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Postgraduate course, Monsoon semester, 2020

VMC 609: Techniques in microbiology and immunology

Topic: *Concentration and purification of animal viruses (PART II)*

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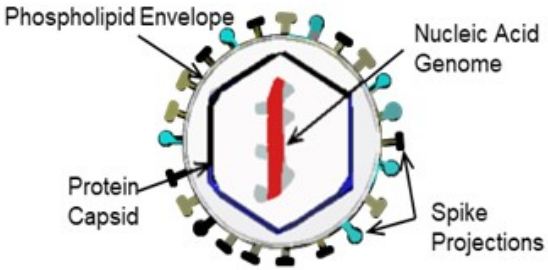
Virus particle- Challenges for vaccine production



- **Complexity**
- **Genetic material**
  - DNA or RNA
- **Protein and other components**
- **Surface diversity:** Heterogenicity in protein distribution, glycosylation pattern, charge variants
- **Other contaminants**
  - Debris, impurities from host cell, media components
- **Instability:** Loss of structure, genetic material

Viral vaccine process can be split into three sections:

- Upstream
  - (production and clarification)
- Downstream processing
  - (purification involving ultrafiltration, chromatography and chemical Treatments
- Formulation (finish-fill operation).

Purification

Purification (initial removal of undesirable materials) plays a critical role in defining a robust purification process

- Purification step primarily removes:
  - whole cells
  - cell debris
  - colloids
  - large aggregates.

- Purification also reduces:
  - insoluble impurities
  - host cell proteins (HCPs)
  - host cell nucleic acids.

- Multistep process
- Requires careful optimisation for virus titre and protein yield & purity
- Various methods are employed for purification

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Traditional methods of virus purification

- Ultracentrifugation
- Precipitation
- Ultrafiltration
- Chromatography

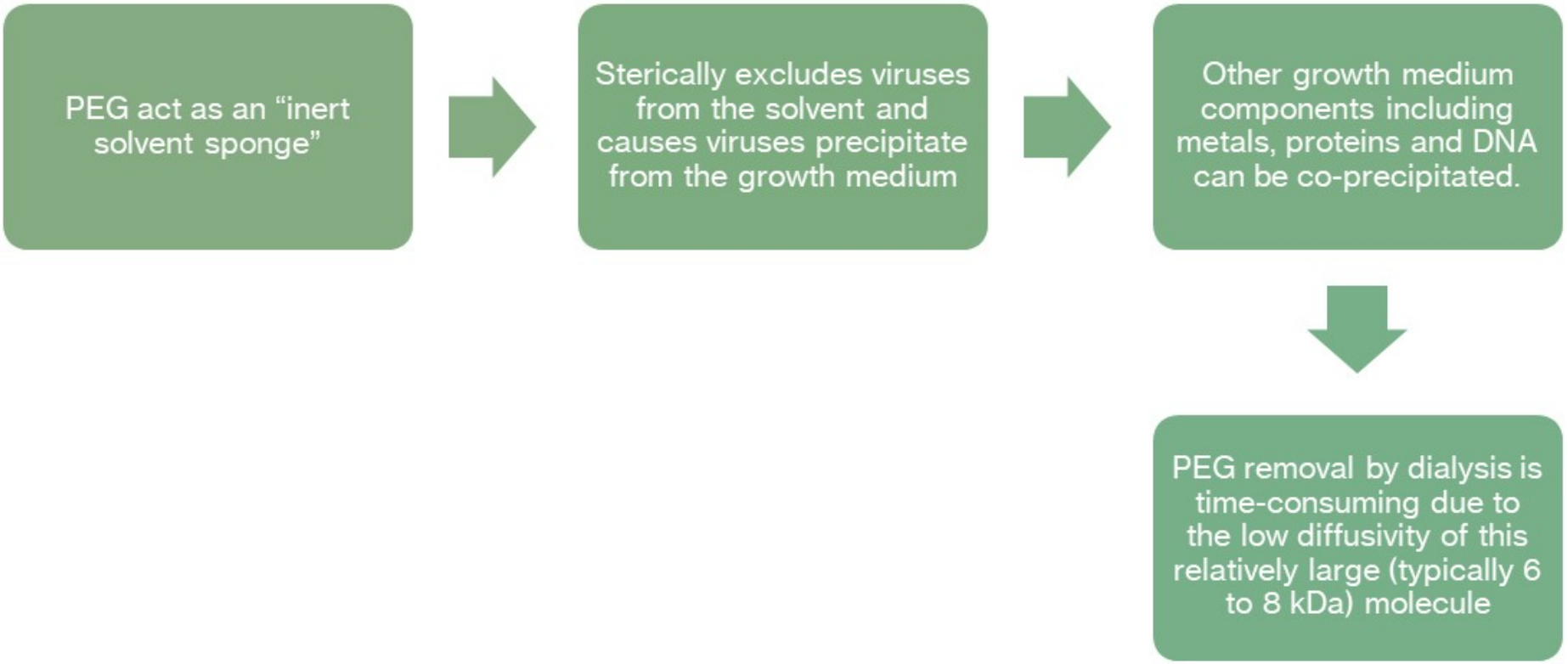
Purification steps

- **Primary purification** - aimed at removing larger particles.
- **Secondary purification** - removing colloids and other sub-micron particles.

