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Platform purification of clinical quality bacteriophage

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BIA Separations products and services

Convective Interaction Media (CIM®) Pre-packed monolithic columns

CIMac™ Analytical and CIMmultus™ Preparative columns

Services, Process development and Technical Support

Development of processes and methods for separation/concentration/purification of large biomolecules.
Custom immobilization, product development,..

Process Analytical Technology (PATfix™)

At-line PAT HPLC suite for **faster process development** and enhanced process control

Integrated Capability from Cell Culture Production through Downstream Processing

Bioprocess scale-up from laboratory to pilot
Managing interface between upstream and downstream
Vero cell bank



BIA Separations State-of-the-Art production facility > 30M USD investment



Expansion to increase production capacity by 5 till end of 2020 and by 30 till end of 2023 in progress

Certifications & Approvals

- DMF for DEAE, QA, SO3, C4 HLD, OH CIM[®] monoliths and CIMmultus housing on file, others pending
- Partners audits: Baxter-Shire, Novartis-Sandoz, Novartis-AveXis, Octapharma, Boehringer Ingelheim, Teva, Agilent, and many at present still confidential partners.
- As key supplier to the Partner FDA audited to meet USA GMP regulations
- ISO 9001: 2008





Convective Interaction

Media (CIM)

monolithic columns

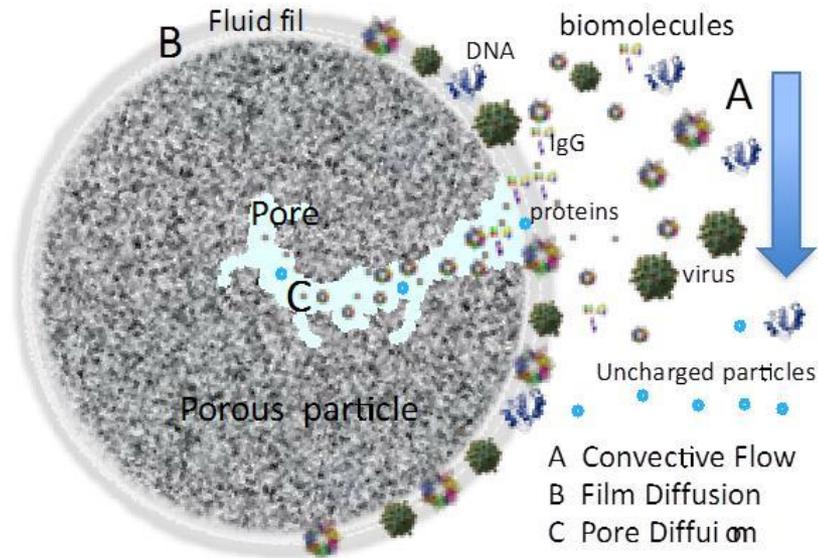


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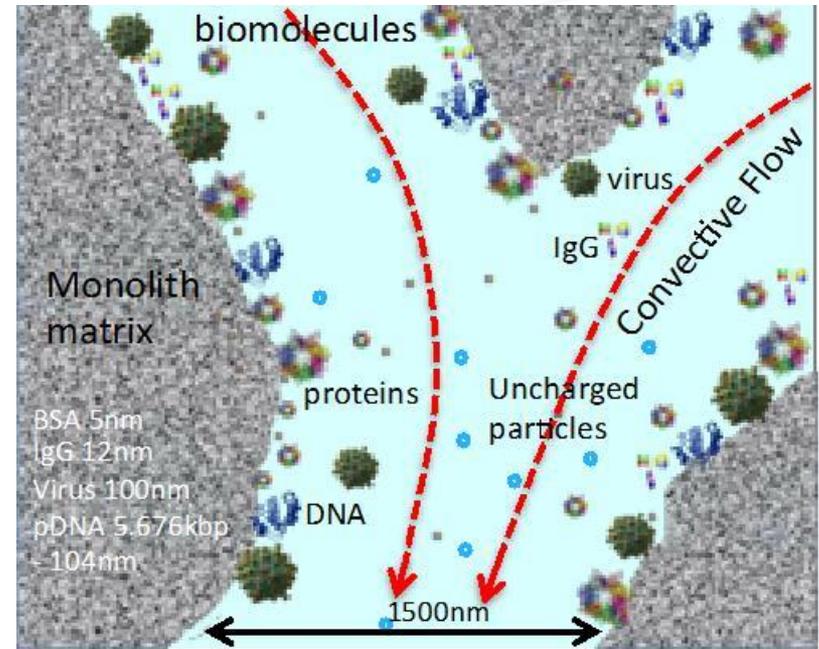
Instant chromatography and enzymatic processes using monolithic resins – no diffusion constraints

