CIM Monolith Technology: Enabling Economic Vaccines Production

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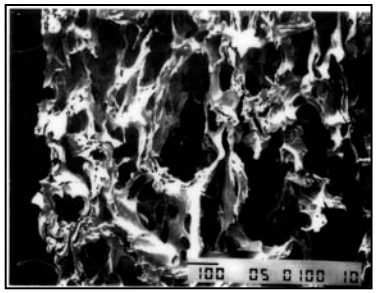
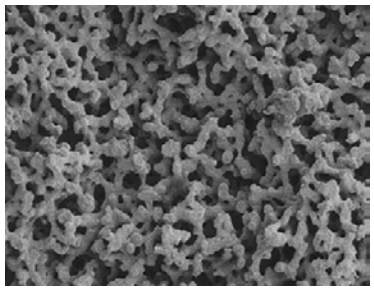
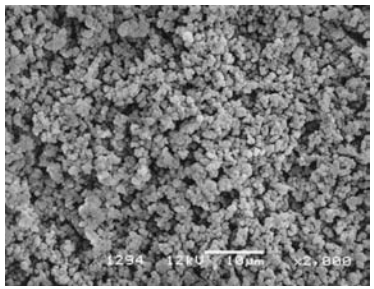
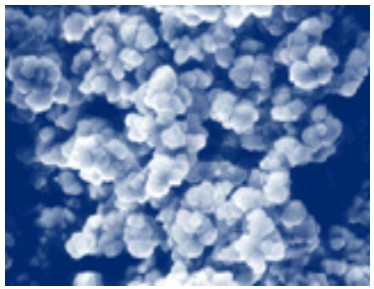
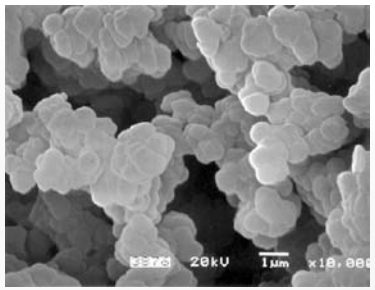
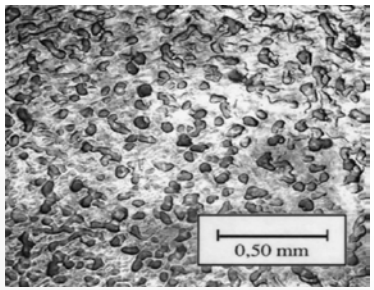
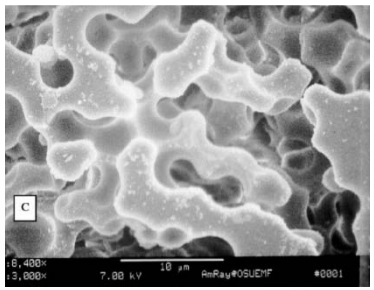
Nuevo Vallarta, Mexico

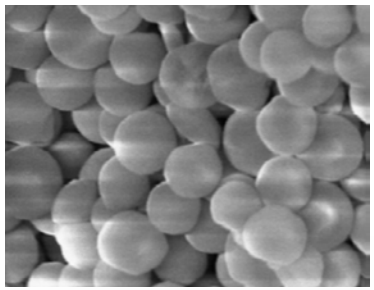
Monoliths

Monoliths are chromatography media that are cast as a single block and inserted into a chromatography housing. They are characterized by a highly inter-connected network of channels, sometimes likened to a sponge.

