Manufacturing of Biotech products

Lecture 6

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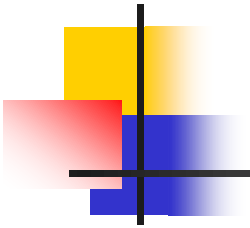
Sequences of manufacturing

1. Freeze–thaw (melt) of the bulk drug substance (therapeutic protein)
2. Formulation (dilution and addition of excipients)
3. Filtration for removing any particulate matter and/or sterilization
4. Filling of drug product in sterile (vials or syringes).
5. Inspection of filled vials or syringes for the presence of any particulate matter (clarity test).
6. Labeling and packaging
7. Storage and shipment of drug product
8. Use of a delivery device for drug administration to the patient

Many of these processes may affect formulation stability (As incompatibilities).

For example:

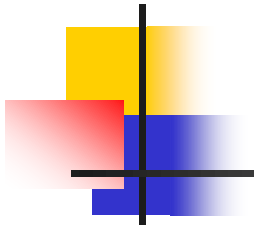
- Exposure to light and shear during inspection and transportation can lead to the formation of micro-bubbles in the formulation, which can increase the tendency toward protein aggregation and oxidation.



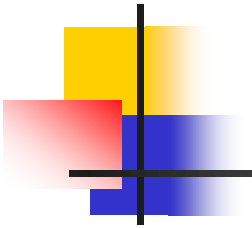
- Protein may interact with silicone oil typically used in syringes for smooth barrel movement, leading to instability of syringe-filled protein formulations.
- Protein loss may occur due to adsorption to manufacturing equipment and filter membranes.
- In addition, leaching of metal ions from manufacturing vessels into the protein formulation can lead to protein instability.

Formulation of biotech products

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



- Most proteins and peptides are not absorbed to any significant extent by **the oral route**, and most available protein pharmaceuticals are therefore administered by parenteral routes (non oral).
- Parenteral protein formulations are typically administered by **IV, IM, or SC injections**.
- In addition, some proteins can also be delivered by pulmonary or nasal routes.



- Parenterally administered proteins are rapidly cleared from circulation by the reticuloendothelial systems (RES).(**How avoid?**)
- Proteins are metabolized by peptidases, leading to rapid loss of their biological activity.(**How avoid?**)
- Recombinant DNA technology has significantly reduced the immunogenicity and antigenicity of particular proteins, but it has little effect upon the stability or blood circulation time of the proteins.
- Thus, protein aggregation can lead to perception of foreign epitope by host cells and generation of antibodies against the injected protein.

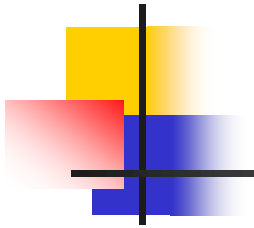
Selection of the type of protein formulation depends on several factors, such as:

- Disease condition. For example, requirement of patient self-administration versus administration by a nurse in a hospital setting. (**as in injectable insulin products**)
- Patient population. For example, age of the patient significantly affects what kind of delivery devices may be used.
- Route of delivery, such as IM, IV, SC, intra-peritoneal, topical, inhalation, or nasal.
- Drug dose, solubility, stability, and other physicochemical properties.

Notes:

- IV route is preferred for rapid onset of administration whereas the SC route provides prolonged action. **Thus, sustained drug delivery devices such as polylactide co-glycolide (PLGA) entrapped drugs are often designed for SC administration.**
- Compared to the SC route, IM injection is exposed to much greater blood supply and, thus, faster absorption.
- Greater injection volumes may be administered by the IM (2–5 mL) than the SC (up to 2 mL) route.
- In cases where patient self-administration of a drug is required, IM or SC injections are needed over IV.