Mass Transport - Porous Particle Media



Traditional approach - Porous particle:

1. Diffusive mass transport – slow process or **lower resolution**
2. Pores too small – very **low capacity**
3. Counter current flow - shear forces – **lower yields**

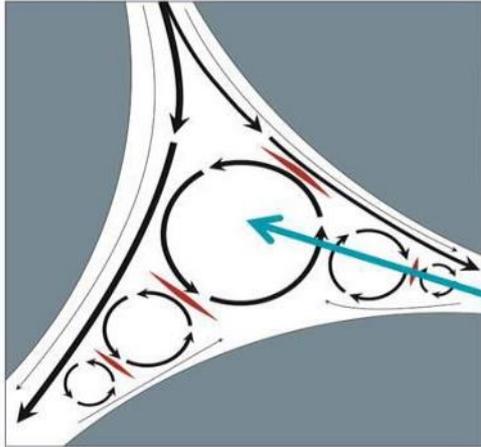


Novel UNIQUE approach – Monolithic columns:

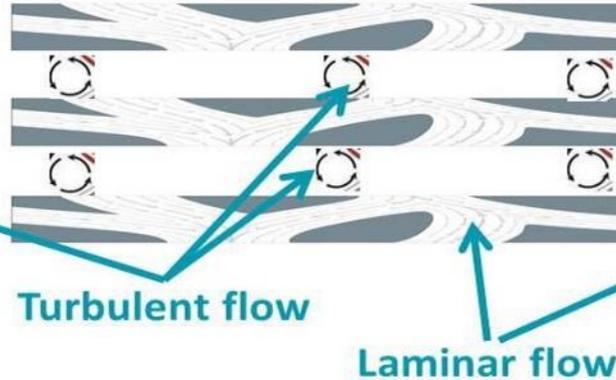
1. Convective mass transport – **flow independent resolution and capacity**, very fast processes
2. Accessible surface for big molecules – **high capacity**
3. Laminar flow - No shear forces – **better yields of e.g. IgM, Lenti, Adeno, Vaccinia, Flu,...**
4. **And better resolution** due to lack of diffusion and no turbulent mixing

Shear stress is very low in non-porous monoliths

Particle based columns



Membrane adsorbers



Turbulent flow

Laminar flow

Monolithic columns



Particle columns and membranes are dominated by turbulent shear stress

Turbulent shear stress in porous particle columns and membranes is directly proportional to flow rate and mobile phase viscosity. Both increase the grinding intensity at countercurrent interfaces and **destroy shear sensitive products.**

Flow through monoliths is almost exclusively laminar. Lack of turbulent shear contributes to higher recovery of infective / potent virus and better purity due to low back mixing.