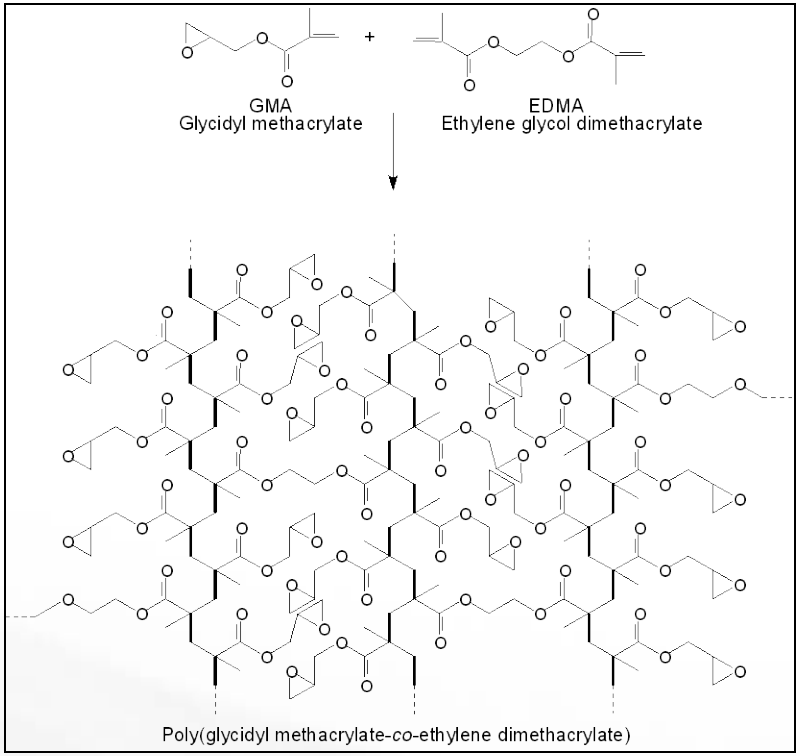






CIM Convective Interaction Media Monoliths

Made of highly cross-linked porous rigid monolithic *poly(glycidyl methacrylate-co-ethylene dimethacrylate)* or *poly(styrene-divinylbenzene)* polymers