Concentration by Precipitation

- Precipitation is commonly used method for protein concentration
- typically performed by the salting out of proteins with the high concentration of salts such as
  - Ammonium sulphate [$(NH_4)2SO_4$]
  - Sodium sulphate (Na_2SO_4)
- Precipitation by polyethylene glycol (PEG) has been used for virus concentration and purification for viruses
  - higher PEG concentrations can concentrate, but not purify virus.
  - lower concentrations of PEG can purify virus by precipitating virus
  - salt precipitation can affect the virus integrity
- Precipitation is typically followed by centrifugation or filtration to either remove or recover the precipitated species.

Virus precipitation using PEG



Precipitation

Advantage

- Cheap
- Simple to operate
- Suitable for microcarrier based process

Disadvantage

- Unreliable
- Time consuming
- Product loss

Protocol- PEG based purification

- Add PEG 6,000 into virus stock with gentle stirring to reach the final concentration of 10% (w/v)
- Add NaCl with the final concentration of 0.5 M.
- Upon addition of PEG and NaCl, virus stock become turbid.
- Store virus stock in the dark at 4°C with gentle stirring overnight.
- Centrifuged stock at 10,000 g for 30 min at 4°C.
- Discard the supernatant and resuspend the pellet via overnight nutation with 1 mM NaCl (pH 5.7, unadjusted)
  - Filter NaCl through a 0.22 µm pore size membrane prior to use.

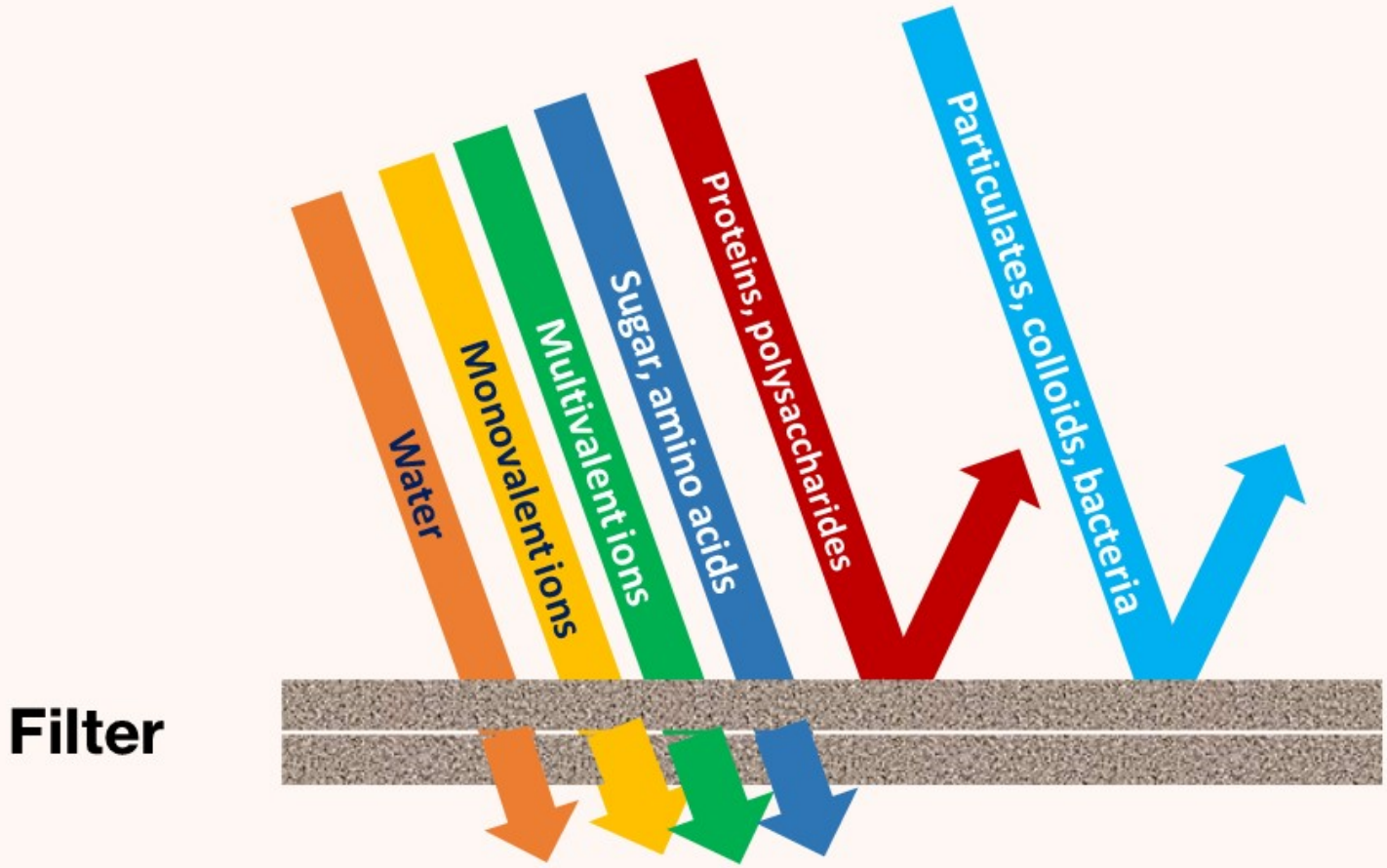
Contd...