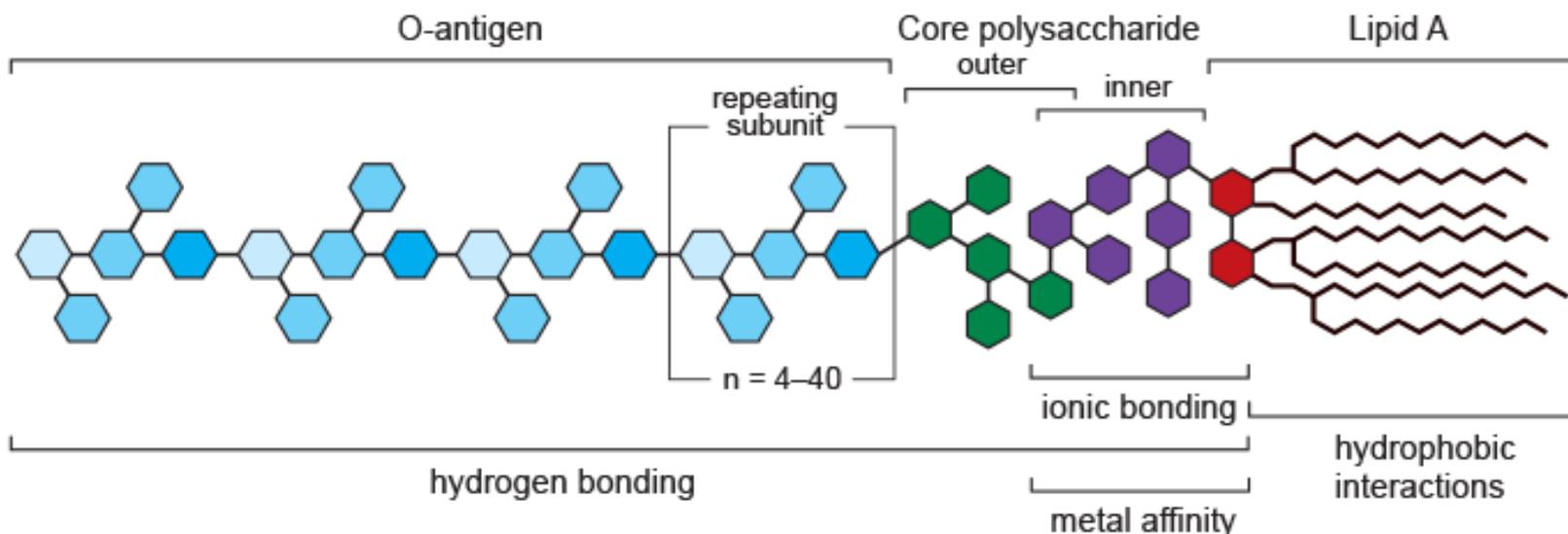


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Monolith chemistries for phage purification

The monolithic chemistries for phage purification were designed to exploit endotoxin chemistry in specific ways.

Different regions of endotoxins express different chemical character.



The O-antigen is uncharged. The core polysaccharide is negatively charged. The lipid A region is extremely hydrophobic. Note the similarity to detergent structure: hydrophobic head, hydrophilic tail.



Clinical grade Phage Industrial Purification Platform Process



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Monolith chemistries for phage purification

- Bacteriophage capture on OH monoliths uses high concentrations of precipitating salts or polyethylene glycol.
- These **solvents promote formation of endotoxin micelles**, with the hydrophobic lipid A region internalized.
- This leaves the hydrophilic O-antigen externalized so that the endotoxins do not interact with weakly hydrophobic surfaces.
- **Proteins are too weakly hydrophobic to bind and are mostly eliminated during sample loading.**
- Phages bind well because of their large size. Meanwhile, **the low hydrophobicity of the monoliths surface minimizes stress and contributes to high recovery of infective phage, typically 80–100%.**
- Stable **hetero-aggregates of phages with host-DNA are removed by washing with 1 M NaOH (CIP and SIP).**