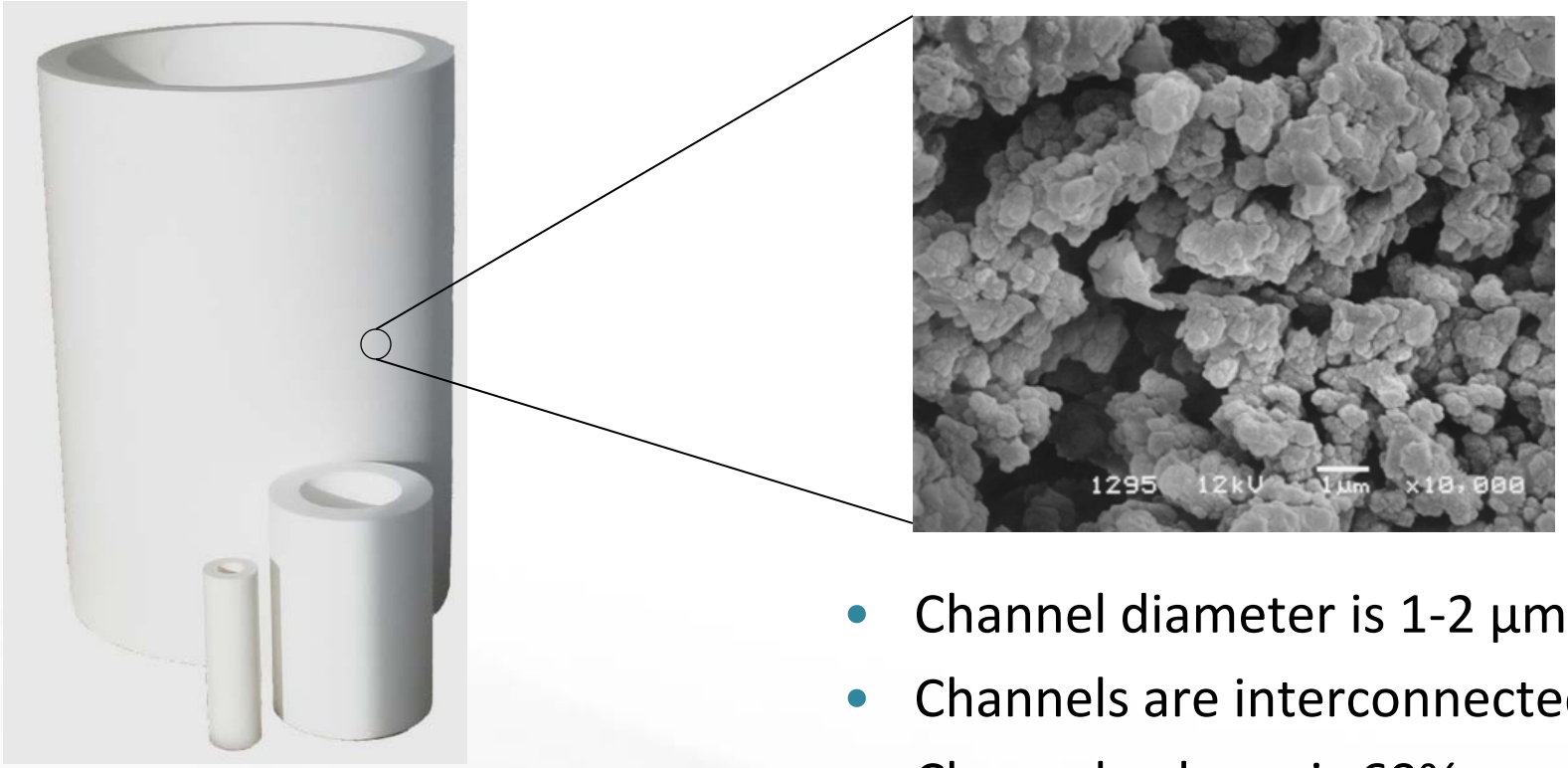


Why CIM Monoliths?

- Vaccines are based on large biomolecules and large composite biomolecules such as viruses
- Monoliths support high capacity and high resolution for such molecules
- Monoliths avoids generation of shear forces

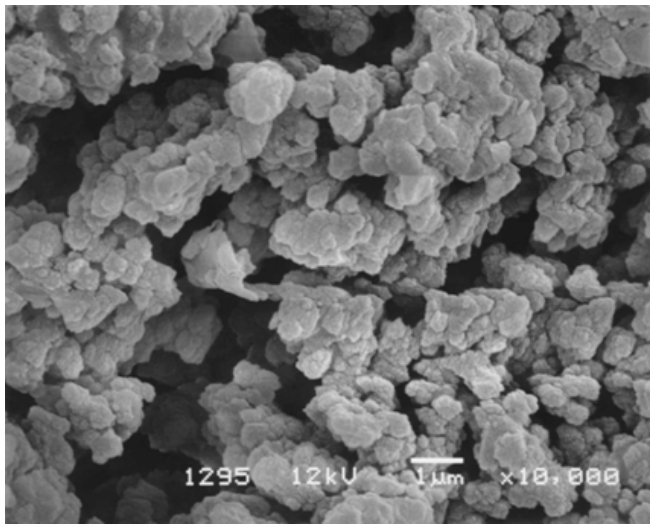
CIM Monoliths Structure

The architecture of monoliths is fundamentally different from packed particle columns.



- Channel diameter is 1-2 µm
- Channels are interconnected
- Channel volume is 60%

