Formulation of Biotech products

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- Most of the biotech products are proteins, but some may soon be smaller peptide-like molecules.
- Proteins are inherently unstable molecules, and their degradation profile can be quite complex.
- They differs from conventional small molecules drug products.(h.w)?

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- Pharmacists involved in compounding with biologically active proteins will be interested in their stabilization, formulation and delivery.
- Protein drugs are extremely potent and are generally used in quite low concentrations.

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Formulation of Biotech products: Includes:

- Microbiological considerations
- Preformulation considerations
- Pharmaceutical considerations
- Biopharmaceutical considerations

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Microbiological considerations: include

- Sterility
- Viral decontamination
- Pyrogen removal

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Sterility:

- Most proteins are administered parenterally and have to be sterile.
- Sterilization of the end product in not possible using autoclaving, gas or ionizing radiation sterilization methods.
- Then the production under aseptic conditions, equipment and excipients are treated separately and autoclaved or sterilized by dry heat (>160°C), chemical treatment or gamma radiation to minimize the bio-burden.

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- Filtration techniques are used for removal of micro-bacterial contaminants.
- Pre-filters remove the bulk of the bio-burden and other particulate materials.
- The final sterilizing step before filling the vials is filtration through 0.2 or 0.22 µm membrane filters (low-protein-binding filters).
- High efficiency particular air (HEPA) filters found in rooms of production.

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- Human factor is a major source of contamination.
- Well trained operators wearing protective cloths (mask, hats, gowns, gloves or head-to-toe overall garments) should operate the facility.

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Viral decontamination:

- Recombinant DNA products (grown in microorganisms) must be free of viruses.
- No unwanted viral material (considered as antigens) should be introduced during the manufacturing process.

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- The viral contaminants can be inactivated by (heat treatment, radiation, dehydration, denaturizing agents and neutralization) and removed by (chromatography, filtration and preciptation).
- Also, excipients like a blood-derived human serum albumin should be tested.

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Pyrogen removal:

- Are components that induce fever, derived from bacterial, viral or fungal sources.
- Bacterial pyrogens are mainly endotoxins shed from G-ve bacteria, which are lipopolysaccharides with high negative electrical charge and adsorption tendency on the surfaces.
- They are stable under standard autoclaving conditions, but break down when heated in the dry state. For this reason, equipment and container are treated at temperatures above 160°C for prolonged periods (e.g. 30min. Dry heat at 250°C).

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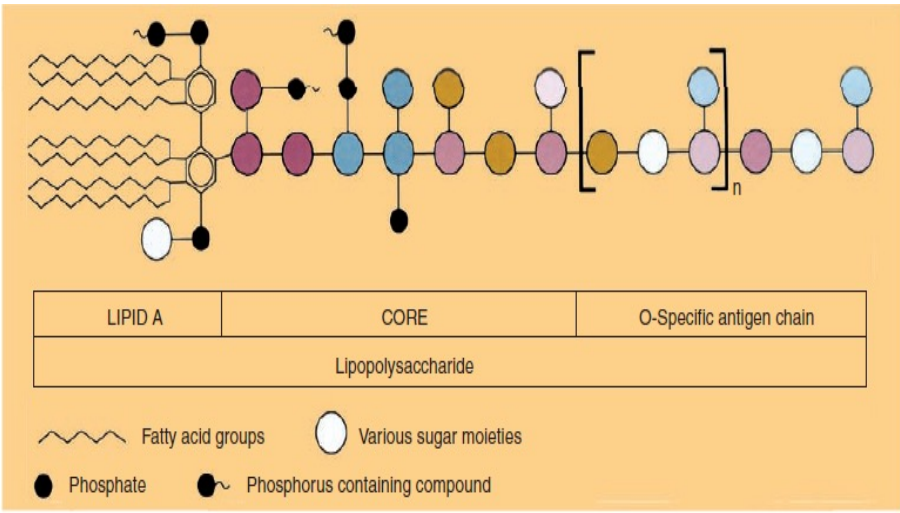

Generalized structure of endotoxins

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- Ion exchange chromatography can effectively reduce endotoxin levels in solution.
- Excipients used in the protein formulation should be essentially endotoxin free. For solutions like (Water for injection) is freshly distilled or produced by reverse osmosis RO. Where, the aggregated endotoxins cannot pass through the RO membrane.

