FORMULATION CONSIDERATION OF BIOLOGICAL DRUGS

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 means that sterilization of the end product is not possible.
- Therefore, Protein pharmaceutical have to be assembled under aseptic 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.
- **Cleaning 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.

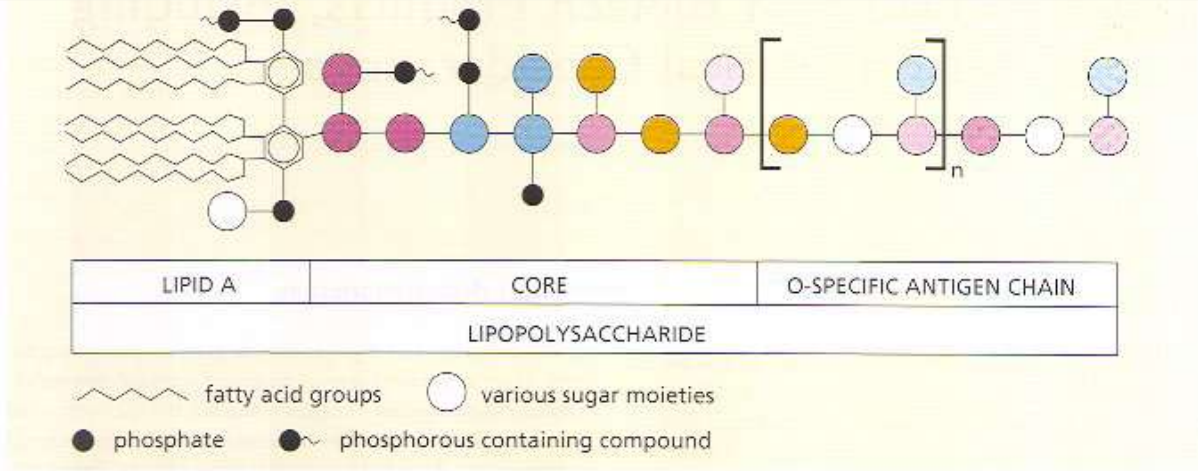

Figure 4.1. Generalized structure of endotoxins. Most properties of endotoxins are accounted for by the active, insoluble 'Lipid A' fraction being solubilized by the various sugar moieties (circles with different colors). Although the general structure is similar, individual endotoxins vary according 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:

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

1. 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 nonglycosylated IL-2, can also help to increase the solubility.*

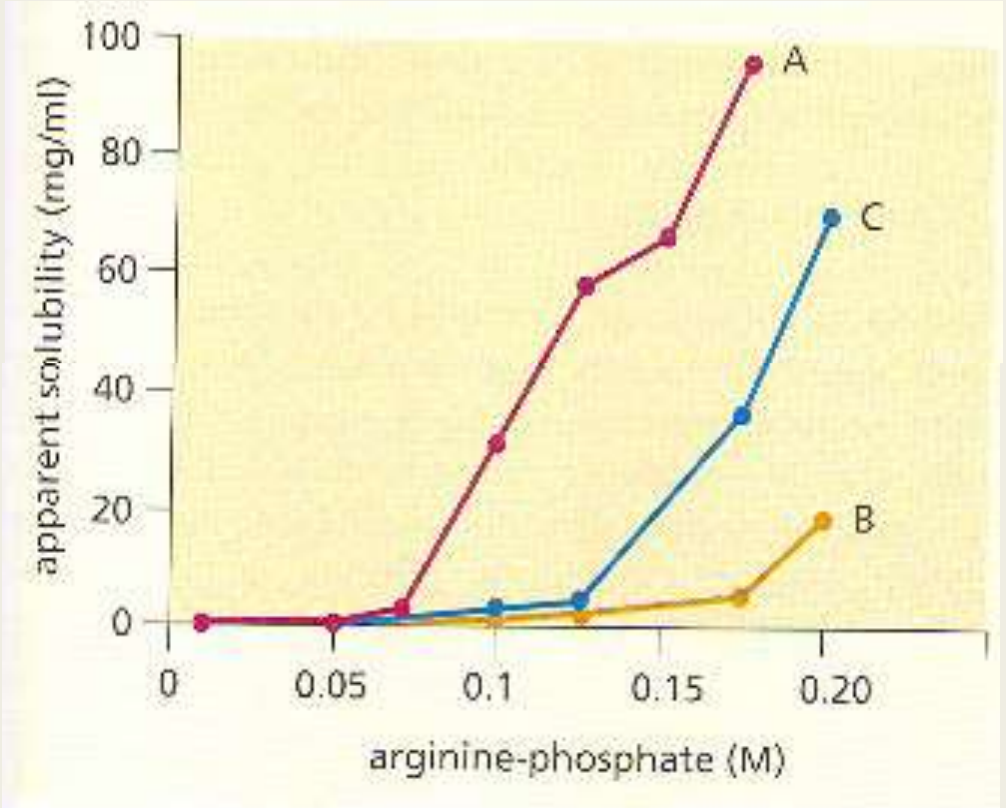

Figure 4.2. Effect of arginine on type I and type II alteplase at pH 7.2 and 25 ° C. A, type I alteplase; B, type II alteplase; C, 50:50 mixture of type I and type II alteplase. From Nguyen and Ward,1993.