CIM Monoliths Properties



- Convective transport
- High surface accesibility
- Low pressure drop

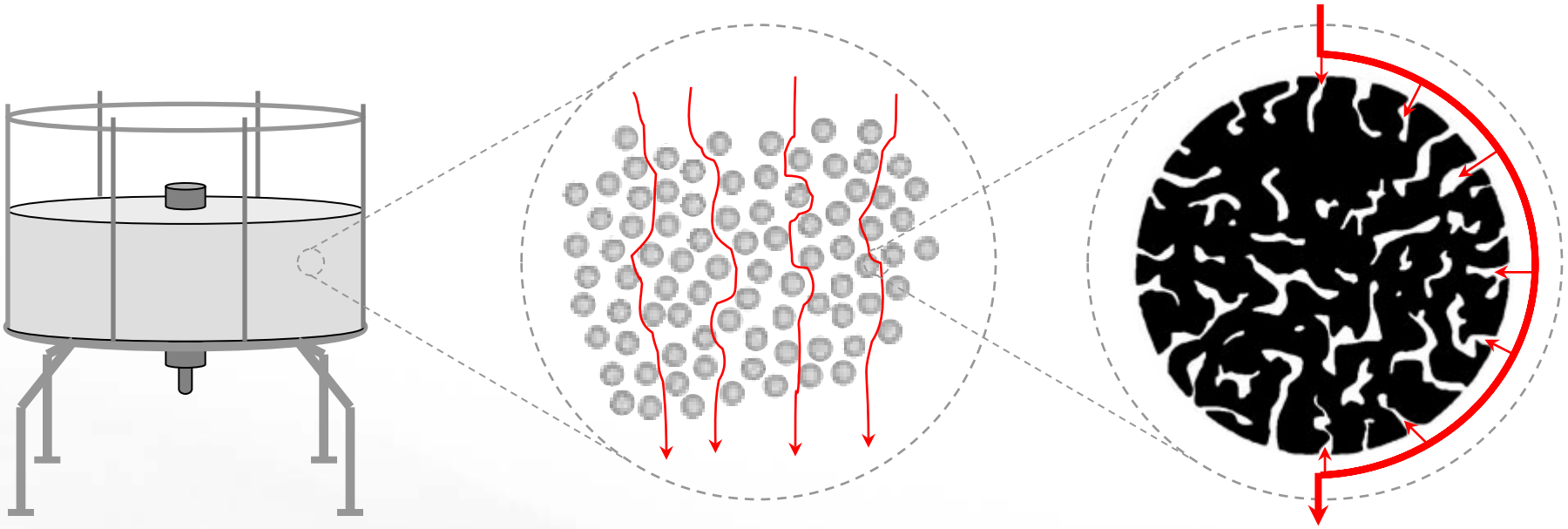
Mass Transport in Chromatographic Supports

Mass transport refers to the way solutes move through a chromatography column.

- Diffusion
  - Migration of the solutes from an area of high to an area of low concentration
- Convection
  - Movement indused by external force

Mass Transport by Diffusion

Mass transport in packed porous particle columns is a combination of convective transport through the void volume, and diffusive transport from particle surfaces into the pores.

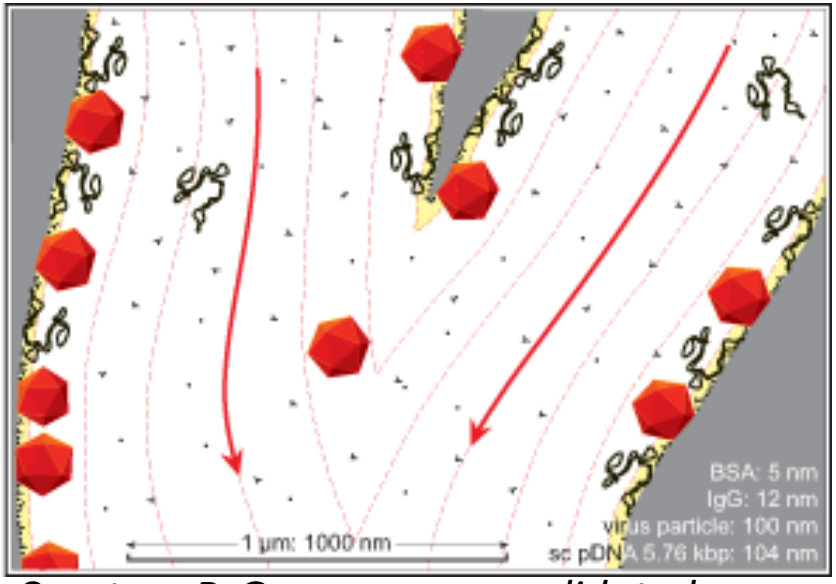


Molecular Mass: Diffusivity

molecule	MW	D (cm^2/s)
H^+	1 Da	1×10^{-4}
NaCl	58 Da	1.4×10^{-5}
BSA	66 kDa	6.1×10^{-7}
IgG	150 kDa	4.2×10^{-7}
TMV	40 000 kDa	5×10^{-8}
DNA	4.4 kbp	1.9×10^{-8}
DNA	33 kbp	4×10^{-9}

The larger the solute, the more slowly it diffuses. The more slowly it diffuses, the longer the time required for it to enter or exit from a pore.

