LECTURE-2

Formulation of Biotech Products, Including Biopharmaceutical Considerations

Microbial considerations

1. Sterility

- Most **proteins** are administered **parenterally** and it should be **sterile**

(E.g.: Recombinant/purified protein vaccines consist of protein antigens like Hepatitis B vaccine I.M.)

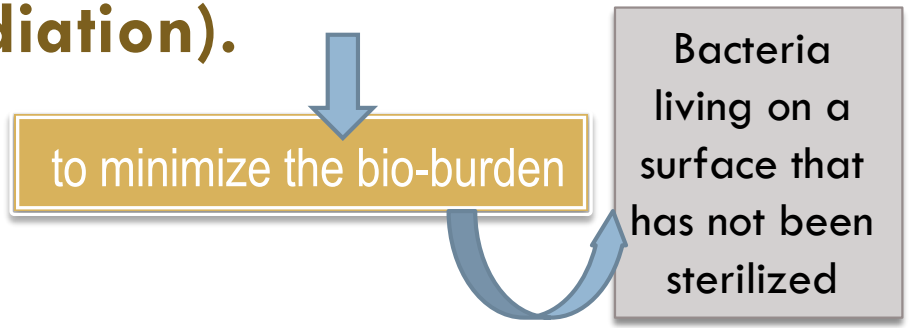
(But are **sensitive to heat and other sterilization treatments**) so **cannot** withstand **[autoclaving, gas sterilization, or sterilization by ionizing radiation]**.

Solution!!

Protein pharmaceuticals assembled under aseptic conditions, following established and evolving rules in the pharmaceutical industry for aseptic manufacture.

Rules in the pharmaceutical industry for aseptic manufacture

1. **Equipment and excipients (autoclaved, or sterilization by dry heat (>160°C), chemical treatment or gamma radiation).**



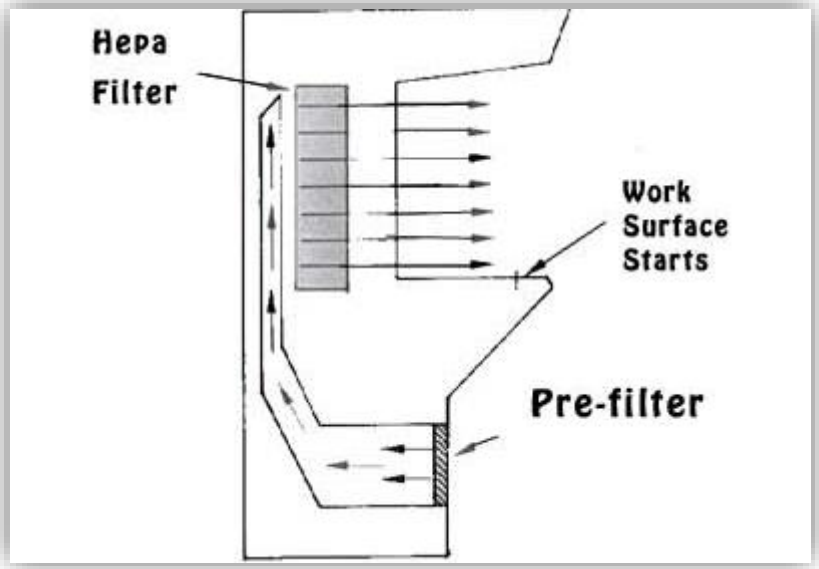
2. **Filtration techniques**

used for the removal of micro-bacterial contaminants .

A. **Pre-filters** (remove the bulk of the bio-burden and other particulate materials).

B. The final 'sterilizing' step is **filtration** through 0.2 or 0.22 µm membrane filters.


HATMAN


Hepa
Filter
Work
Surface
Starts
Pre-filter


MILLIPORE
Express™PLUS
0.22 µm

Specification of filters used for filtration during preparation of proteins

Product is done in class 100 (maximum 100 particles per cubic foot [≥ 0.5 µm]) rooms with laminar air flow that is filtered through HEPA (high efficiency particulate air) filters.

U.S. Federal Standard 209E Cleanroom Standards*

Class	Maximum Particles / ft³					ISO Equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥5 µm	
1	35	7	3	1		ISO 3
10	350	75	30	10		ISO 4
100		750	300	100		ISO 5
1,000				1,000	7	ISO 6
10,000				10,000	70	ISO 7
100,000				100,000	700	ISO 8

Cleanroom classifications

Cleanrooms classified according to the number and size of particles permitted per volume of air. Large numbers like "class 100" or "class 1000" denote the number of particles of size 0.5 μm or larger permitted per cubic foot of air.

