Pharmaceutical Biotechnology

Microbiological consideration in formulating biotech products

Sterility

- Most proteins are administered **parenterally** and have to be **sterile**.
- In general, proteins are sensitive to heat **(Why? What can happen?)** and other regularly used sterilization treatments.
- Proteins **cannot withstand** autoclaving, gas sterilization, or sterilization by ionizing radiation.
- Consequently, sterilization of the protein end product is **not possible**.
- Therefore, protein pharmaceuticals have to be **assembled under aseptic conditions**, following the established and evolving rules in the pharmaceutical industry for aseptic manufacture.

Making “sterile” biotech products

- **Equipment** and **excipients** are treated separately from the protein.
- These equipment and excipients can be sterilized by:
  - Autoclaving (121 °C for 15-20 minutes),
  - dry heat (>160 °C for 1-2 hours),
  - chemical treatment **(H.W. chemicals used in chemical sterilization?),**
  - or gamma radiation

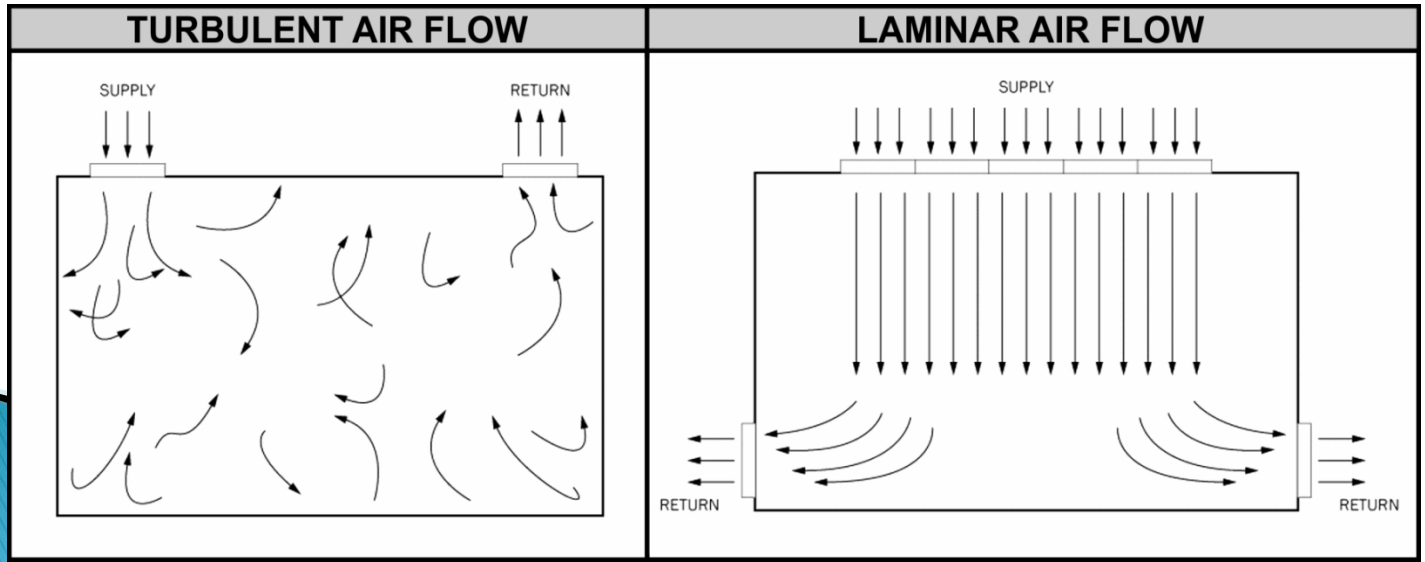
to minimize the bioburden (bioburden is defined as the number of bacteria living on a surface that has not been sterilized).

- Filtration techniques are used for removal of microbacterial contaminants.
  - **Pre-filters** remove the bulk of the bioburden and other particulate materials.

  - The final “sterilizing” step before filling the vials is filtration through **0.2 or 0.22 µm** membrane filters.

- Assembly of the product is done in **class 100 rooms** with **laminar air flow** that is filtered through HEPA (high efficiency particulate air) filters.

particle/ft³						
Class	**0.1 µm**	**0.2 µm**	**0.3 µm**	**0.5 µm**	**1 µm**	**5 µm**
1	35	7	3	1		
10	350	75	30	10	1	
100		750	300	100	10	1
1,000				1,000	100	10
10,000				10,000	1,000	100
100,000				100,000	10,000	1,000



- **The human factor**: represents a major source of contamination.
- Therefore, a well-trained operators wearing protective cloths (face masks, hats, gowns, gloves, or head-to-toe overall garments) should operate the facility

- Regular exchange of filters, regular validation of HEPA equipment, and thorough cleaning of the room plus equipment are critical factors for success in producing sterile products.