Convective Mass Transport

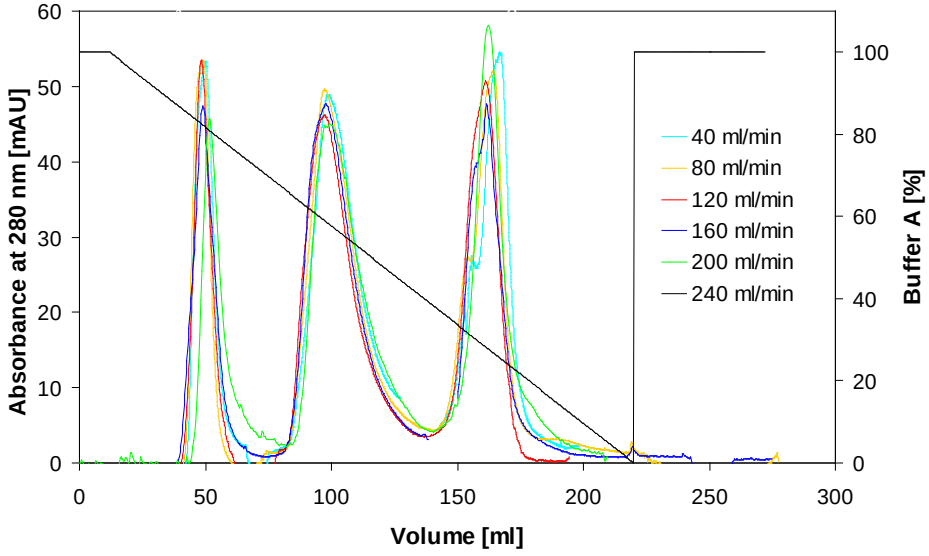


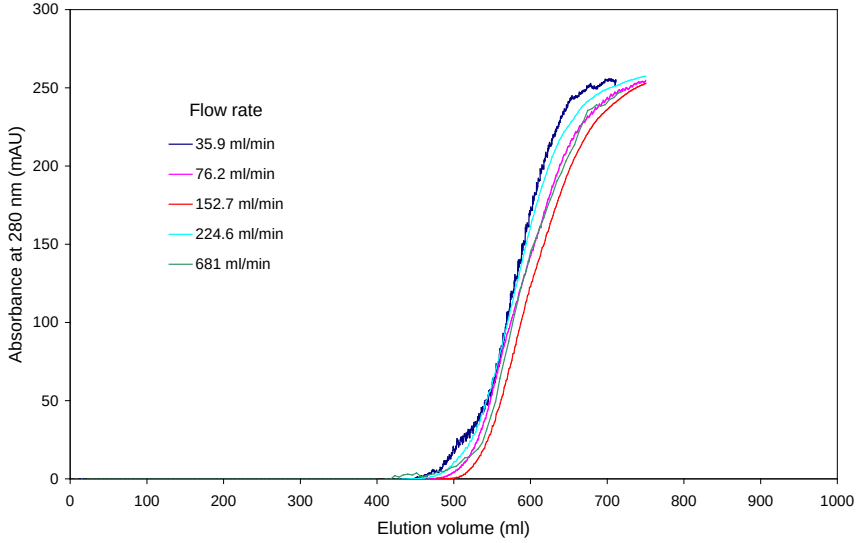
Courtesy P. Gagnon www.validated.com

Laminar flow prevents the eddy formation that causes dispersion and shear in packed particle columns. In further contrast, the axis of flow in a monolith is determined by local channel orientation. This prevents formation of “flow-shadows” that occur below the abaxial particle surfaces in packed columns