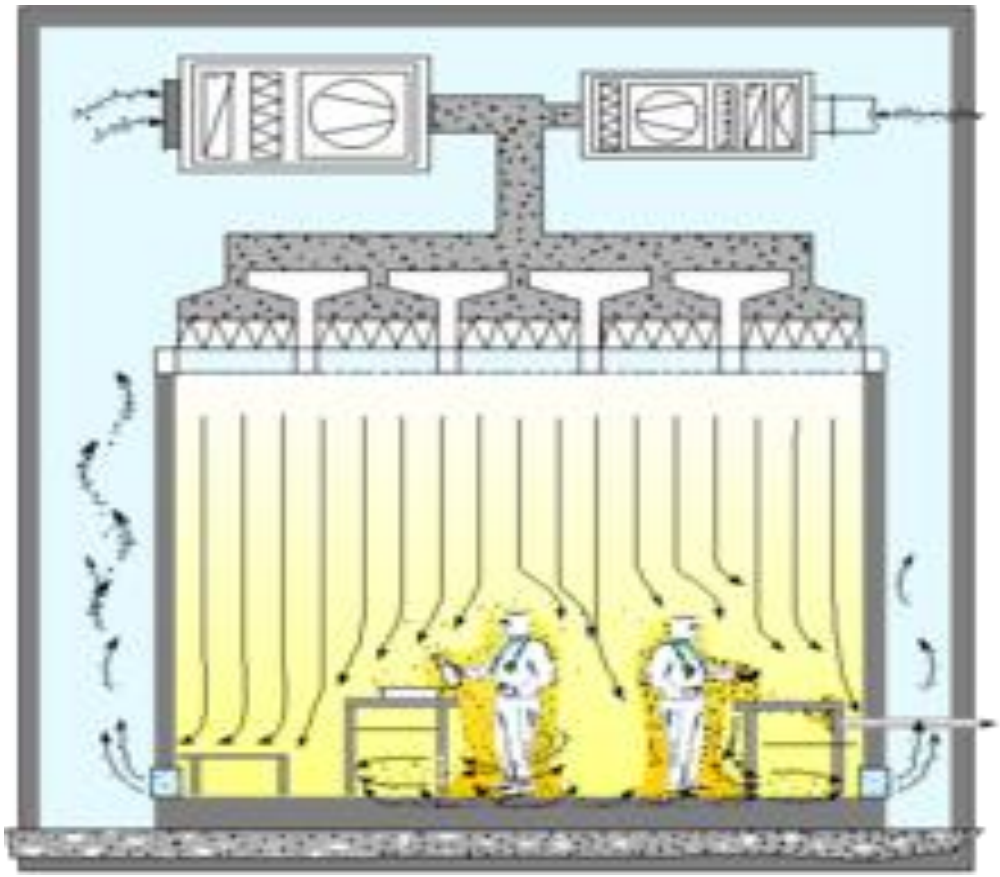
Notes

- Cleanrooms maintain particulate-free air through the use of either HEPA or ULPA filters employing **laminar or turbulent air flow** principles.

- The air entering a cleanroom from outside is filtered to exclude dust, and then the air inside is constantly recirculated through high-efficiency particulate air (HEPA) and/or ultra-low penetration air (ULPA) filters to remove internally generated contaminants.

Air flow principles

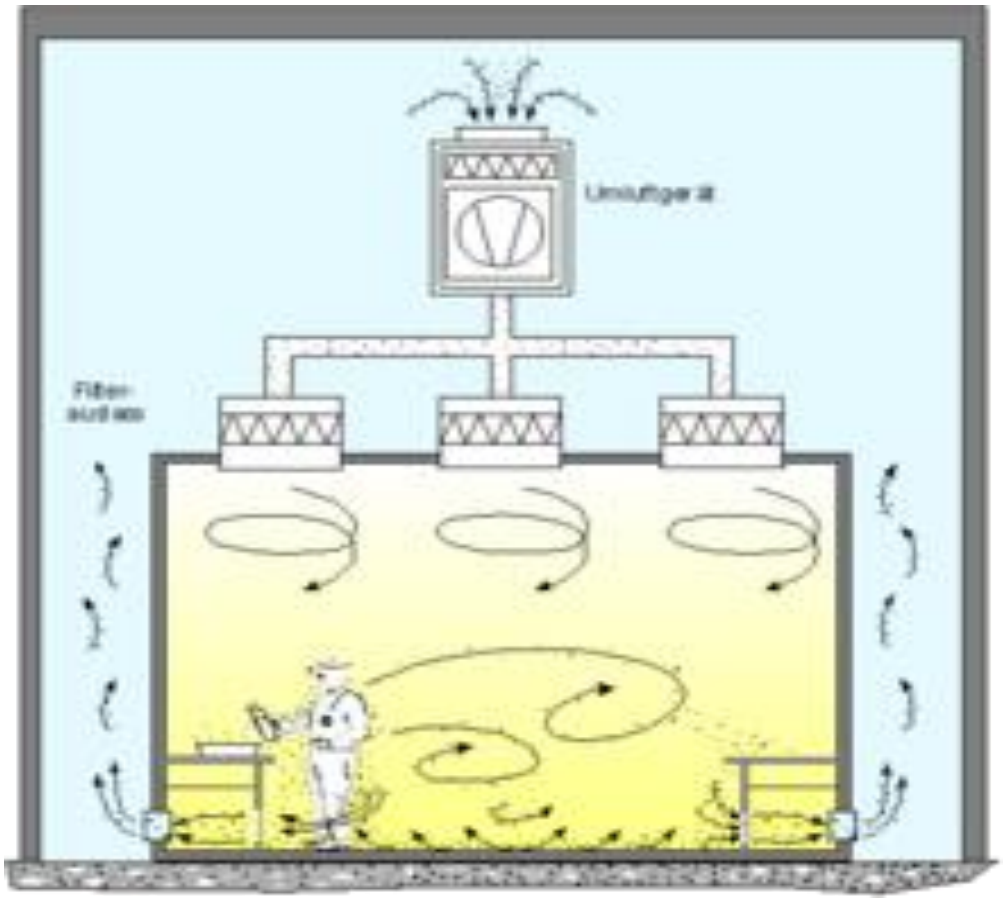
Air flow pattern for
"Laminar Flow Cleanroom"



