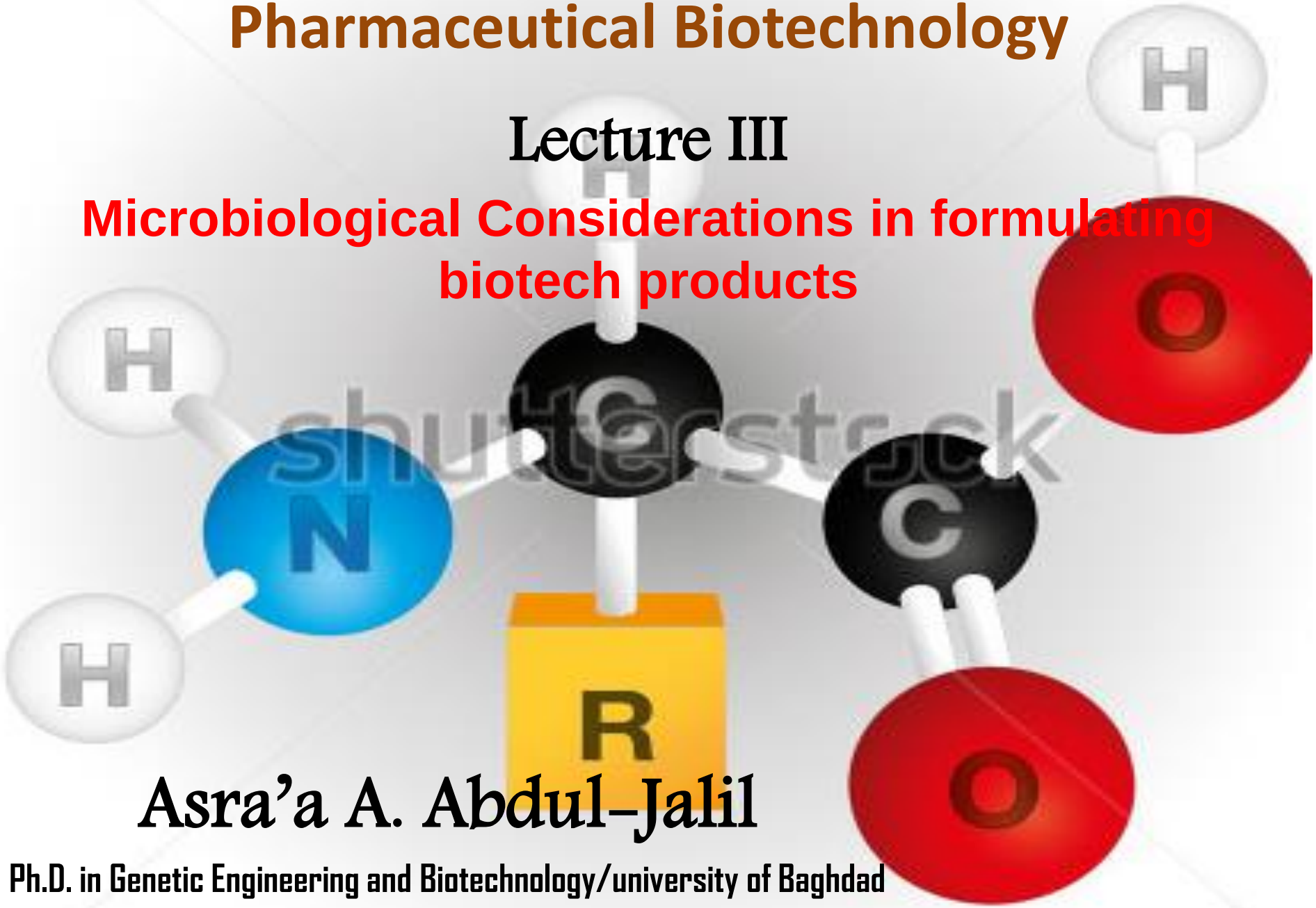
Pharmaceutical Biotechnology

Lecture III

Microbiological Considerations in formulating biotech products



Asra'a A. Abdul-Jalil

Ph.D. in Genetic Engineering and Biotechnology/university of Baghdad

MICROBIOLOGICAL CONSIDERATIONS

- **Sterility**
- Most proteins are administered **parenterally** and have to be sterile.
- In general, proteins are sensitive to heat(Why?) and other regularly used sterilization treatments; they cannot withstand autoclaving, gas sterilization, or sterilization by ionizing radiation.
- Consequently, sterilization of the protein end product is **not possible**

Sterility

- protein pharmaceuticals have to be assembled under aseptic conditions.

- Equipment and excipients are treated separately and autoclaved, or sterilized by dry heat (> 160C). chemical treatment (example:H.W) or gamma radiation to minimize the bioburden.

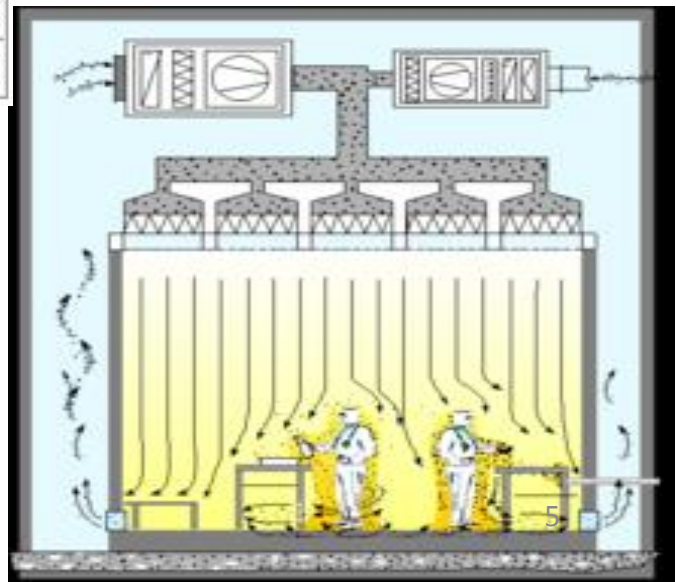
Sterility

- Filtration techniques are used for removal of microbial 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 airflow that is filtered through high efficiency particulate air (HEPA) filters