Convective Transport: Consequences

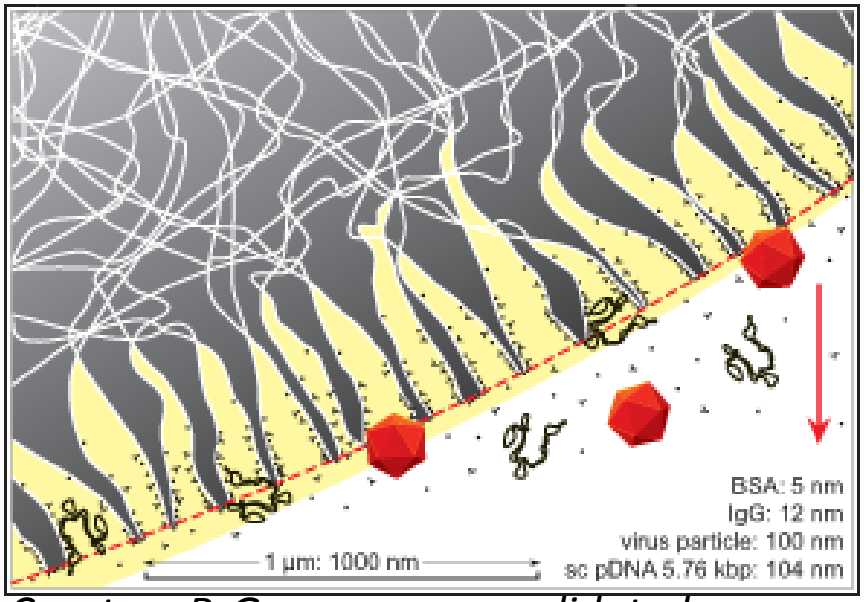
- Flow independent properties





Podgornik et al., Anal. Chem. 72 (2000) 5693

Molecule Size: Surface Accesibility



Courtesy P. Gagnon www.validated.com

Molecule	nm
Proteins	1-3
IgM	25
Plasmids	150-250
Rotavirus	130
Poxvirus	200 x 500
T4	220 x 85

Most porous particle chromatography media are optimized for protein applications. Average pore size among different products ranges from about 60 to 100 nm. Many plasmids and virus particles are larger and cannot enter such pores. Since most of the surface area resides within the pores, this dramatically reduces binding capacity