- Laminar air flow clean rooms utilizes **HEPA filters** to filter and clean all air entering the environment.
- Laminar filters are often composed of **stainless steel or other non-shed materials** to ensure the amount of particles that enter the facility remains low.
- These filters usually compose roughly 80 percent of the ceiling space.
- Cleanrooms employing laminar air flow are typically referred to as **Unidirectional Airflow Cleanrooms.**

Air flow principles

Air flow pattern for "Turbulent Cleanroom"



Non-unindirectional airflow cleanrooms utilize turbulent airflow systems to clean particulate air and maintain a clean environment.

Turbulent airflow filters designed to use laminar flow and random, non-specific velocity to keep the air particle-free.

Turbulent airflow can cause particles movement that are difficult to separate from the rest of the air, thus non-unidirectional airflow systems count on this random movement to move particles from the air through the filter.

- Additionally, the 'human factor' is a major source of contamination, and well-trained operators wearing protective cloths (face masks, hats, gowns, gloves, or head-to-toe overall garments) should operate the facility.

- The regular exchange of filters, regular validation of HEPA equipment and the thorough cleaning of the room and equipment are critical factors for success.

2.Viral Decontamination

A. **Recombinant DNA products** are grown in **microorganisms**, these organisms should be tested for viral contaminants (like using microscope) and appropriate measures should be taken if viral contamination occurs:

1. **To get rid of viral materials** in the final product (**by using filtration, precipitation**).
2. **Inactivation of viral contaminants** in the final product (**by using heat, radiation**).

B. **Excipients with a certain risk factor** (**such as blood-derived human serum albumin**) should be carefully tested before use and their presence in the formulation process should be minimized.

3. Pyrogen removal

Pyrogens are compounds that induce fever, that are of two types **endogenous pyrogens and exogenous pyrogens.**

- **Exogenous pyrogens** (pyrogens introduced into the body, not generated by the body itself) can be derived **from bacterial, viral or fungal sources.**

- Bacterial pyrogens are mainly **endotoxins shed from gram negative bacteria** (They are lipopolysaccharides [cell wall from bacteria]).

Fever inducing mechanism

Endotoxin pyrogen enters blood stream and **binds to lipopolysaccharide binding PTNs**

Then bind to reticuloendothelial system (**receptor cells of circulate Mononuclear and Polynuclear cells: CD-14 0f macrophages**)

Production and release of proinflammatory cytokines (IL-1 and IL-6) of **endogenous pyrogen**

Inflammation and Fever (due to activation of arachidonic acid pathway).