particles						
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



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

❖ Viral Decontamination

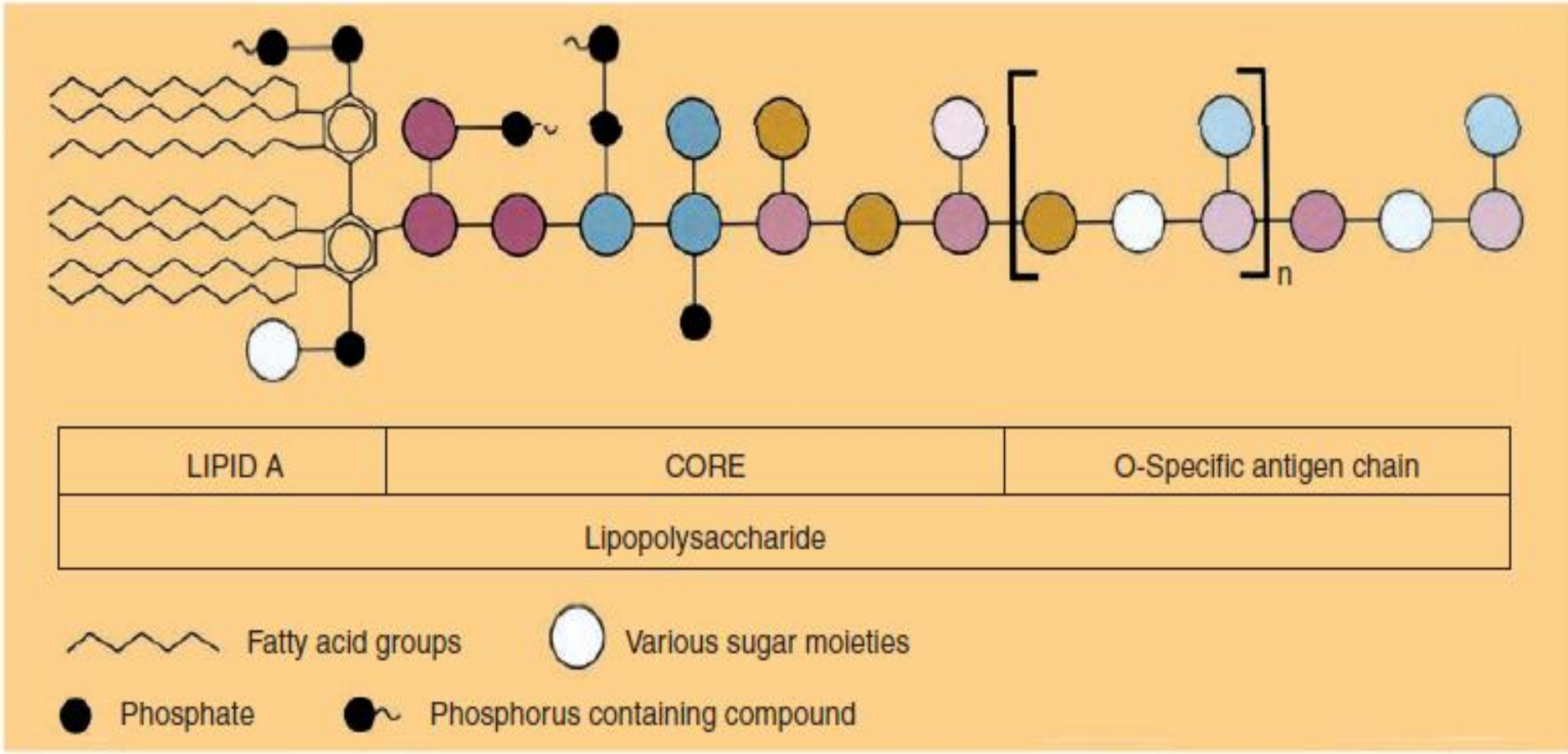
As recombinant DNA products are grown in microorganisms, these organisms should be tested for viral contaminants and appropriate measures should be taken if viral contamination occurs. In the rest of the manufacturing process, no (unwanted) viral material should be introduced. Excipients with a certain 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.

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 4 Methods for reducing or inactivating viral contaminants.

❖ Pyrogen Removal

- Pyrogens are compounds that induce fever.
- 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.
- Humans are sensitive to pyrogen contamination at very low concentrations (**picograms/mL**).
- Another general property shared by endotoxins is their high, negative electrical charge. Their tendency to aggregate and to form large units with MW of over 10^6 in water and their tendency to adsorb to surfaces indicate that these compounds are amphipathic in nature.



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 (different colored circles). Although the general structure is similar, individual endotoxins vary according to their source and are characterized by the O-specific antigenic chain. Source: Adapted from Groves, 1988.

❖ Pyrogen Removal

- ✓ 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 160C for prolonged periods(e.g., 30 minutes dry heat at 250C).

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

- ✓ Endotoxins can also be inactivated on utensil surfaces by oxidation (e.g., peroxide) or dry heating (e.g., 30 minutes dry heat at 250C).

Thank you