- In terms of formulation requirements, the needed volume of injection is determined by **the drug dose and solubility??**.
- If solubility is inadequate, solubilization approaches may be needed.
- Preparation of concentrated protein solutions can, however, lead to high viscosity which could make deaeration upon agitation and deliverability through a syringe difficult.
- For example, SC injections typically use lower diameter (25–30G) syringe compared to IM injections (20–22G).



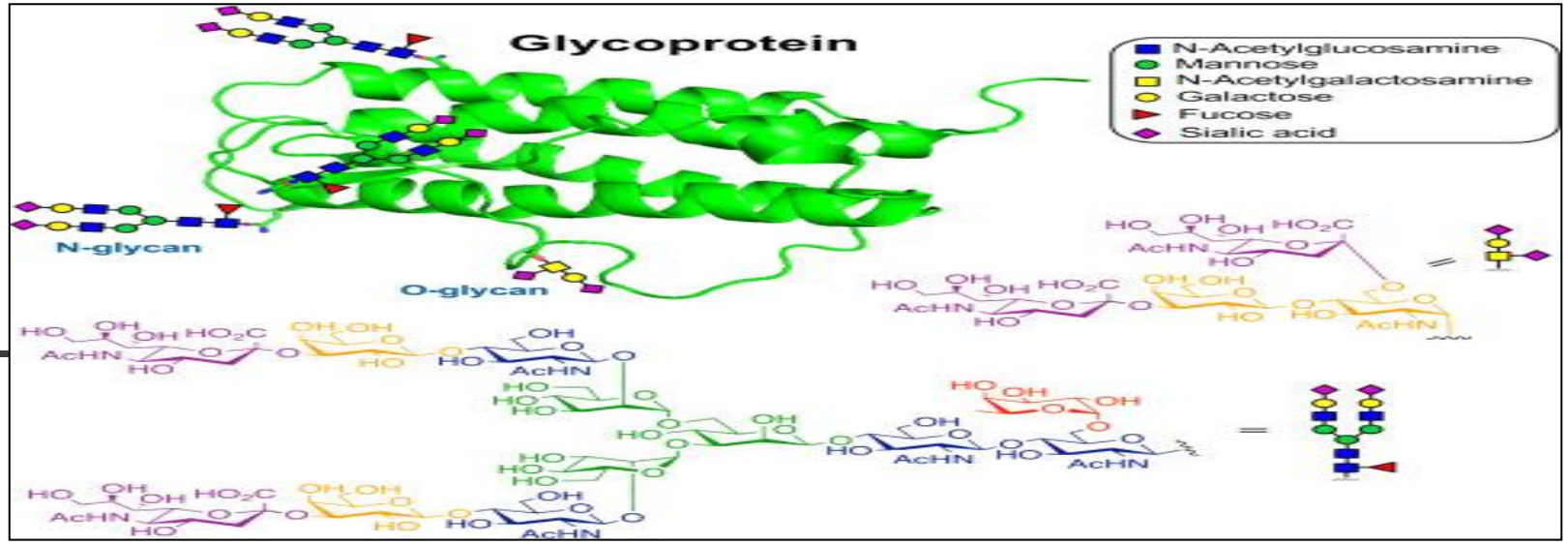
- Proteins and peptides for parenteral administration are typically formulated as **ready-to-use aqueous solutions or as lyophilized solid mass** that is reconstituted into a protein solution by dilution with water, isotonic dextrose solution, or isotonic sodium chloride solution immediately before administration.
- Proteins and peptides for inhalation and nasal routes of administration are typically formulated as **dry powders and sometimes as liquid formulations**.

Formulation components

- The development of a suitable pharmaceutical formulation of a protein usually involves the screening of a number of physiologically acceptable buffers, salts, chelators, antioxidants, surfactants, cosolvents, and preservatives.

- 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)

Table 1 Components found in parenteral formulations of biotech products. All of the above are not necessarily present in one particular protein formulation.