Potential contaminants in recombinant protein products derived from bacterial and nonbacterial hosts are listed below

Origin	Contaminant
Host-related	Viruses
	Host-derived proteins acid DNA
	Glycosylation variants
	N- and C-terminal variants
	Endotoxins (from Gram-negative bacterial hosts)
Product-related	Amino acids substitution and deletion
	Denatured protein
	Conformational isomers
	Dimers and aggregates
	Disulfide pairing variants
	Deamidated species
	Protein fragments
Process-related	Growth medium components
	Purification reagents
	Metals
	Column materials

H.W. mechanism of protein or peptide deamidation?

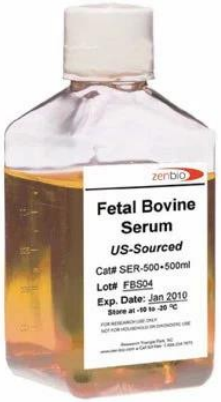
Viral decontamination

- **Viruses can be introduced by:**

  1) **Nutrients** for cells producing recombinant protein (The most frequent source of virus introduction is **animal serum**!)

  2) **An infected production cell line**

  3) And or **human handling** during the production process.



- In addition, animal serum can introduce other unwanted agents such as bacteria, mycoplasmas, prions, fungi, and endotoxins.
- Cell banks and growth medium constituents should be strictly screened for viruses and other adventitious agents.

- **Viruses can be inactivated by physical and chemical treatment of the product such as:**

● Heat ● irradiation, ● sonication, ● extreme pH, ● detergents, ● solvents, ● certain disinfectants.

H.W.: Extreme pH is a preferred method in monoclonal antibodies viral decontamination, why?

- These procedures can be harmful to the product (remember, protein products) and should therefore be carefully evaluated and validated.

More on viral decontamination…

- **Nanofiltration** (PDA 2005) is an elegant and effective technique to remove viruses. Filtration through **15 nm membranes** can remove even the smallest non-enveloped viruses like bovine parvovirus.

- **More methods are shown in the table:**

Category	Types	Example
Inactivation	Heat treatment	Pasteurization
	Radiation	UV-light
	Dehydration	Lyophilization
	Cross linking agents, denaturating or disrupting agents	β-propiolactone, formaldehyde, NaOH, organic solvents (e.g., chloroform), detergents (e.g., Na-cholate)
	Neutralization	Specific, neutralizing antibodies
Removal	Chromatography	Ion-exchange, immuno-affinity, chromatography
	Filtration	Nanofiltration
	Precipitation	Cyroprecipitation

Table 3.5 ■ Methods for reducing or inactivating viral contaminants.

Pyrogen Removal

- **Pyrogens** are compounds that induce fever.
- Pyrogens can be derived from **bacterial**, **viral**, or **fungal** sources.
- Bacterial pyrogens are **mainly endotoxins** shed from gram-negative bacteria. They are lipopolysaccharides (LPS) which are **negatively charged**.
- Humans are sensitive to pyrogen contamination at very low concentrations (**picograms/mL**).
- Simple 0.2 µm filtration **does not** remove pyrogens.
- Endotoxins are **stable** under standard autoclaving conditions.
- Endotoxins can be inactivated on utensil surfaces (not the biotech product) by **oxidation** (e.g., peroxide) or **dry heating** (e.g., 30 min dry heat at 250 °C).

General structure of endotoxin

Characterized by:

- Conserved lipid A moiety
- Negatively charged (phosphates groups)