- Treat the solution with the resuspended virus by adding chloroform (1:1 v/v) to remove remnant PEG.
- Vigorously vortex resuspension upon addition of chloroform for 30 s and then centrifuged at 1,700 g for 30 min.
- The aqueous fraction above the white layer should be carefully aspirated into fresh tube as stock without disrupting the white layer.
- Finally, dialyzed the virus stock against 1 mM NaCl (pH 5.7, unadjusted) and stored at 4°C.

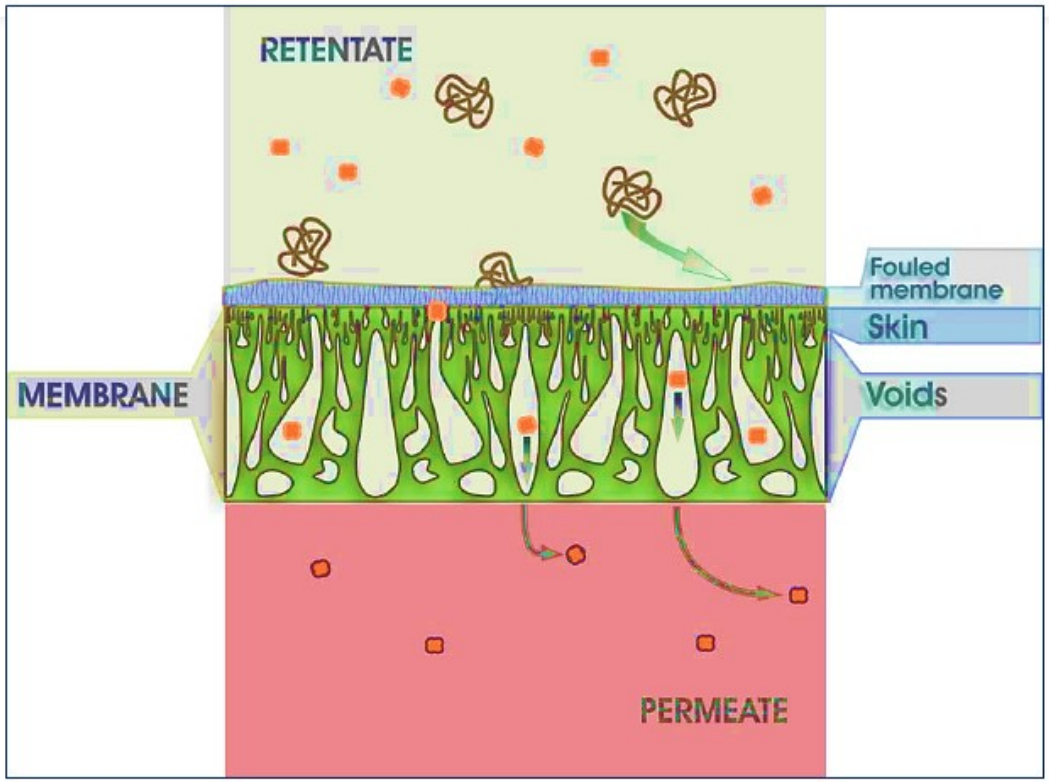
Filtration

- Concentration and/or purification step is frequently required for
  - investigations on infectious viral diseases
  - development of vaccines or antiviral drugs
- Filtration is used both as a concentration and separation method for viral products
- Membrane based separation and purification methods are typically applied as size-based separations.
- Current membrane based separation methods typically use ultrafiltration membranes
- Combinations of polypropylene, cellulose and glass fiber materials are efficient