Monolith chemistries for phage purification

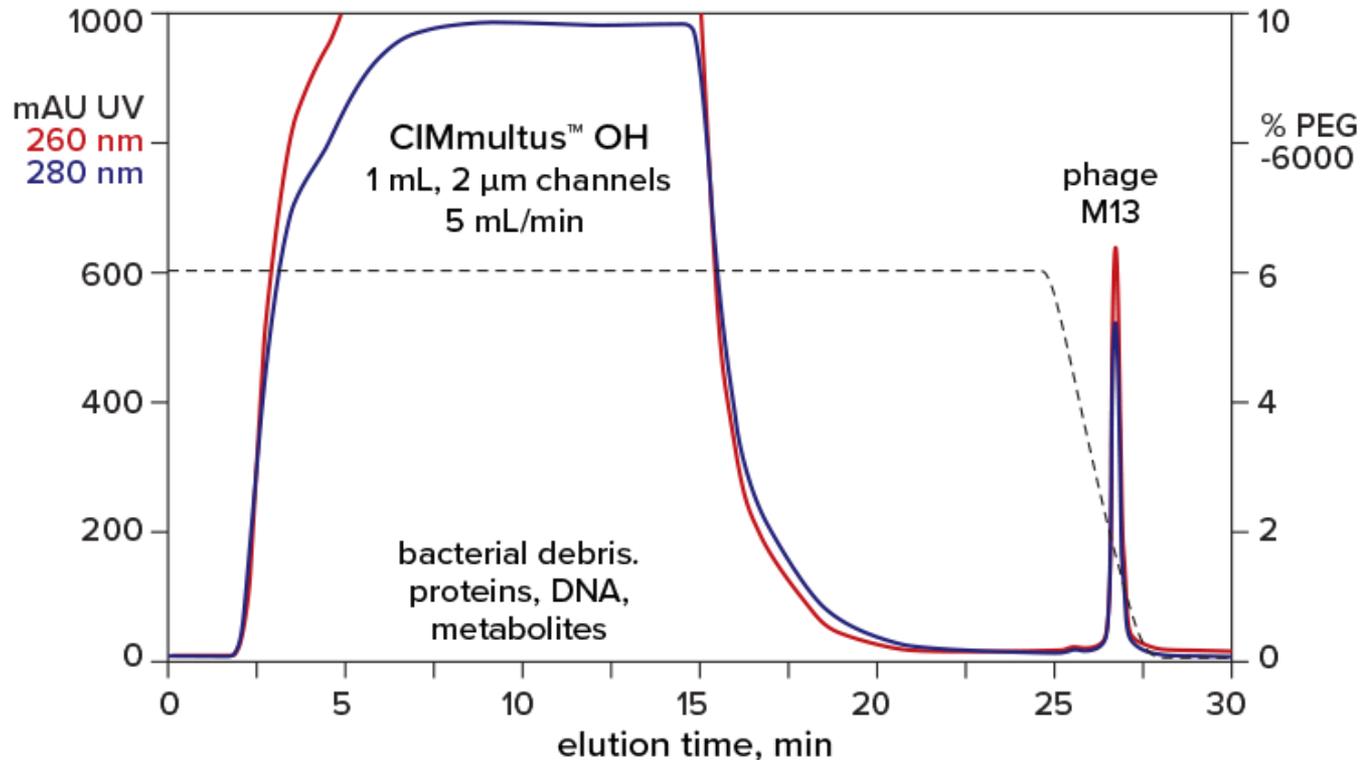
- Anion exchange chromatography is well known for phage purification. It polishes out low-level protein and DNA contamination.
- **Endotoxins bind anion exchangers by their negatively charged core polysaccharide regions.**
- Unfortunately traditional anion exchangers are often overwhelmed by the high endotoxin levels coming from Gram negative production hosts. They commonly reduce endotoxin levels by 2–3 logs but this is not always enough.
- **CIMmultus PrimaS™* was developed to more effectively exploit hydrogen bonding. This enhances endotoxin binding by involving the O-antigen region. Endotoxins are commonly reduced by 3–5 logs.**

*CIMmultus PrimaS™ for Bacteriophages not yet available for sale.



Phage capture with CIMmultus OH

Capture and initial purification of bacteriophage M13 using steric exclusion of polyethylene glycol to promote retention.



Bacteriophage M13, E.coli culture supernatant. Flow rate: 12 CV/min.
Capacity: 10^{13} vp/mL. Host protein reduction: 2 logs. DNA reduction: 2 log.
Infectivity recovery: >95%. Total process time: 30 min.