A general structure is shown in the following Figure

Responsible for harmful and useful activities of endotoxin

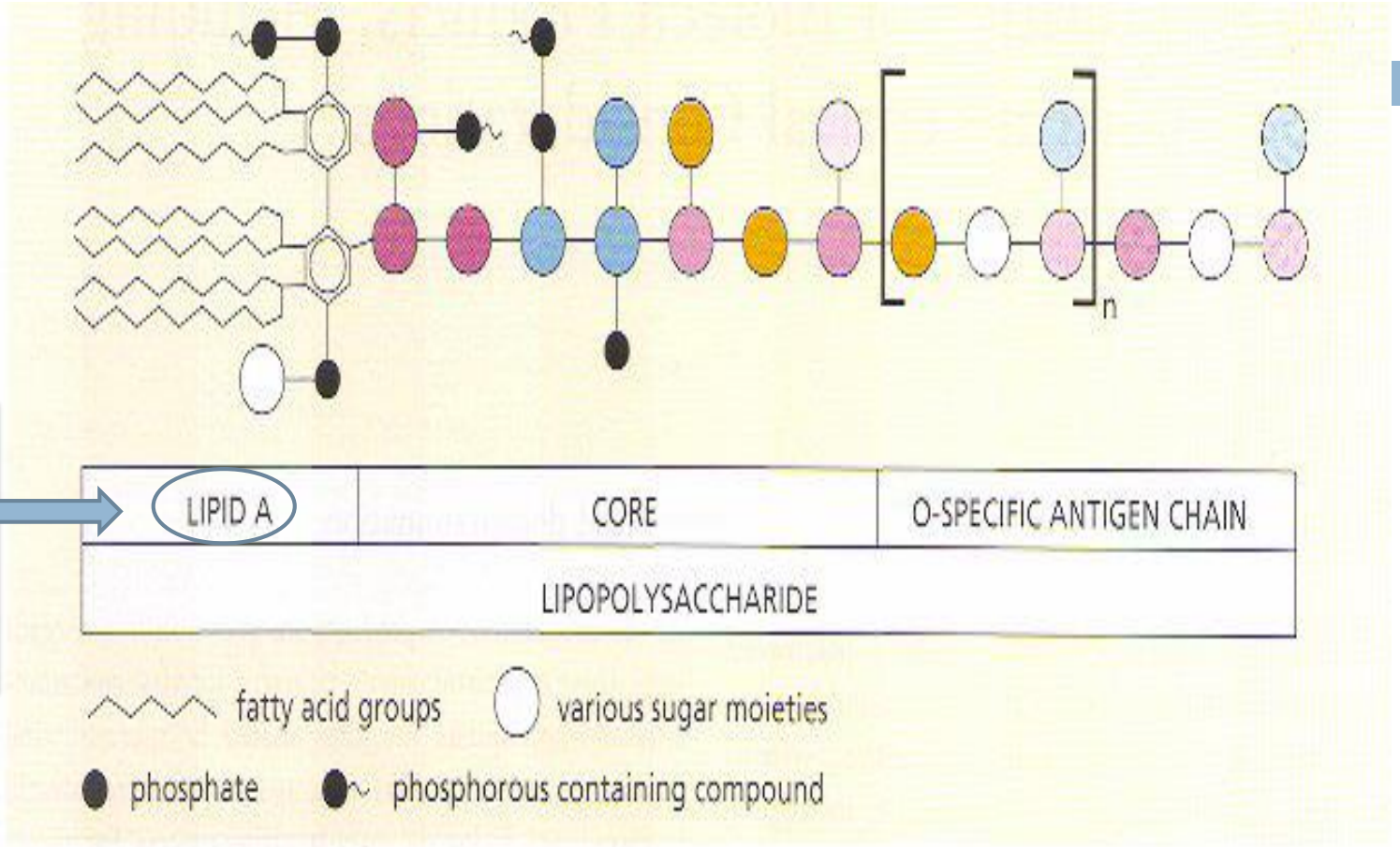
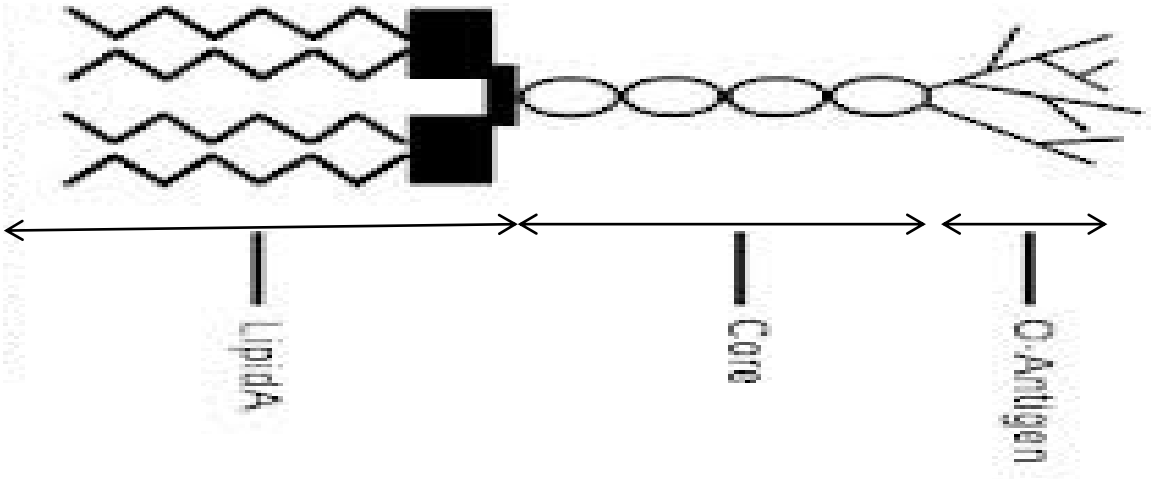