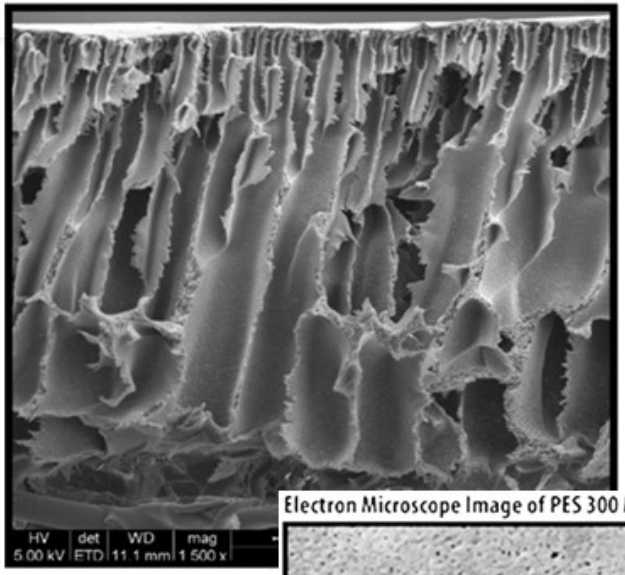
Ultrafiltration



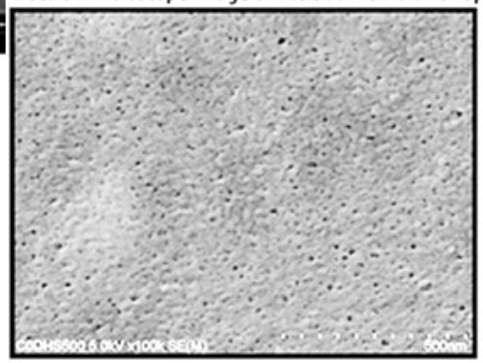
Ultrafiltration- filter structure



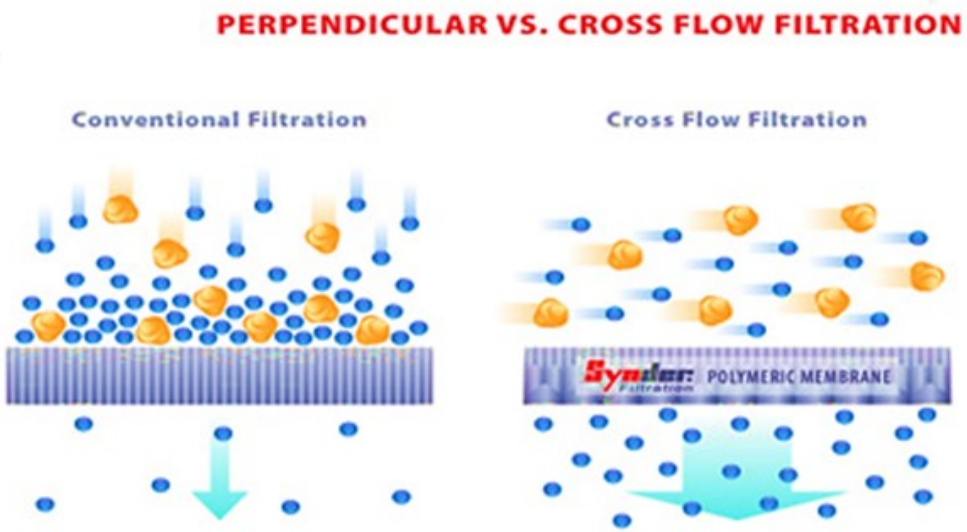
Electron Microscope Image of PES300 Membrane Cross Section



Electron Microscope Image of PES 300 Membrane Top View



- Ultrafiltration membrane range in pore size from 0.5 to 1000 kDa
- 0.65 µm or 0.45 µm microfiltration membrane device are used for secondary filtration.
- Filtration methods are operated in two types of flow
    1. normal flow filtration (NFF) (also called dead end filtration)
    2. tangential flow filtration (TFF)
    - **NFF** → flow is perpendicular to the membrane and particles larger than the membrane pore size are typically withheld by the membrane while smaller particulates pass through the membrane.
    - **TFF** → tangential flow of fluid with applied pressure



Types of filter

Depth filters - contain positively charged material and filter aid that enhance retention of :

cell debris	colloids	negatively charged & unwanted components.

Membrane filters- retain particles by size exclusion and are not of high dirt holding capacity. Suitable for secondary clarification step.

Protocol – Purification by filtration

- Load virus stock into Ultra filter unit and centrifuged at 1,500 g to bring the concentrate volume to ~0.5 mL.
- Add pre-filtered 1 mM NaCl solution (pH 5.7, unadjusted) to the concentrate to fill up the centrifuge tube
- Centrifuged the suspension again at 1,500 g until 0.5 mL sample remained in the concentrate.
- Repeat washing step for at least 10 times to remove media remnants and to complete storage solution exchange.
- Finally, collect the concentrate and use as the purified stock.

Thank you