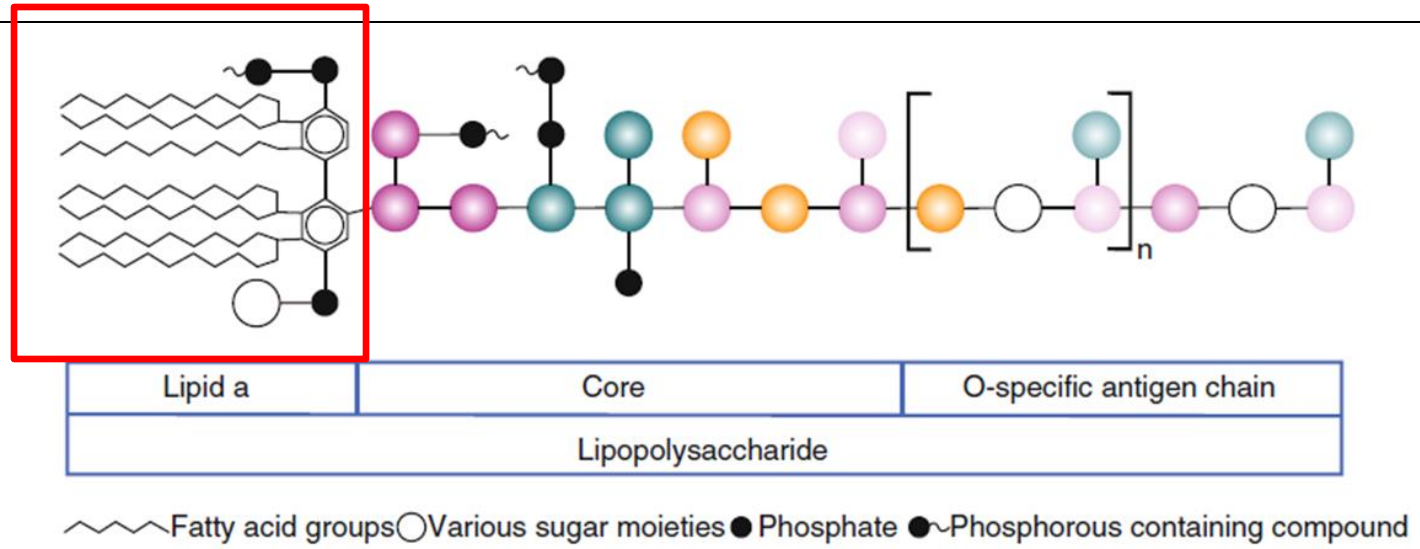
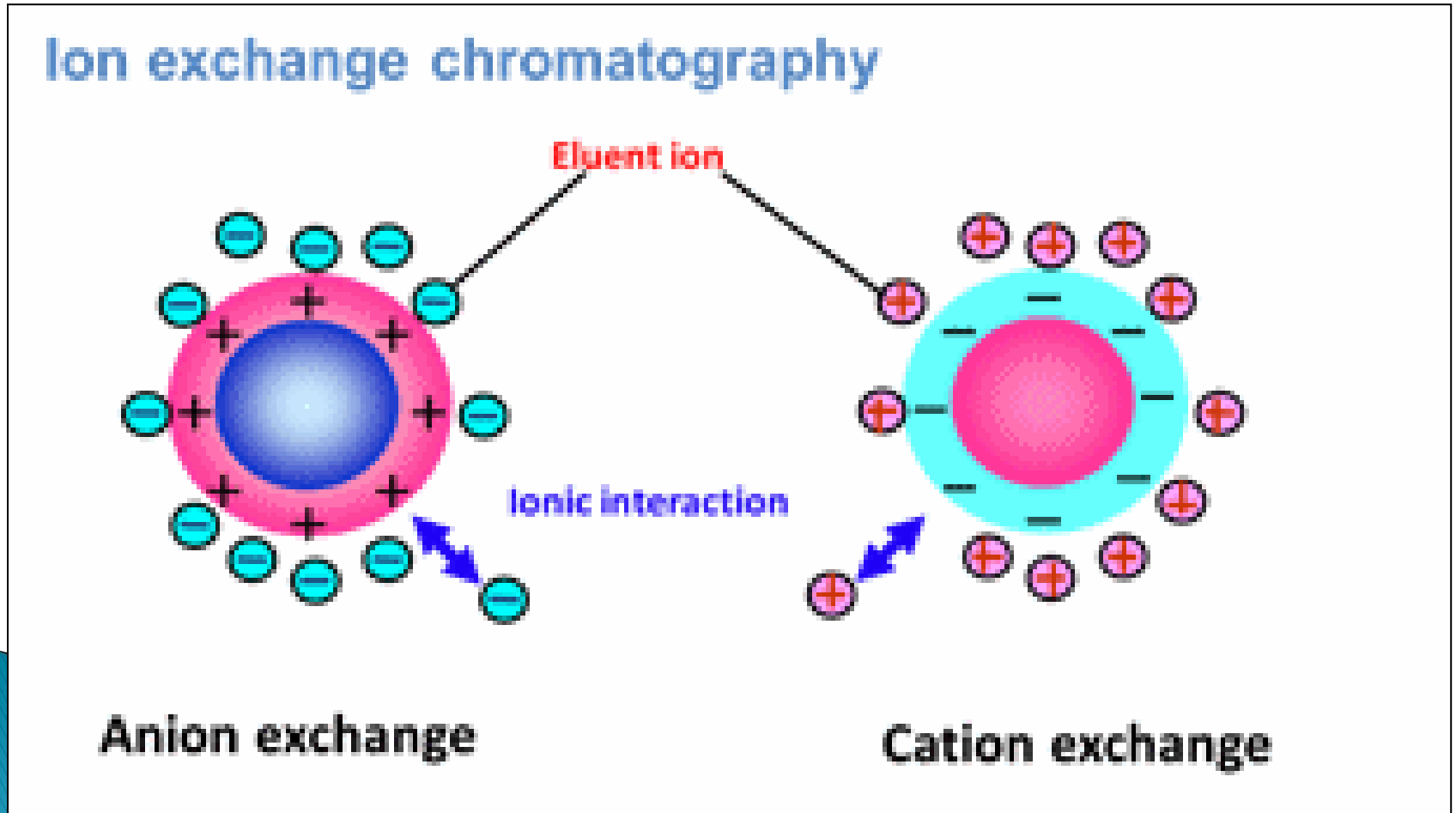