Figure 4.1. Generalized structure of endotoxins. Most properties of endotoxins are accounted for by the active, insoluble 'Lipid A' fraction being solubilized by the various sugar moieties (circles with different colors). Although the general structure is similar, individual endotoxins vary according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

General Structure of Endotoxins



Lipopolysaccharides

General notes on endotoxins

- They aggregate and **form large units with M.wt. of over 10^6 in water** and sharing a general property of **high negative electrical charge**.

- In addition to **their tendency to adsorb to surfaces**, thus indicating that these compounds are **amphipathic (hydrophilic and lipophilic) in nature as they are lipopolysaccharides**.

- **Stable under autoclaving conditions**, **but break down when heated in the dry state** (thus equipment and containers are treated at temperature above 160°C for prolonged periods (e.g. 30 minutes dry heat at 250°C)).

Continuous with Pyrogen removal

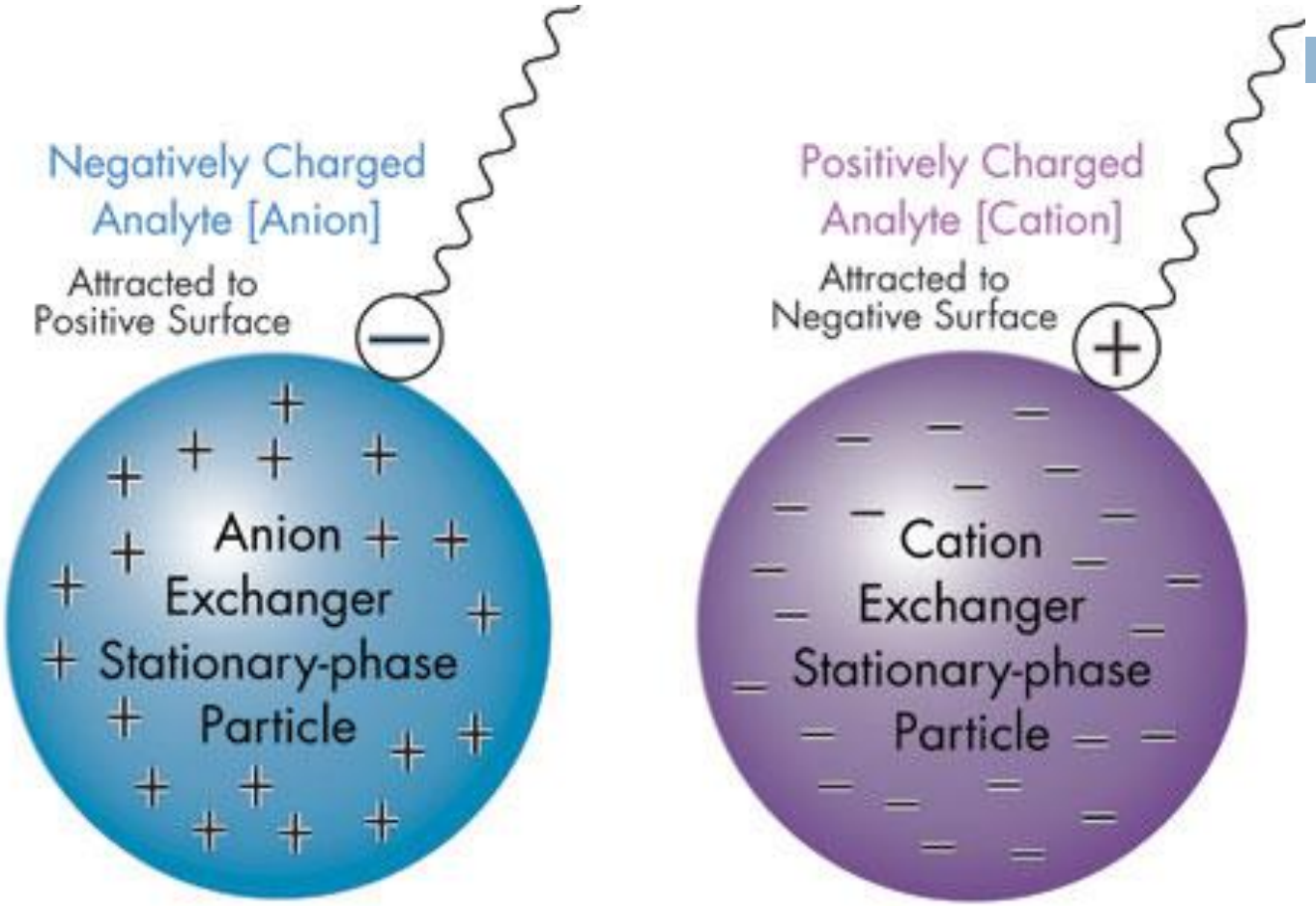
- **Pyrogen removal of recombinant products** derived from bacterial sources should be an integral part of preparation process:

Ion exchange chromatographic procedures (utilizing its negative charge) can effectively reduce endotoxins levels in solution.