Solubility enhancers:

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

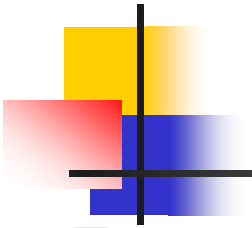
1) Selection of the proper pH and ionic strength conditions.
2) Addition of amino acids such as alanine, lysine or arginine.
3) Use of surfactants such as Polysorbate, sodium lauryl sulfate and poloxamer (Surface active polymer).

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.

Buffer components:

- Buffer selection is an important part of the formulation process, because of 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.

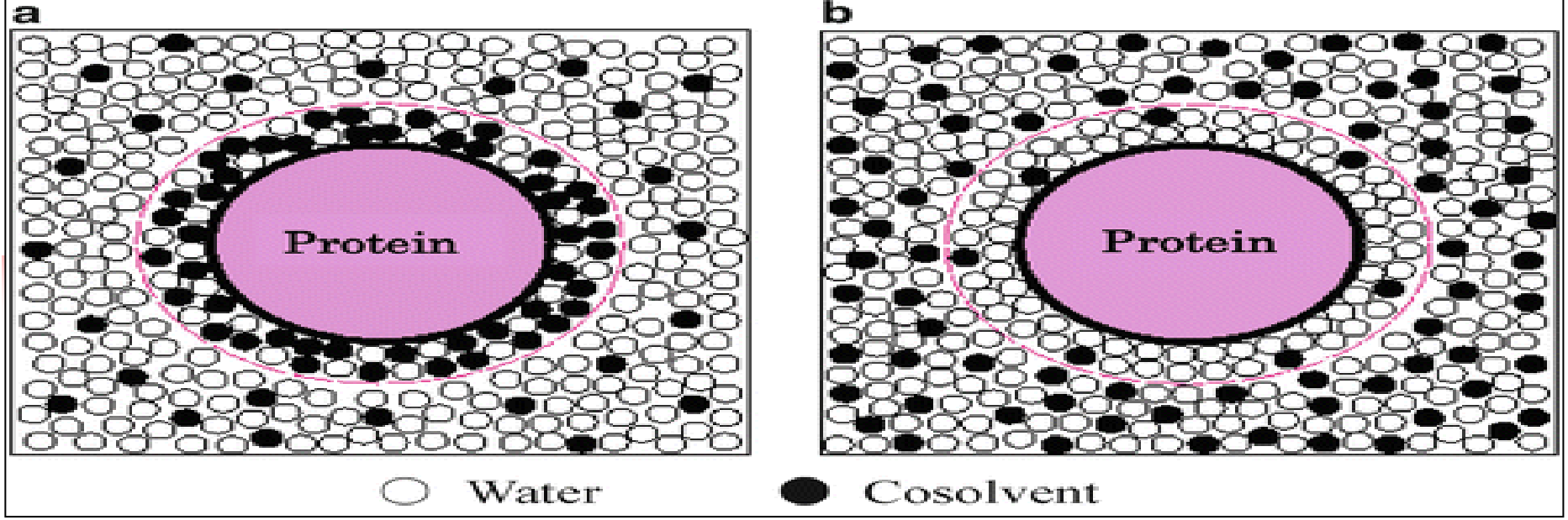


- Even short, temporary pH changes can cause aggregation. These conditions can occur, for example during the freezing step in a freeze-drying process, when one of the buffer components is crystallizing and the other is not.
- In a phosphate buffer, Na_2HPO_4 crystallizes faster than NaH_2PO_4 . This 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.

Stabilizers: (Physical, chemical and microbiological)

- Addition of antioxidant like ascorbic acid, acetyl cysteine, GSH, sodium disulfide and alpha tocopherol, are frequently used at a concentration of about 0.05-0.1%.
- Chelating agents can be incorporated to bind copper, iron, calcium and manganese. Ethylenediamine tetraacetic acid (EDTA) is commonly used at a concentration of about 0.01%-0.05%.