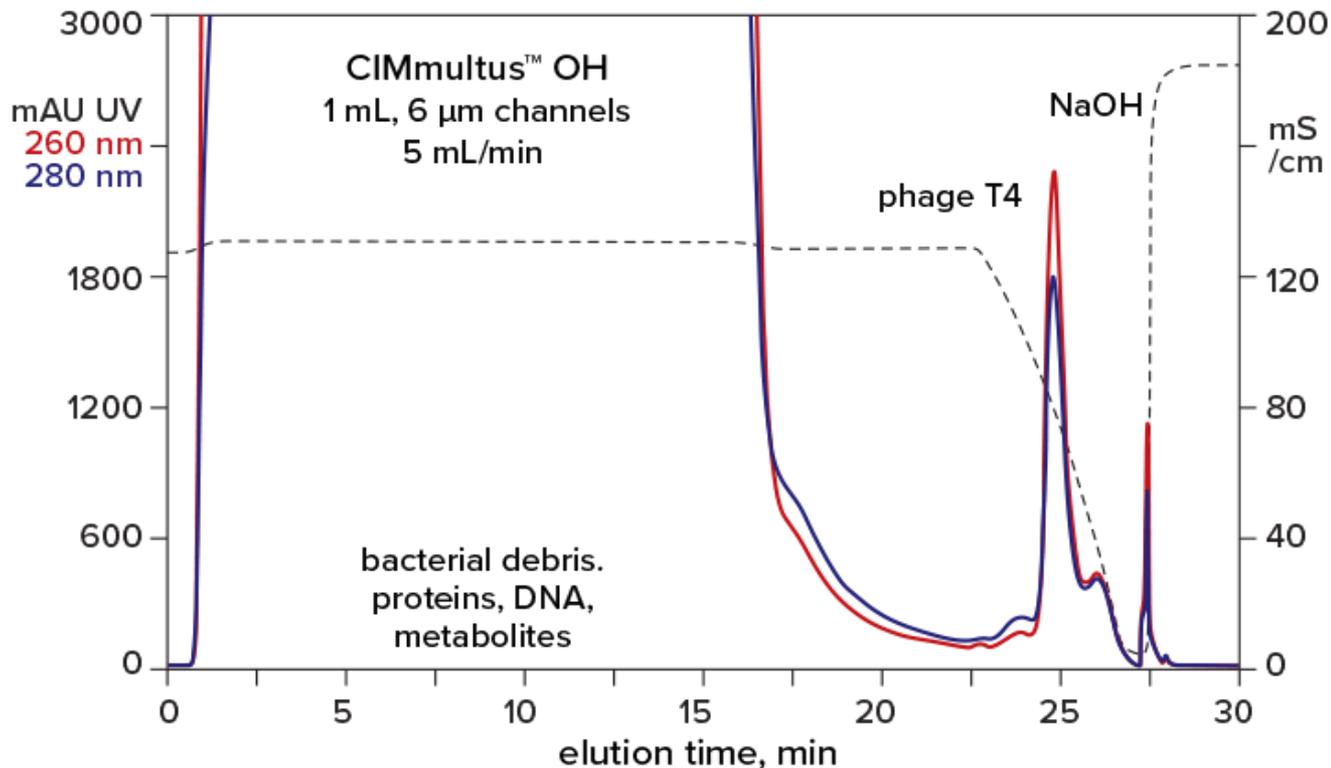


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Figure adapted from Lee et al, J Chromatogr A
1270 (2012) 162–170.

Phage capture with CIMmultus OH

Capture and initial purification of bacteriophage T4 using preferential exclusion of potassium phosphate to promote retention.