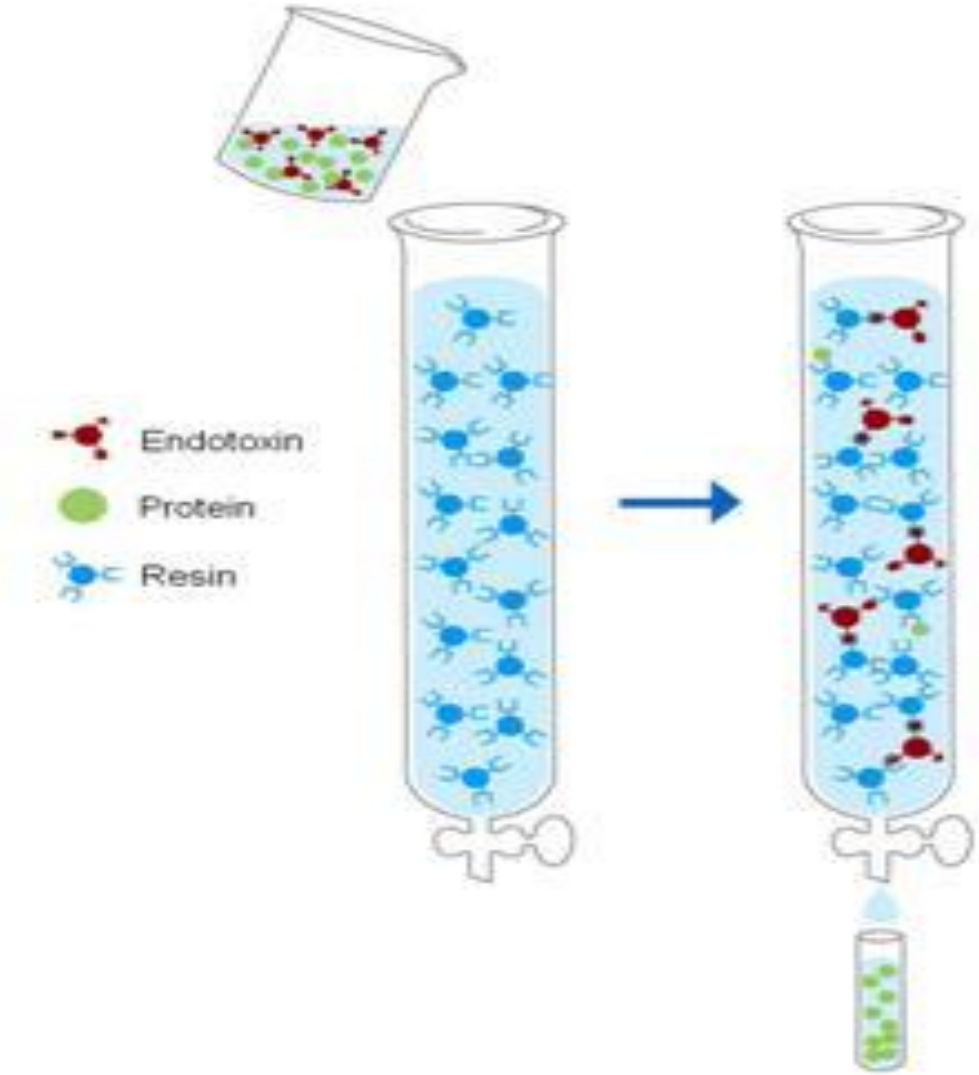
- **Excipients** used in the protein formulation should be essentially **endotoxins-free**.

