Pharmaceutical Biotechnology

Lecture 2-biopharmaceutical consideration

Asra'a A. Abdul-Jalil

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

Abbreviations

MI	Myocardial infarction
LPS	lipopolysaccharide
rDNA	Recombinant deoxyribonucleic acid/recombinant-DNA
HSA	Human serum albumin
IP	Intra peritoneal
LDL	low-density lipoprotein
SOD	Superoxide dismutase
WHO	World Health Organization
IA	Intra-arterial
IC	Intracoronary

Topics

Formulation of pharmaceutical proteins

MICROBIOLOGICAL CONSIDERATIONS

Sterility

MICROBIOLOGICAL CONSIDERATIONS

- **Sterility**
- Most proteins are administered parenterally and have to be sterile.

- In general, proteins are sensitive to heat and other regularly used sterilization treatments; they cannot withstand autoclaving, gas sterilization, or sterilization by ionizing radiation

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 or gamma radiation to minimize the bioburden.

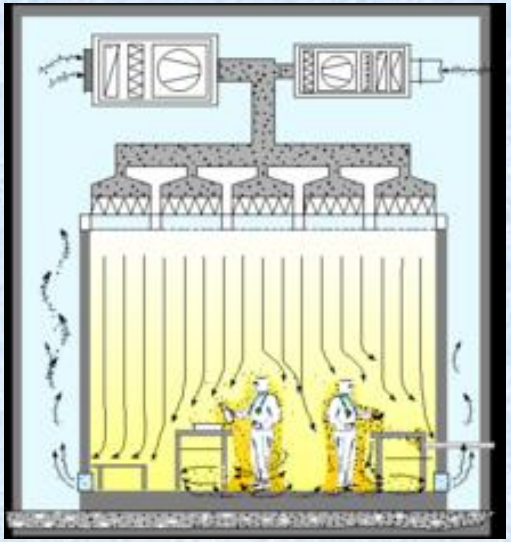
Sterility

Filtration techniques are used for removal of microbial contaminants

Prefilters 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 mm 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

“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)