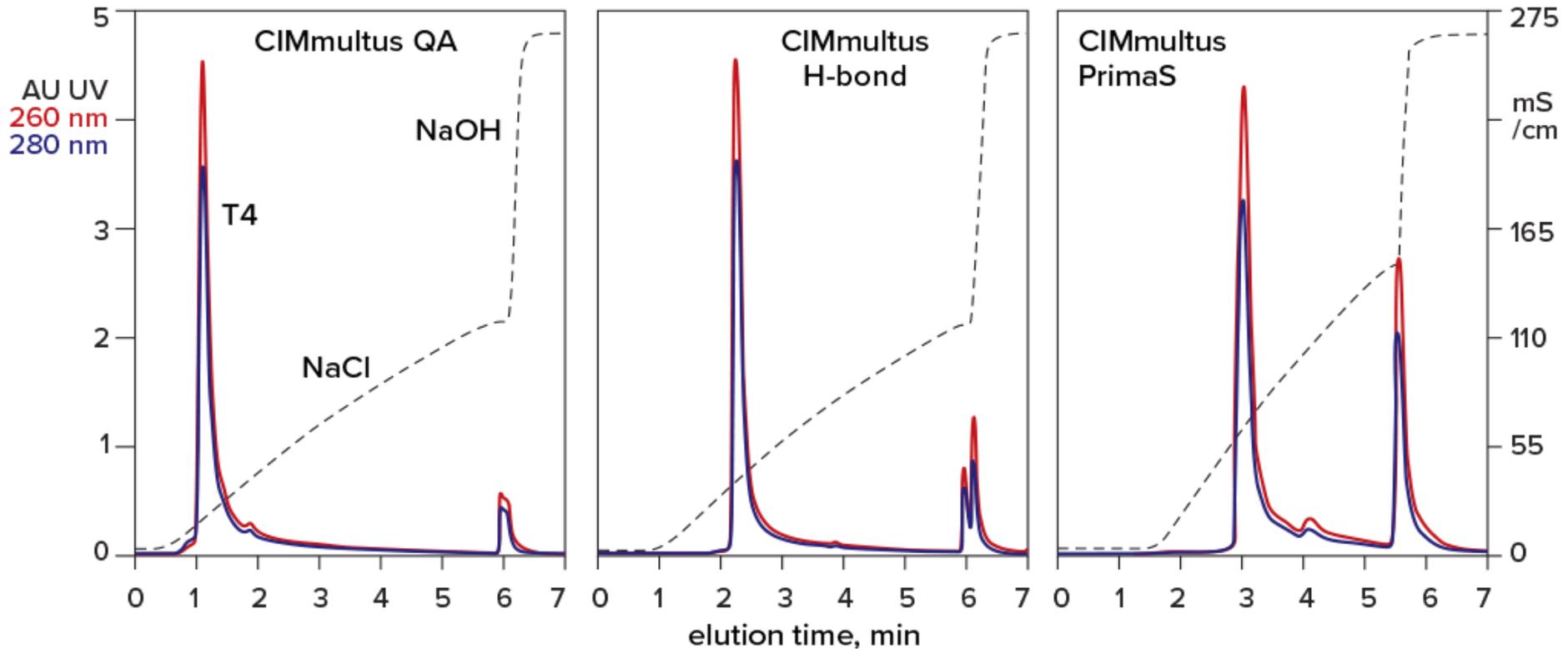


Sample: Bacteriophage T4, E.coli culture supernatant. Flow rate: 6 CV/min
Capacity: 10^{13} vp/mL. Host protein reduction: 4 logs. Infectivity recovery: 100%. Total process time: 30 min.



Polishing by anion exchange chromatography

Several anion exchangers can be used but PrimaS is consistently best



Infective recovery: 40%
Host protein redux: 99%
Endotoxin redux: 2 logs

Infective recovery: 51%
Host protein redux: 99%
Endotoxin redux: 3 logs

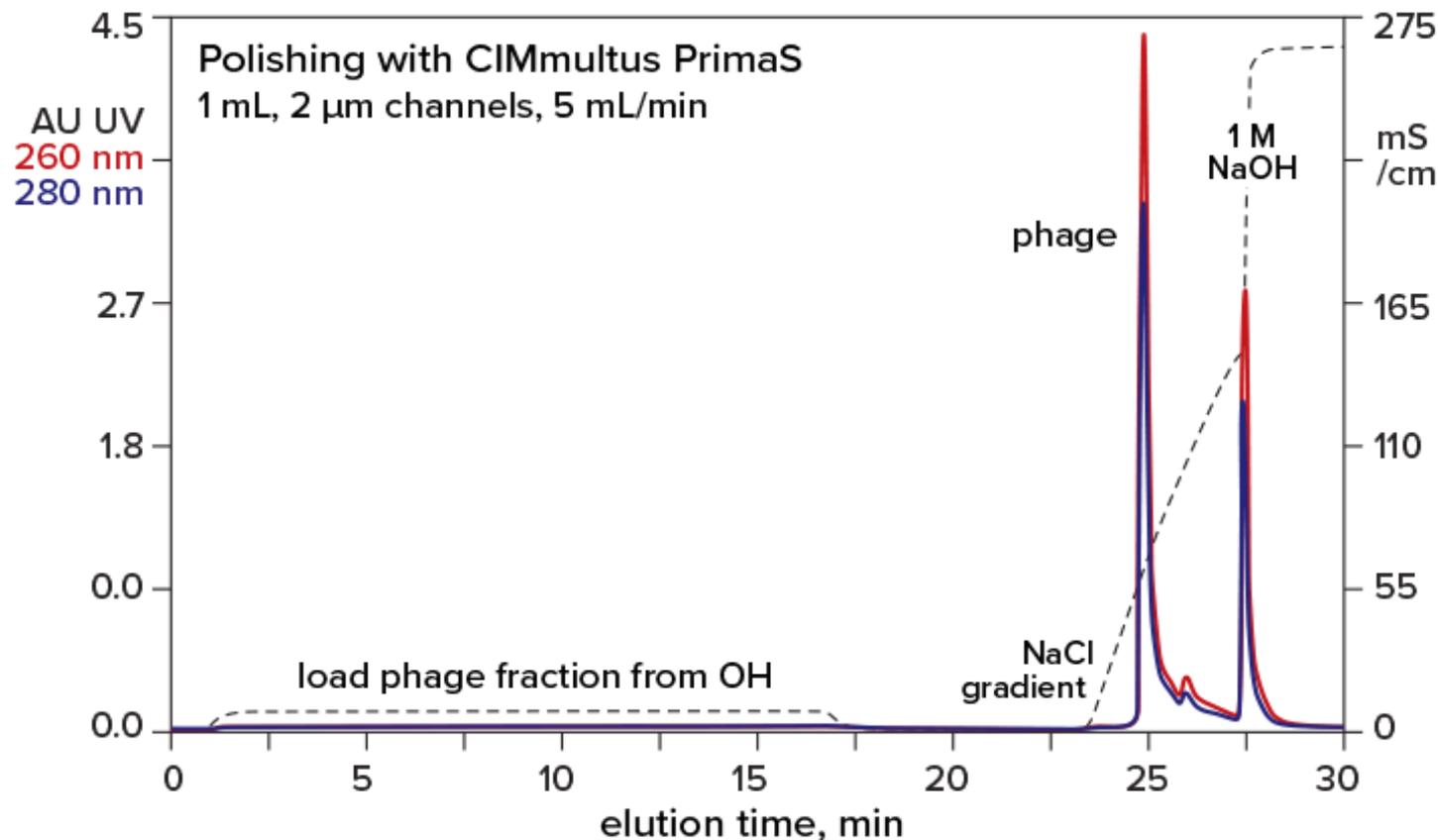
Infective recovery: 73%
Host protein redux: 99%
Endotoxin redux: 5 logs
Zoom out next page



