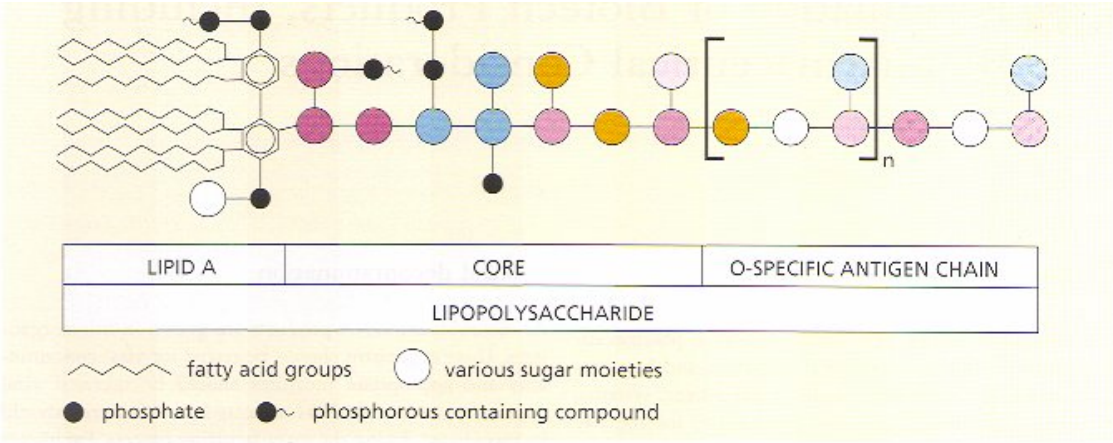

Figure 4.1. Generalized structure of endotoxins. Most properties of endotoxins are accounted for by the active, insoluble 'Lipid A' fraction being solubilized by the various sugar moieties (circles with different colors). Although the general structure is similar, individual endotoxins vary according to their source and are characterized by the O-specific antigenic chain. Adapted from Groves 1988.

Excipients Used in Parenteral Formulation of Biotechnology Products:

- The following components are found in the presently marketed formulation (table 4.1, page 74 in the text book): which will be explained in details later.
  1. Active ingredient
  2. Solubility enhancer
  3. Anti-adsorption and anti-aggregation agents.
  4. Buffer components
  5. Preservative and anti-oxidants.
  6. Lyoprotectants/cake formers
  7. Osmotic agents
  8. Carrier system

Excipients Used in Parenteral Formulation of Biotechnology Products:

- Solubility enhancers:
- The non-glycosylated proteins 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 arginine and lysine which used to stabilize t-PA,
  - Or surfactants, such as SDS to stabilize non-glycosylated IL-2, can also help to increase the solubility.

Excipients Used in Parenteral Formulation of Biotechnology Products:

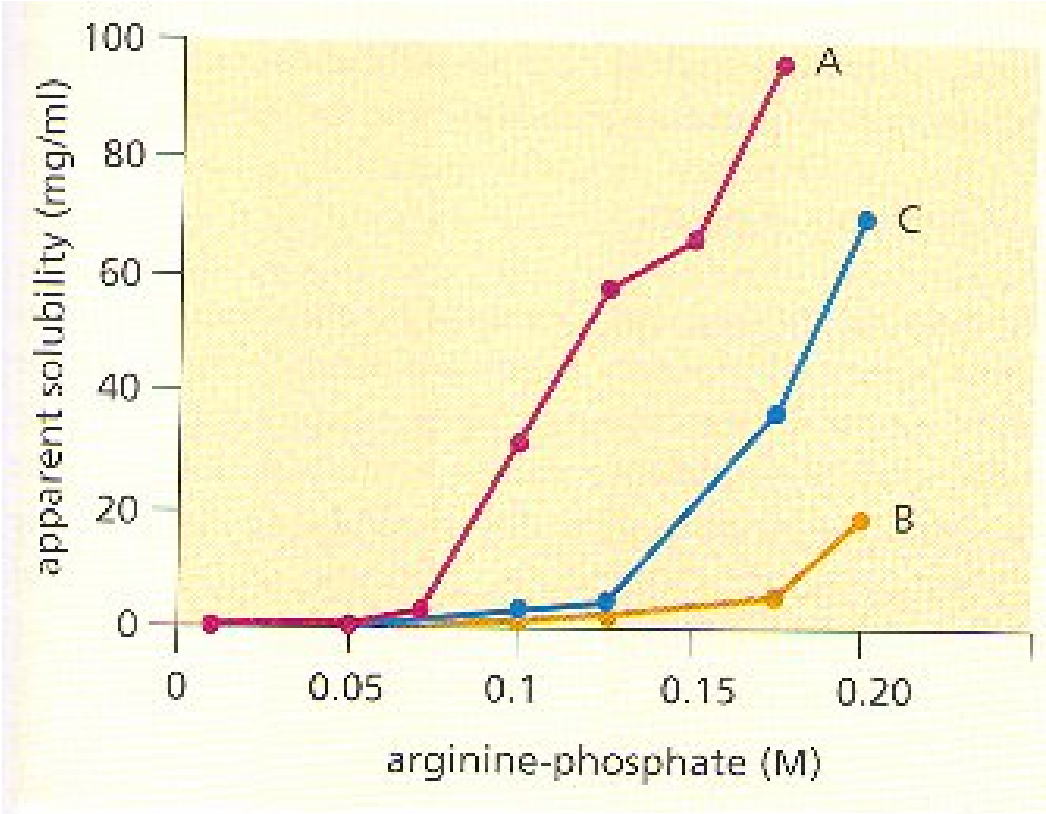

Figure 4.2. Effect of arginine on type I and type II alteplase at pH 7.2 and 25 ° C. A, type I alteplase; B, type II alteplase; C, 50:50 mixture of type I and type II alteplase. From Nguyen and Ward,1993.

Excipients Used in Parenteral Formulation of Biotechnology Products:

2- Anti-adsorption and Anti-aggregation agents

- Anti-adsorption agents are added to reduce adsorption of the active protein to interface.
- Some proteins tend to expose hydrophobic sites, normally present in the core of the native protein structure when an interface is present.
- These interfaces can be water/air, water/container wall or interface formed between the aqueous phase and utensils used to administrate the drug (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 Fig.4.

Excipients Used in Parenteral Formulation of Biotechnology Products:

2- Anti-adsorption and Anti-aggregation agents

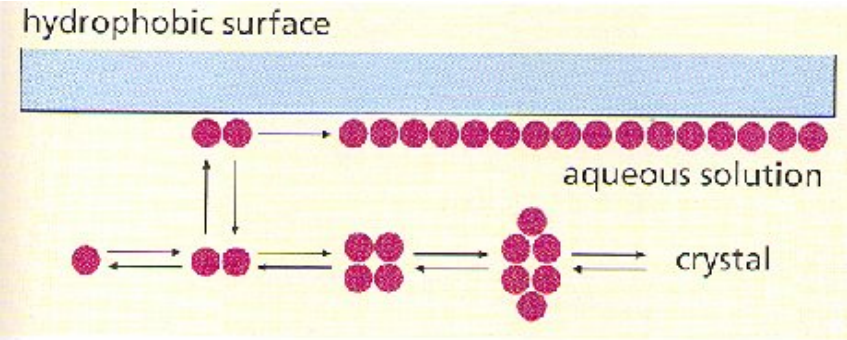

Figure 4.3. Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein film: ● represents a monomeric insulin molecule. Adapted from Thurow and Geisen, 1984.

- 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 Fig.4.

Excipients Used in Parenteral Formulation of Biotechnology Products:

3- 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
  - Phosphate
  - Citrate
  - acetate

Excipients Used in Parenteral Formulation of Biotechnology Products:

3- Buffer components:

- Even short, temporary pH changes can cause aggregation. These conditions can occur, for example, during the freezing step in the freeze drying process, when one of the buffer components is crystallizing and the other is not (In Phosphate buffer, Na2HPO4 crystallizes faster than NaH2PO4).
- This cause a pronounced drop in pH during the freezing step.
- Other buffer components do not crystallize, but form amorphous systems and then pH changes are minimized

Excipients Used in Parenteral Formulation of Biotechnology Products:

4- Preservatives and Anti-oxidants:

- Methionine, cystiene, tryptophane and histedine are amino acids that are readily oxidized.
- Proteins rich in these amino acids are susceptible to oxidative degradation.
- The replacement of oxygen by inert gases in the vials helps to reduce oxidative stress.
- Moreover , the addition of antioxidant, such as Ascorbic acid, Sodium Formaldehyde sulfoxylate, can be considered

Excipients Used in Parenteral Formulation of Biotechnology Products:

4- Preservatives and Anti-oxidants:

- Certain protein are formulated in containers designed for multiple injection schemes.
- After opening the container contamination with different type of micro-organisms may occur to solve this problems different preservative have been used.
- E.g. of such preservative are phenol, benzyl alcohol etc.

Excipients Used in Parenteral Formulation of Biotechnology Products:

5- Osmotic agents:

- For proteins, the regular rules apply for adjusting the tonicity of parenteral product. 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 alcohol 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.