Sterility

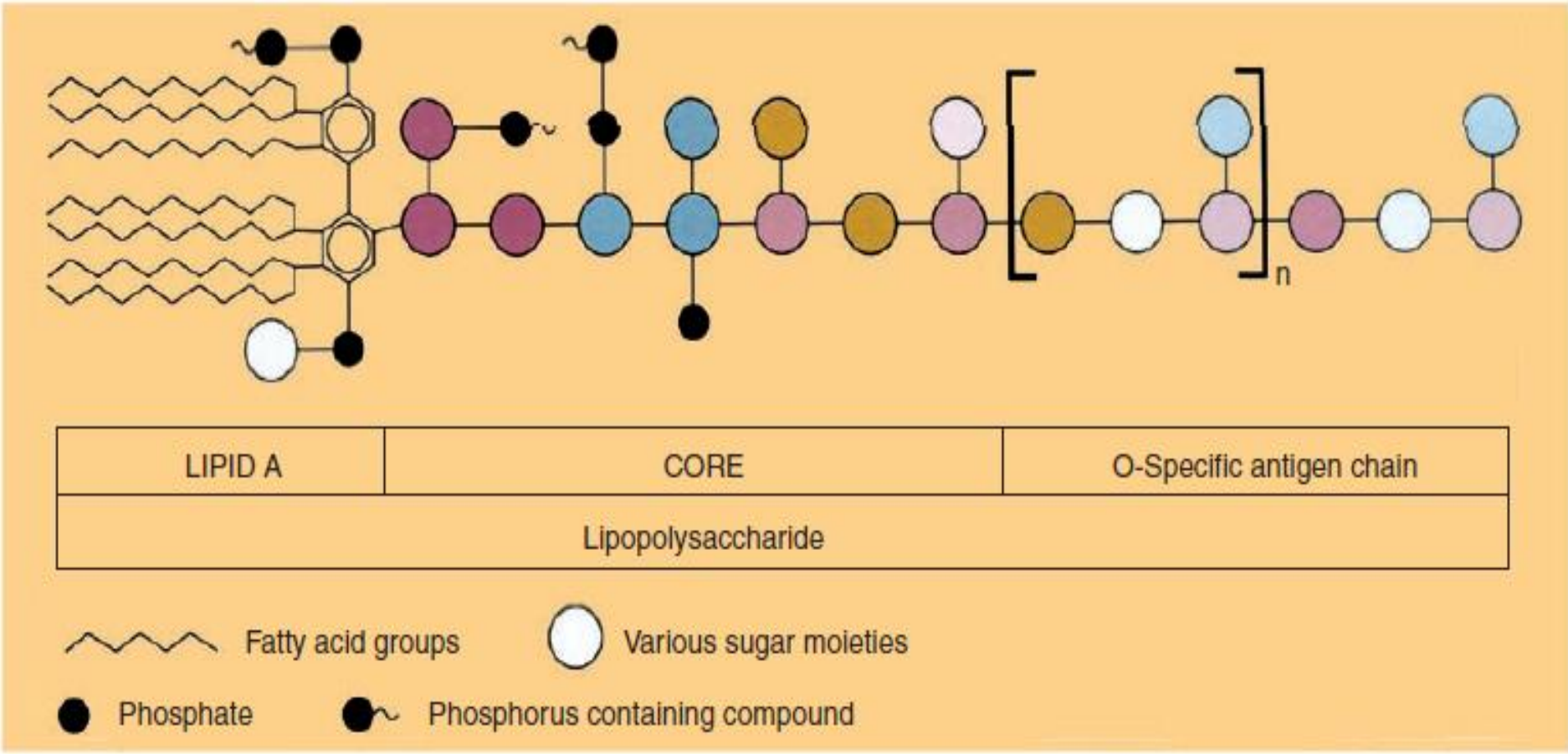
❖Viral Decontamination

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.

Sterility

❖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.



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.

Sterility

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 106 in water and their tendency to adsorb to surfaces indicate that these compounds are amphipathic in nature.

Sterility

- ✓ 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).

EXCIPIENTS USED IN PARENTERAL FORMULATIONS OF BIOTECH PRODUCTS

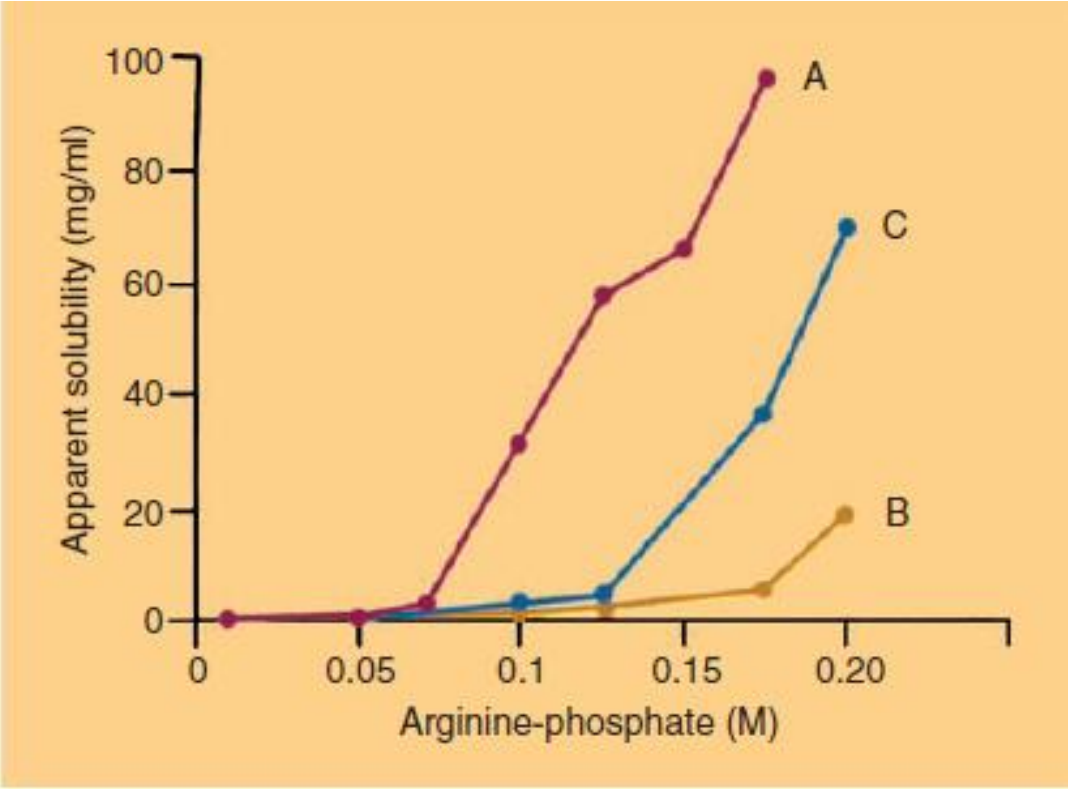
- Active ingredient
- Solubility enhancers
- Anti-adsorption and anti-aggregation agents
- Buffer components
- Preservatives and antioxidants
- Lyoprotectants/cake formers
- Osmotic agents
- Carrier system (see later in this chapter)

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

❑Solubility Enhancers

Proteins, in particular those that are non-glycosylated, may have a tendency to aggregate and precipitate. Approaches that can be used to enhance solubility include selection of the proper pH and ionic strength conditions. Addition of amino acids such as lysine or arginine (used to solubilize tissue plasminogen activator, t-PA), or surfactants such as sodium dodecylsulfate to solubilize non-glucosylated IL-2 can also help to increase the solubility

Solubility Enhancers



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. Source: From Nguyen and Ward, 1993.