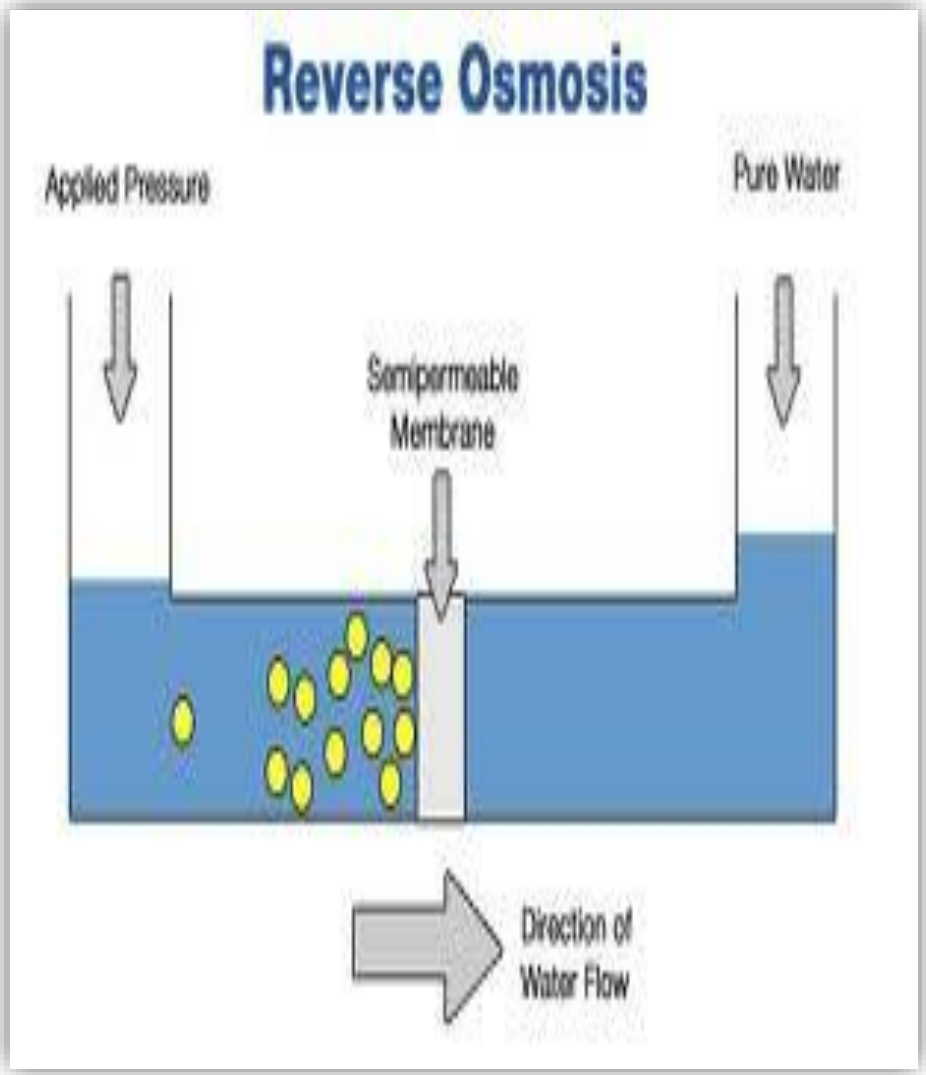
- For solutions, **water for injection** (compendia standards) is (freshly) distilled or **produced by reverse osmosis**.