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T4 Phage polishing with CIMmultus PrimaS

Zoom out



Sample: partially purified phage after capture by CIMmultus OH.
Host protein reduction by ELISA: 4 logs. **Endotoxin reduction: 5 logs.**
Infectivity recovery: 84%. Total process time: 30 min.



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Two-step platform for purification of all phages

0. No need for initial concentration by TFF (speeding up the process).

1. CIMmultus OH, 30 minutes.

Equilibrate column

Dilute sample with binding buffer and load column

Wash

Elute with descending salt gradient

Clean in place with NaOH.

2. CIMmultus PrimaS, 30 minutes.

Equilibrate column

Dilute phage sample from OH to reduce conductivity, then load

Wash

Elute with ascending salt gradient

Clean in place with NaOH.

The dilution factor required to prepare the sample for capture and capture by OH is typically less than the amount of buffer required to perform TFF, and much less than the amount of buffer required for TFF + chromatography.



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More than 15 phages tested for far

Two-step platform for purification of all phages

- CIMmultus columns are available with manufacturing volumes up to 8 L, and by special order up to 40 L.
- Based on an average capacity of $1e13$ infective phage particles per mL, an 8 L OH monolith can yield to $8e16$ phages per cycle.
- Up to 7 complete cycles of the 2-step process can be run per shift. Adjusting for an average 60% recovery across the 2 steps, this amounts a productivity of $> 3e17$ phages per shift.
- With a surge tank between the production system and the first column, purification can be continuous.



Conclusions

- Monoliths support represent rapid high capacity platform purification of bacteriophages with high resolution and low shear.
- CIMmultus OH concentrates the phage while it removes 2–4 logs of host proteins and endotoxins. Average recovery is > 80%.
- CIMmultus PrimaS also concentrates the phage while removing 3–5 logs of host proteins and endotoxins. Average recovery is 60–80%.
- The complete 2-step process can be run in about 1 hour.
- CIMmultus PrimaS can be also used following initial concentration by tangential flow filtration or PEG precipitation, but overall process performance and economics are inferior to OH > PrimaS.



BIA Separations - industry standard for production of Gene Therapy, Phage, Vaccines, Exosomes,.. products

- Platform processes for pDNA, mRNA, AAV, exosomes, Flu, Adeno,
- First drug purified using CIM[®] monoliths on the market, one passed CIII trial, 5 projects in CIII.
- More than 200 projects in CPI – CII trials (various Influenza, various Adenovirus, various AAVs, bacteriophages, various IgMs, Inter- alpha-inhibitors, exosomes,...).
- More than 1000 projects in pre-clinical trials (Influenza A and B virus (eggs, Vero and MDCK cells), Rabies virus, Rotavirus, AAV, various Adenovirus subtypes, Hepatitis A, Vaccinia, Mulv, MVM, Feline calicivirus, Japanese encephalitis, Crimean-Congo hemorrhagic fever, Hantaan virus, VLP (Hepatitis B, HPV, Influenza, Adenovirus), bacteriophages (Lambda, T4, VDX10, Pseudomonas phage), Tomato and Pepino Mosaic virus, pDNA, IgM, various proteins).



Acknowledgements

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