- Certain proteins are formulated in multiple injection systems, then contamination with M.O. may be occurred after administration of first dose, therefore preservatives are needed.
- **Preservatives used in concentrations that are bacteriostatic rather than bactericidal in nature (Why?).**
- Examples: thimerosal, p-hydroxy benzoic acids, phenol, benzyl alcohol and chlorobutanol



- Stabilization of protein conformation can be achieved by addition of co-solvents such as glycerol or polyethylene glycol (PEG), which may decrease the protein surface area in contact with the solvent.

- Electrostatic interactions in proteins may be modulated by the alteration of the solvent polarity and dielectric constant to change protein electrostatic interactions in solution and, thus, reduce the association tendency of a protein.

Tonic agents:

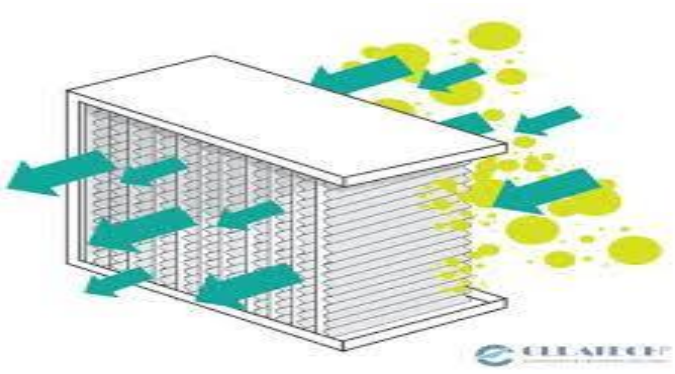
- Are added for adjusting the tonicity like saline (NaCl), KCl, $CaCl_2$, $MgCl_2$, $ZnCl_2$, sodium gluconate and sulfate salts.

Manufacturing methods:

- Methods used in manufacturing of biotech products:
- Sterilization
- Viral decontamination
- Pyrogen removal

Sterilization:

- Most proteins are administered by injectable routes and have to be sterile.
- Sterilization of the end product is not possible using autoclaving, gas or ionizing radiation sterilization methods (Why?).
- 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.

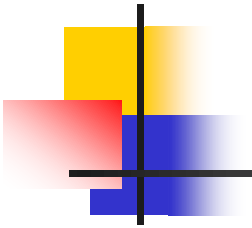


- Filtration techniques are used for removal of micro-bacterial contaminants.
- Pre-filters remove the bulk of the bio-burden and other particulate materials.
- The final sterilizing step before filling the vials is filtration through 0.2 or 0.22 µm membrane filters (low-protein-binding filters).
- High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

- 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.

Viral decontamination:

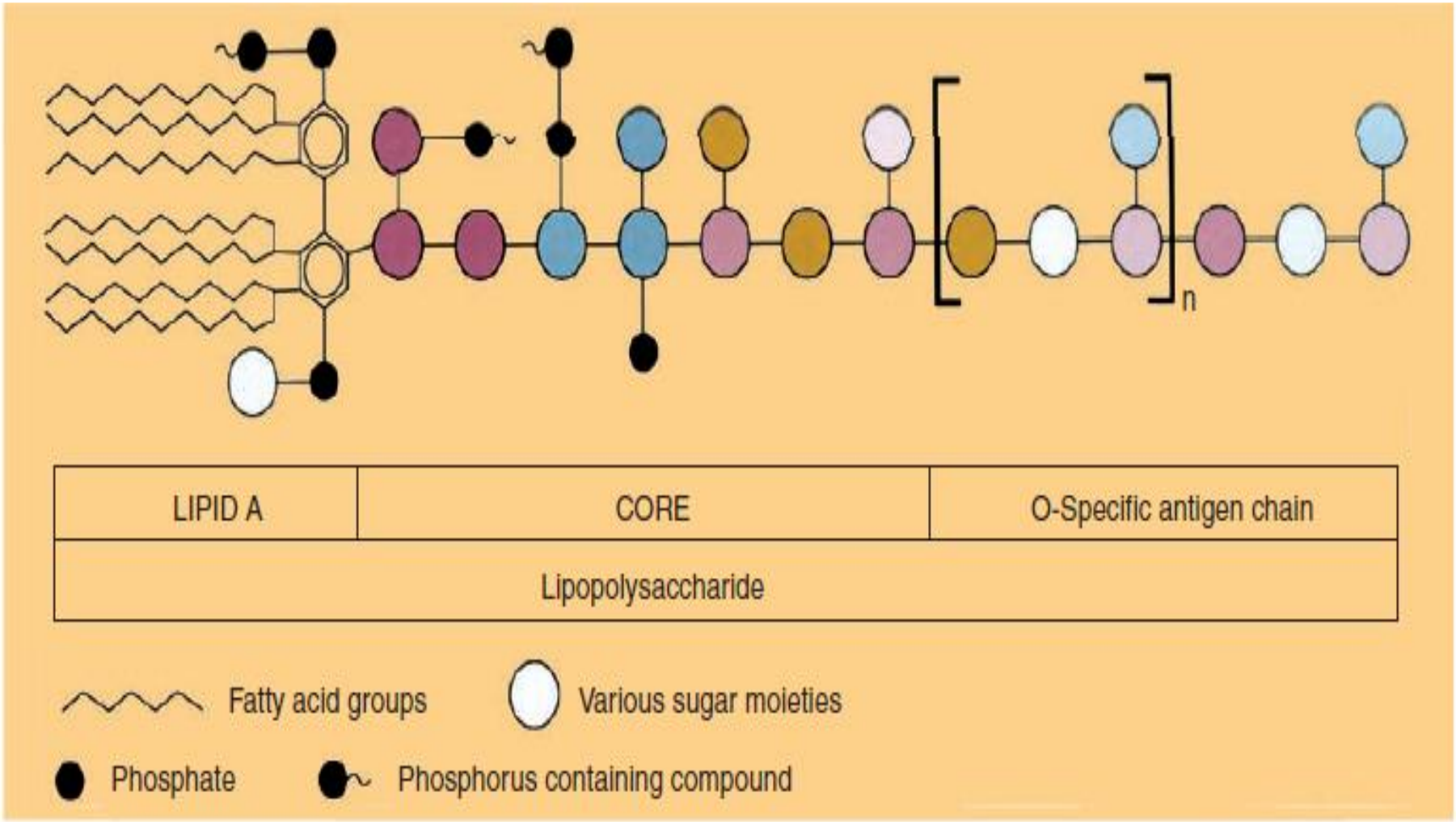
- Inactivation or removal of viral contaminants.
- Recombinant DNA products (grown in microorganisms) must be free of viruses.
- No unwanted viral materials (considered as antigens) should be introduced during the manufacturing process.



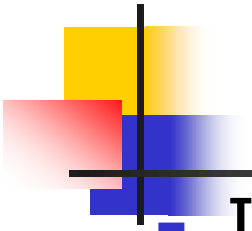
- The viral contaminants can be **inactivated** by (heat treatment, radiation, dehydration, denaturizing agents and neutralization).
- They can be **removed** by (chromatography, filtration and precipitation).
- Also, excipients like a blood-derived human serum albumin should be tested for the same purpose.

Pyrogen removal:

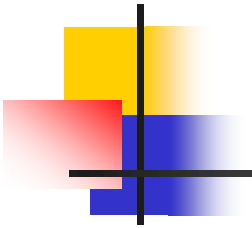
- Are components that induce fever, derived from bacterial, viral or fungal sources.
- Bacterial pyrogens are mainly endotoxins shed from G-ve bacteria, which are lipopolysaccharides with high negative electrical charge and adsorption tendency on the surfaces.
- They are stable under standard autoclaving 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).



Generalized structure of endotoxins



- 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.



- 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.