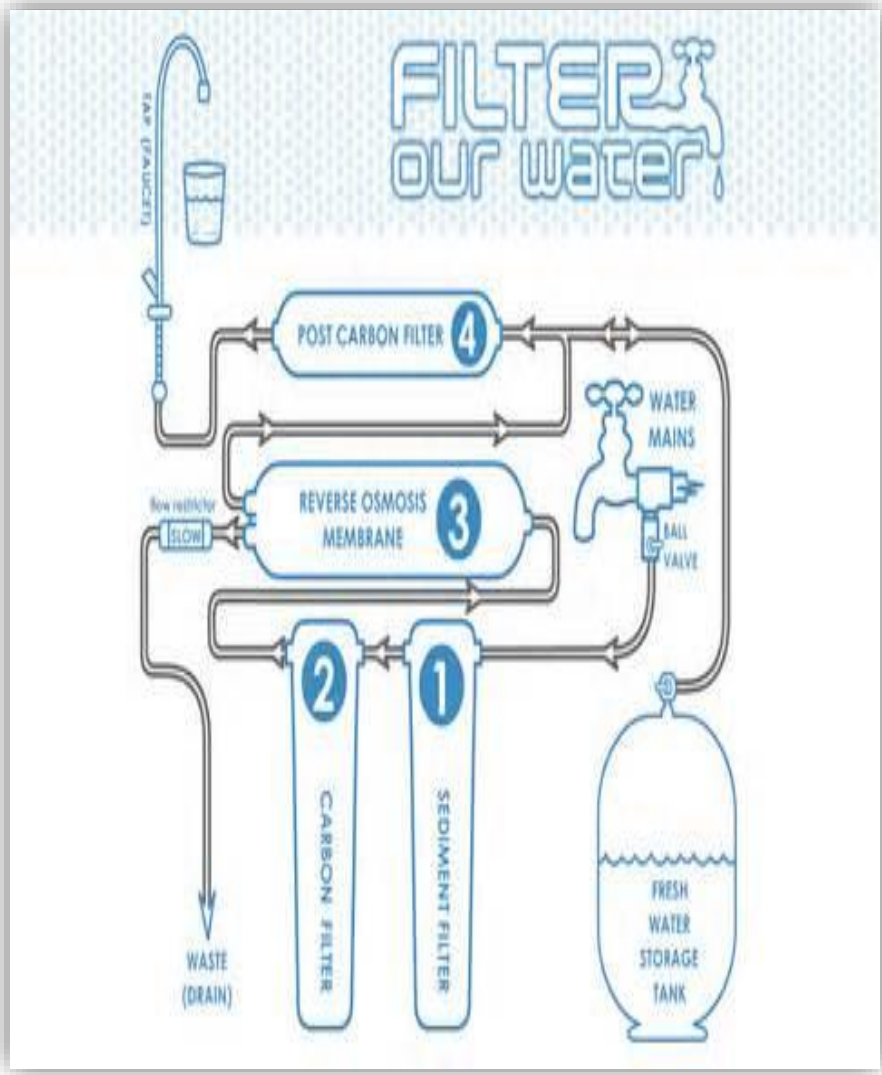
Ion exchange chromatography



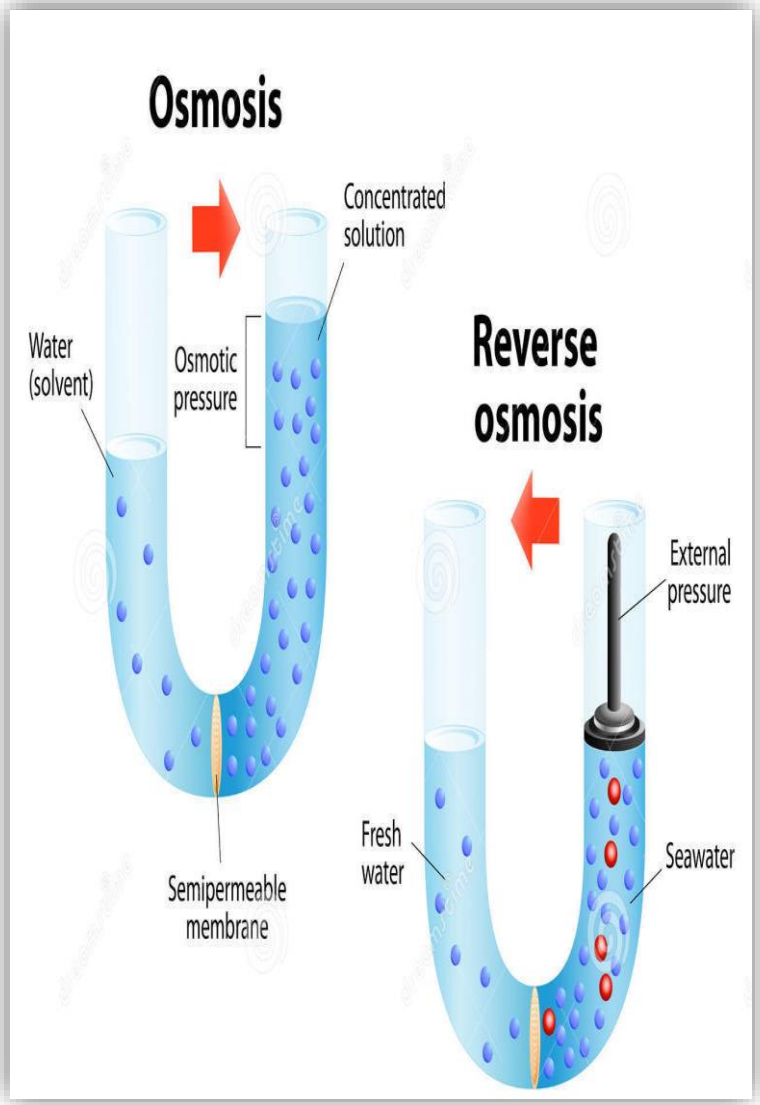
Ion exchange chromatography




Reverse Osmosis
Applied Pressure
Pure Water
Semipermeable Membrane
Direction of Water Flow


FILTER our water
TAP (FAUCET)
POST CARBON FILTER 4
WATER MAINS
REVERSE OSMOSIS MEMBRANE 3
flow restrictor
SLOW
BALL VALVE
2
1
CARBON FILTER
SEDIMENT FILTER
WASTE (DRAIN)
FRESH WATER STORAGE TANK

- The aggregated endotoxins cannot pass through the reverse osmosis membrane.
- Removal of endotoxins immediately prior to the filling of the final container can be accomplished by using activated charcoal or other materials with large surfaces **offering hydrophobic interactions.**
- Endotoxins can also be inactivated on utensil surfaces by oxidation (e.g. peroxide) or dry heating (e.g. 30 minutes dry heat at 250°C).




THANK
YOU