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).

Excipients Used in Parenteral Formulation of Biotechnology Products

2. Anti-adsorption and Anti-aggregation agents

- 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

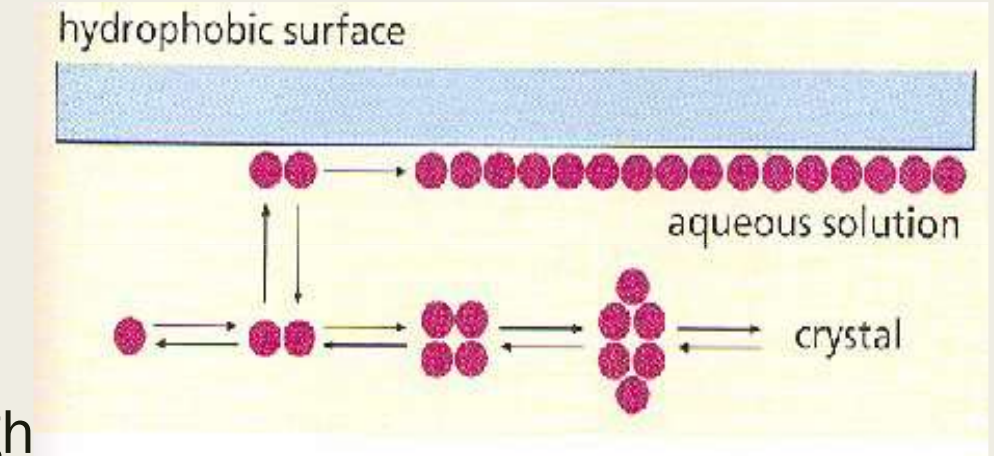

Figure 4.3. Reversible self-association of insulin, its adsorption to the hydrophobic interface and irreversible aggregation in the adsorbed protein film: ● represents a monomeric insulin molecule. Adapted from Thurow and Geisen, 1984.

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, Na2HP04 crystallizes faster than NaH2P04).
- 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, cysteine, tryptophan and histidine 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

Storage of Biopharmaceuticals

- Biotech products have unique storage requirements when compared to the majority of products that pharmacists normally dispense.
- The shelf life of these products is considerably shorter than for traditional compounds. For example, **interferon-α2a** is only stable in a refrigerator in **the ready-to-use solution for 2 years**.
- **After the first dose, cartridges may be stored at less than 25oC for up to 28 days** although refrigeration is recommended. Since most of these products need to be kept at refrigerated temperatures, some pharmacies may need to **increase cold storage space** in order to accommodate the storage needs.

Storage of Biopharmaceuticals

- Since biotech products are primarily proteins, they are subject to **denaturation when exposed to extreme temperatures.** In general, most biotech products are shipped by the manufacturer in **gel ice containers** and need to be stored at 2C to 8C
- **Once reconstituted**, they should be stored under **refrigeration** until just prior to use. There are a few exceptions to this rule. For example, **alteplase** (tissue plasminogen activator) **lyophilized powder** is stable at room temperature for **several years** at temperatures not to exceed 30C. However, **after reconstitution**, the product should be used within **8 hours.**

Storage of biopharmaceuticals

- granulocyte-colony stimulating factor **(G-CSF, filgrastim)** and **erythropoietin**, which are stable in **ready-to- use form** at room temperature for **24 hours and 14 days**, respectively.
- Granulocyte macrophage-colony stimulating factor **(GM-CSF, sargramostim)** is packaged as **a lyophilized powder**, but still requires **refrigeration** and once **reconstituted** is stable at room temperature for **30 days or in the refrigerator for 2 years**.
- **Aldesleukin** (interleukin-2) is stable for **48 hours at room temperature or under refrigeration**. **Betaseron** (interferon-β1b) must be **stored in a refrigerator** and should be used within **3 hours after reconstitution**.
- While most products require refrigeration to maintain stability, extreme cold such as **freezing** may be just as harmful to most products.