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- Removal of endotoxins immediately before filling the final container can be occurred by using activated charcoal or other materials with large surface offering hydrophobic interactions.
- They are also inactivated on utensil surfaces by oxidation (e.g. peroxide) or dry heating (e.g 30min. Dry heat at 250°C)

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Preformulation considerations

- Includes mainly the physicochemical considerations.
- like, the molecular weight (high), potential for aggregation and adsorption, immunogenic potential, solubility, protein-excipient incompatibility, the structure, isoelectric point, stability, polymorphism, stereoisomers, surface denaturation and filtration media compatibility.

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- The solubility depends on a number of factors, including chemical structure, pH, and temperature.
- Stability (physical and chemical) depends on pH, temperature, agitation and the overall environment of container.

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Pharmaceutical considerations

- The aim of formulation (liquid or dry), is to get therapeutically effective, stable and safe products. So different excipients are used to get that.

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Types of excipients:

- Solubility enhancers.
- Anti-adsorption and anti-aggregation agents
- Buffer components
- Stabilizers
- Osmotic agents/tonicity adjusting agents
- Lyo-protectants/cake formers
- Carrier system

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Solubility enhancers:

- **Added due to the aggregation and precipitation properties of proteins (specially non glycosylated types).**
- **As approaches used are:**

1) **Selection of the proper pH and ionic strength conditions.**
2) **Addition of amino acids such as alanine, lysine or arginine (used with tissue plasminogen activator t-PA).**
3) **Use of surfactants such as sodium dodecyl sulfate to solubilize as ex. non-glycosylated IL-2.**

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Note:

- Proteins are generally more soluble in their native environment or medium in presence of sodium chloride, trace elements, lipids and other proteins in an aqueous medium.

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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 in the 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).

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- These adsorbed, partially unfolded protein molecules form aggregates, leave the surface, return to the aqueous phase, form larger aggregates and precipitate. (As in aqueous insulin solutions).
- Native insulin in solution is in an equilibrium state between monomeric, dimeric, tetrameric and hexameric forms.

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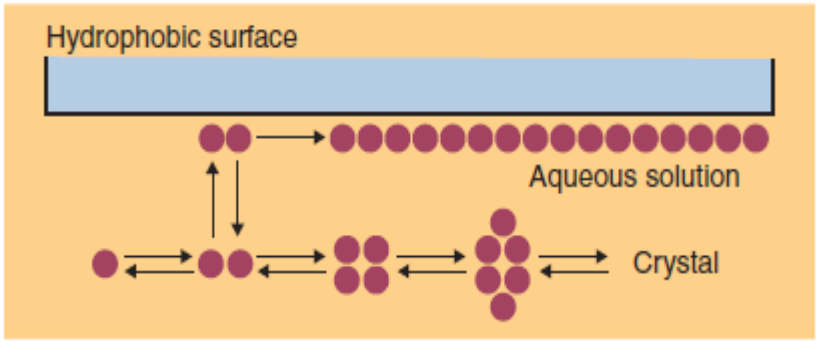

Figure 3 Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein films. Each circle represents a monomeric insulin molecule. *Source*: Adapted from Thurow and Geisen,

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- The relative abundance of the different aggregation states depends on the pH, insulin concentration, ionic strength and specific excipients (e.g., Zn^{2+} and phenol).
- Aggregation is physical in nature, i.e. based on hydrophobic and/or electrostatic interactions between molecules.

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- Also, aggregation based on the formation of covalent bridges between molecules through disulfide bonds and ester or amide linkages (chemical reaction).
  - To avoid that, albumin can be used as anti-adhesion agent (by competition on the binding sites), leucine can be used as antiaggregation.
  - Low concentration of phospholipids and surfactants used for decreasing of insulin fibrillation (ppt.).

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- The selection of proper pH.
- Surfactants like poly-sorbate 20 or 80 can also prevent adhesion to interfaces and ppt., these molecules readily adsorb to hydrophobic interfaces with their own hydrophobic groups and render this interface hydrophilic by exposing their hydrophilic groups to the aqueous phase.
- Polymers like PEG and povidone can be used.

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Note

- The adsorption can be decreased by **Siliconization**, soaking or rinsing of the glass vials in a silicon solution or emulsion. Then the drained containers should be placed in an oven at about 250°C for 5-6 hours.

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Buffer components:

- Buffer selection is an important part of the formulation process, because of the pH dependence of protein solubility and stability (physical and chemical).
- Buffer systems used may be phosphate, citrate and acetate.
- An increase in buffer concentration means an increase in pain on injection.

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- Even short, 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 pronounced drop in pH during the freezing step. Other buffer components do not crystallize, but form amorphous systems and then pH changes are minimized.

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