Adsorptive and Mechanical Mechanisms of Fluid Purification Using Charge Modified Depth Filtration Media

Dr. Robert Conway, Ph.D.
Depth Filter Cartridge Construction
Zeta Plus Media Materials of Construction

- Filter Aids
  - High Purity Diatomaceous Earth, Perlite, Precipitated Silica
  - Activated Carbon: Three Porosities; Five Carbon Types

- Binding Resin
  - Three Amine Functionalities
  - Provides Wet Strength to Media

- Cellulose
  - Inert (Size Exclusion Matrix)
Zeta Plus® Depth Filters

• High Internal Surface Area
• Charge-modified via wet strength resin
• Filter aid options available

SEM Showing Bacterial Removal by Adsorption

Diatomaceous Earth

Fluid Purification
Depth Filtration

- Proper grade selection results in effective use of the depth of the filter media and not just the surface. Improper selection results in only surface filtration, which leads to short filter life.
Depth Filter Selection Criteria

- Throughput
  - volume to plugging
- Protection
  - Increased throughput of downstream membranes
- Filtrate turbidity
  - Reduced turbidity
- Absorbance
  - Removal of soluble species
Depth Filter Resin Chemistries vs. Adsorptive Profile

Charge Capacity (mg Metanil Yellow Dye/cm²)

pH

3.5  5  6  7  9

60ZA  60SP  60CP  60HT
Electrokinetic Double Layer

- Positively charged particle/fibre
- Strongly bound layer
- Diffuse layer
- Bulk Solution
- Two filters tested
  - 1. non-charge modified
  - 2. charge modified

- Both perform equally at removal of large particles - mechanical sieving

- Charge-modified filter more efficient at removal of sub-micron particles through adsorption.

*Capture of Latex Beads, Bacteria, Endotoxin, and Viruses by Charge Modified Filters; Applied and Environmental Microbiology, Nov. 1980, K. Hou, et al*
Depth Filter Applications

- Post fermentation cell/ cell debris clarification
- Pre chromatography clarification
- Post chromatography debris clarification
- Concentrated protein clarification
- DNA, virus, HCP removal
5000 Liter Process for Protein Production from Mammalian Cells

www.lonza.com
Options for Cell Harvest Purification

- Bioreactor → Centrifuge → Depth Filtration → Sterile Filtration 0.2 µm (0.45) → Purification

- Bioreactor → TFF → Depth Filtration → Sterile Filtration 0.2 µm (0.45) → Purification

- Bioreactor → Depth Filtration → Depth Filtration → Sterile Filtration 0.2 µm (0.45) → Purification
Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal during Monoclonal Antibody Purification

Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal during Monoclonal Antibody Purification

- Focus on Protein A chromatography platform purification process
- Addresses co-elution of host cell protein (HCP)/ aggregates with monoclonal antibody peak
- Investigates means to reduce contaminating HCP/ aggregates
Protein A Column Elution Profile

HCP/aggregate peak

280 nm (blue) = protein
410 nm (red) = turbidity
Pretreatment of Protein A Column Cell Harvest Load

Figure 3. Effect of pretreatment of cell culture harvest prior to Protein A chromatography. Overlaid absorbance traces at 410 nm showing the elution part of the Protein A chromatogram. Cell culture harvest samples were pretreated in the following ways prior to loading on the Protein A column: (i) Control (no pretreatment); (ii) Millistak A1HC depth filter at 150 L/m² loading, 50 LMH flux; (iii) Cuno 90ZA depth filter at 150 L/m² loading, 50 LMH flux; (iv) Anion-exchange flowthrough on a Fractogel TMAE column at 50 mg/mL loading (~50 mL harvest/mL column volume).

Effect of Single or Double Stage Depth Filtration on HCP/aggregate Breakthrough

![Graph showing area under Protein A elution peak at A410mm vs. volumetric loading (L/m²)](image)
Mechanism of HCP/Aggregate Protein Removal by Depth Filtration

**Figure 8.** Effect of mobile phase additives on BSA adsorption to Cuno 90ZA depth filters. Mobile phase: 10 mM Tris, pH 7.5. Flux: 50 LMH.

Adsorptive Depth Filtration: Conclusions

- Depth filtration reduces HCP/ aggregate protein contamination of Protein A column eluate

- Increased loading of depth filters result in breakthrough of HCP/ aggregates

- Use of series (redundant) depth filtration significantly reduces HCP/ aggregate contamination of Protein A column load

- Removal of HCP/ aggregates by depth filters results from electrostatic and hydrophobic mechanisms
Adsorptive Depth Filtration: Conclusions

- Depth filtration is an effective platform technology for mammalian cell harvest clarification
- Depth filters remove particulate and soluble contaminants
- Depth filters remove contaminants by size exclusion and adsorptive mechanisms
- Adsorptive removal by depth filters is often overlooked in selection and scale up evaluations
Depth Filter Skid Assembly