Storage

- Most biotech products **may adhere** to either **plastic or glass containers** such as syringes, polyvinyl chloride (PVC) intravenous bags, infusion equipment, and glass intravenous bottles.
- The **effectiveness** of the product may be reduced by **three- or four-fold** due to adherence.
- **human serum albumin (HSA)** is usually added to the solutions. The relative loss through adherence is concentration dependent.
- Some products that require the addition of HSA include **filgrastim** (2mg/ mL of HSA to the final solution is required for concentrations of 5 to 15 mg/mL). 1 mg of HSA/1mL 0.9% Sodium Chloride injection is added to achieve a final concentration of 0.1% HSA for sargramostim concentrations of <10 mg/mL. For aldesleukin (0.1%, HSA is required). For erythropoietin (2.5mg/ml HSA for single-dose and multi-dose vial). 1 mg/mL of HSA is added to interferon-α in single dose and multidose vials and pens).

- Biotech product **stability** may vary when stored in different types of **containers and syringes**.
- Some products are **only stable in plastic syringes, e.g., somatropin and erythropoietin**, while others are stable in **glass, polyvinyl chloride and polypropylene**, e.g., **aldesleukin**. Batch prefilling of syringes is possible. However, it is important to make sure that the product you wish to provide in pre-filled syringes is stable in the type of syringe you wish to use.

- **G-CSF** is stable in Becton **Dickinson (B-D) disposable plastic syringes for up to 7 days while erythropoietin is stable for up to 14 days**. Aldesleukin is recommended to be administered in PVC although glass has been used in clinical trials with comparable results.

- Solutions are stable for 48 hours when refrigerated. GM-CSF and G-CSF can be administered in either PVC or polypropylene

- Many biotech products are **sensitive to light**. Manufacturer's information usually suggests that products be protected from strong light until the product is used.

- **Dornase-a** is packaged in **protective foil pouches** by the manufacturer to protect it from light degradation and should be stored in these original light protective containers until use. For **patients who travel, the manufacturer will provide special travel pouches** on request

- **Alteplase** in the **lyophilized** form also needs to be **protected** from light but is **not light sensitive when in solution**

- Pharmacists must be aware of the **specific storage requirements** with respect to light for each of the products stocked in the pharmacy.

Handling

- **Mixing and Shaking**
- Improper handling of protein products can lead to denaturation. Shaking and severe agitation of most of these products will result in degradation.
- Once the diluent is added to the container, the vial should be swirled rather than vigorously shaken.
- Some shaking **during transport** may be **unavoidable** and proper inspection of products should occur to make sure the products have not been damaged during transit.
- When a product is **affected by excessive shaking, physical separation or frothing** within the vial of liquid products can usually be observed.
- For **lyophilized** products, agitation is **not harmful** until the product has been **reconstituted**.

Handling

- **Travel Requirements**
- When patients travel with these products, certain precautions should be observed. The drugs should be stored in insulated, cool containers (ice packs).
- When traveling in subfreezing weather, the products should be protected from freezing (temperatures below 2C).
- Keeping biotech drugs at proper temperature during automobile travel may present a problem with temperatures inside a parked car often exceeding 37C on a warm day. Patients and delivery personnel must take care not to leave products that are not in insulated containers inside the car while shopping or making deliveries.
- When ice is used, care should be taken **not to place the product directly on the ice**.
- **Dry ice should be avoided** since it has the potential for freezing the product. When **traveling by air**, biotech products should be taken onto the plane in **insulated packages** and not placed in a cargo container. Airplane **cargo containers** may be

Administration of biopharmaceuticals

- Prior to administering these products, pharmacists will need to use caution in reviewing dosage regimens.
- A potential source of medication error is the variation in units as some products are dosed in micrograms/kilogram (µg/kg) rather than milligrams/kilogram (mg/kg).
- Also, some units of measure may be unique to the product, e.g., Chiron or Roche units.
- Dosage calculations need to be carefully checked to avoid potential errors

Administration of biopharmaceuticals

- Biotech products are primarily administered **parenterally**
- although many other routes of administration are in development. Some products may be given by either the intravenous or subcutaneous route while others are restricted to the subcutaneous or intramuscular routes.
- The manufacturer should always be consulted in order to obtain supporting evidence for a particular route that is not approved, but may be more convenient for the patient.
- For example, **G-CSF** should be administered by the SC or IV route only, while **GM-CSF** is given by IV infusion, over a two-hour period. Aldesleukin is approved for IV administration only. However, subcutaneous administration, while not approved, has been used
- **Erythropoietin** should only be administered by the IV or SC routes, while **alteplase** is only approved for the IV route.
- **Alteplase** has also been administered by the intracoronary, intra-arterial, and intraorbital routes as well.