Figure 4.1 ■ Generalized structure of endotoxins. Most properties of endotoxins are accounted for by the active, insoluble "lipid A" fraction being solubilized by the various sugar moieties (*circles* with different colors). Although the general structure is similar, individual endotoxins vary according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

Eliminating endotoxins

- **Excipients** used in the protein formulation should be essentially endotoxin-free.
- For solutions “water for injection” (compendial standards) is (freshly) distilled or produced by **reverse osmosis**. (The aggregated endotoxins can not pass through the reverse osmosis membrane).
- Removal of endotoxins immediately before filling the final container can be accomplished by using **activated charcoal** or other materials with large surfaces offering **hydrophobic interactions**.
- **Ion exchange chromatographic procedures** (utilizing its negative charge) can effectively reduce endotoxin levels in solution.
- Question: type of ion exchange chromatography used here?

- Anion exchangers bind negatively charged molecules and cation exchangers bind positively charged molecules.
- The type of the column needed is determined by the properties of the analyte to be purified (e.g., **isoelectric point** and **charge density**).



Excipients used in parenteral formulation of biotech products

Introduction

- Molecular Biologist can produce
  - More recombinant protein drugs
  - Produce Pure Products

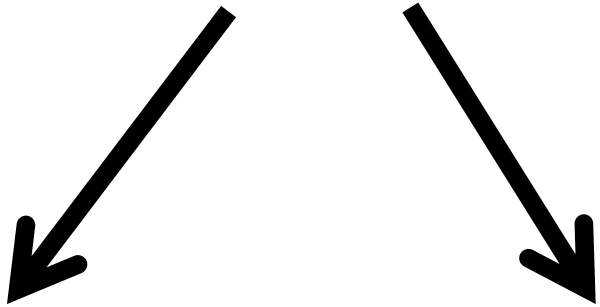
- Purification process of proteins strips away carbohydrates, salts, lipids and other proteins which are in natural environment.

- This purification process could make the protein drug less stable.

- Protein is sensitive to process such as
  - Shear
  - Agitation
  - Enzymatic and Chemical Degradation
  - Aggregation

Biotech products

(composition)



Active substance

Excipients

Goal of protein formulation

- The formulation should stabilize protein from chemical and physical degradations.

- Goals of Formulation
  - Easily administered
  - Efficacious
  - Adequate stability for shelf life for marketing.
  - Simple
    - excipient has potential interaction with protein drug which could block activity.

- To stabilize the protein using
  - Chemical Additives (Excipients)
  - Physical Methods (Lyophilization or Spray Drying)

- For parenteral formulation, several factors should be considered such as sterility, isotonicity, pH, and preservatives.

- Container and delivery devices are important
  - protein can be adsorbed to surfaces.
  - protein can be destabilized by extractable compounds from the packaging material.

Components found 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

Excipients

** Not necessarily all of the above are present in one particular protein formulation