Filtration

- Filtering biotech products is not generally recommended since most of these **proteins will adhere to the filter**.
- Some hospitals and home infusion companies routinely use in-line filters for all intravenous solutions to minimize the introduction of particulate matter into the patient.
- In the case of biotech products, they should be infused below the filter to avoid a potential decrease in the amount of drug delivered to the patient

Flushing solutions

- Pharmaceutical biotechnology products are usually flushed with either **saline or dextrose 5%** in water.
- The **product literature should be consulted** and care should be taken to assure that the proper solution is used with each agent
- In general, biotech drugs should not be administered with other drugs since, in most cases, **data do not exist** that demonstrate whether biotech products are **compatible with other drugs or fluids**

FDA Guidelines of biologicals

- To demonstrate biosimilarity between protein drug and reference, it should include:
  - *Structural characterization*
  - *Functional characterization*
  - *Animal toxicity*
  - *Human pharmacokinetics*
  - *Human Pharmacodynamics*
  - *Clinical immunogenicity*
  - *Clinical safety and effectiveness*

Protein structure characterization

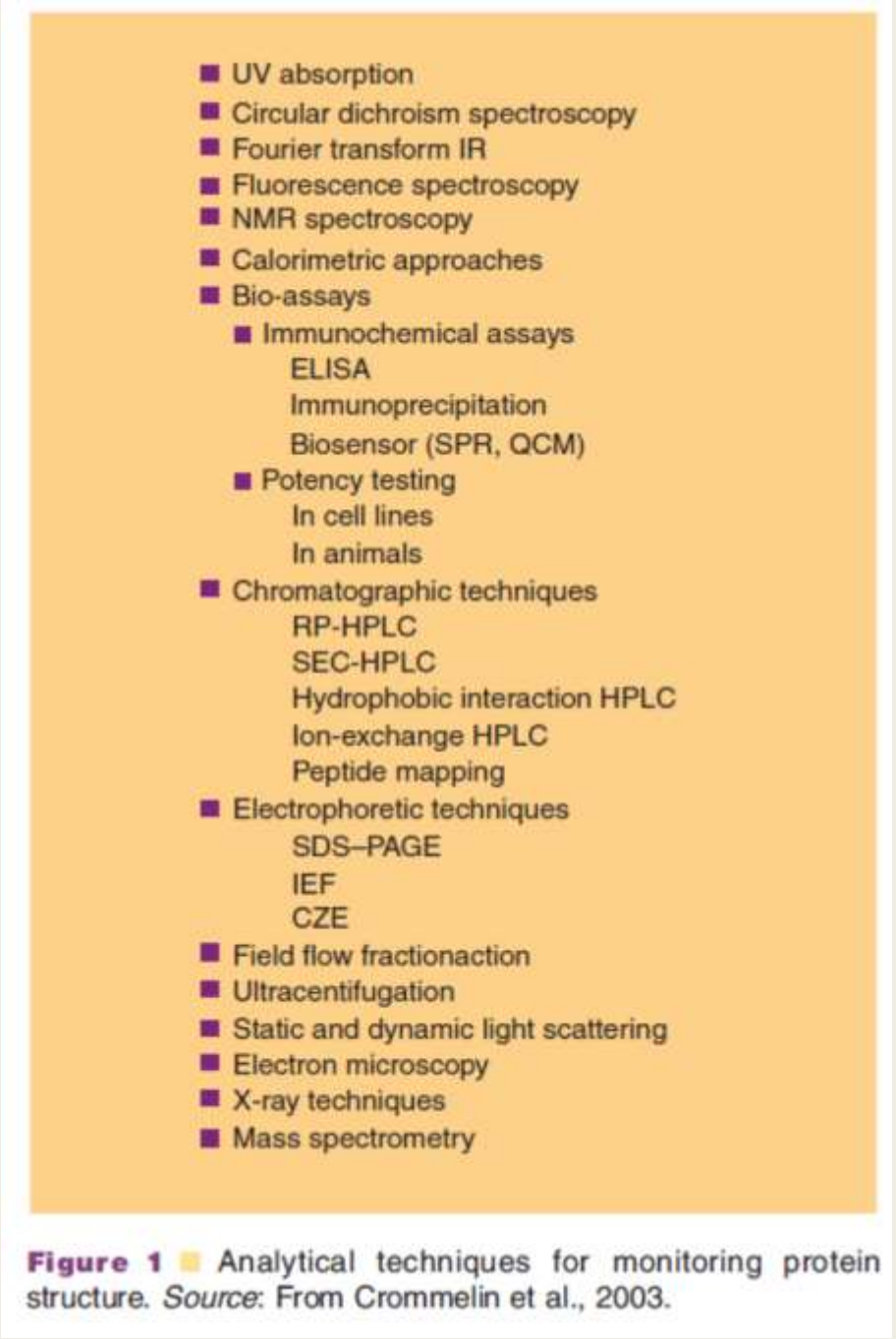

Figure 1 Analytical techniques for monitoring protein structure. *Source*: From Crommelin et al., 2003.