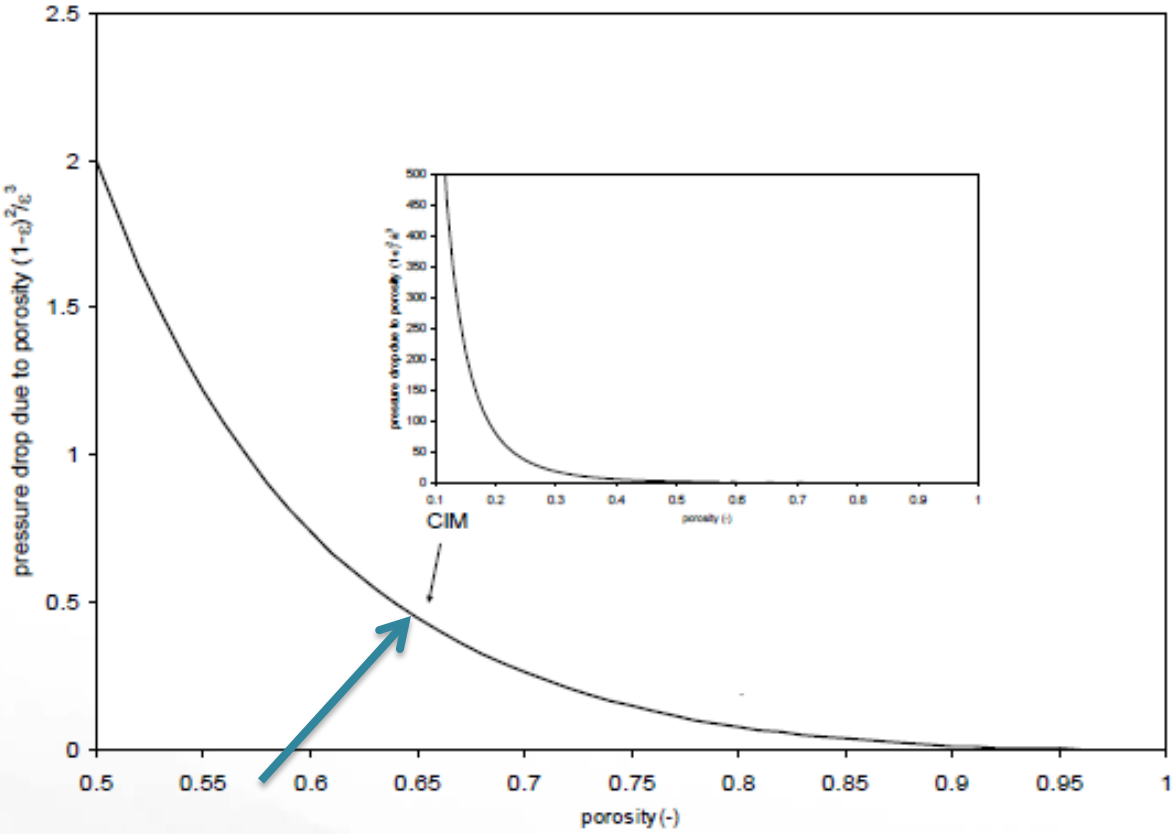
Surface accesibility fo CIM Monoliths

- High capacity for viruses and DNA

Molecule	Column	Capacity
Plasmid DNA	CIM DEAE	8 mg/mL
Genomic DNA	CIM DEAE	15 mg/mL
Endotoxins	CIM QA	>115 mg/mL
ToMV	CIM QA	2.0E+14 vp/mL
Influenza virus	CIM QA	2.0E+10 vp/mL
Adenovirus	CIM QA	3.0E+12 vp/mL
Ad3 VLPs	CIM QA	7.3E+16 VLP/mL

Porosity

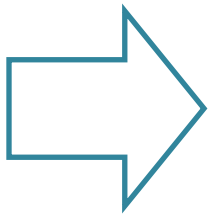
- Low pressure drop



CIM Monoliths Properties

- Flow independent properties
- High capacity for viruses and DNA
- Low pressure drop

- Fast separations
- Low buffer consumption
- High concentration factor

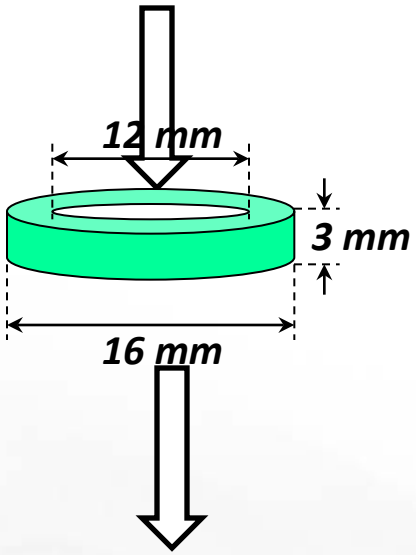


Process economics

CIM Monoliths Bed Configuration

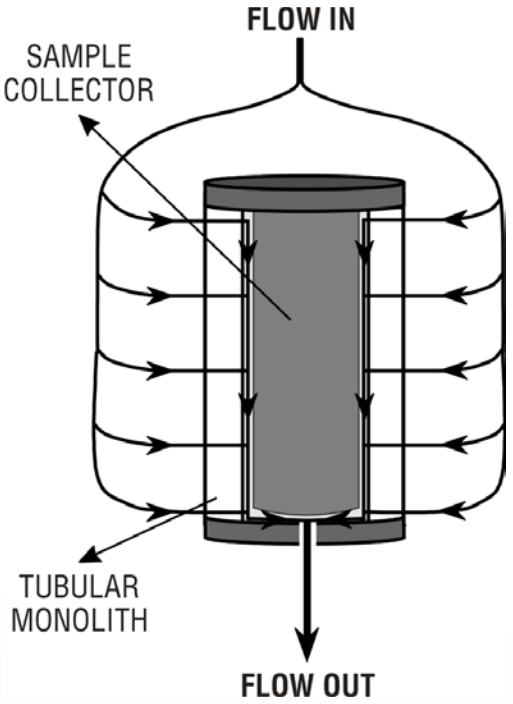
Small scale columns

CIM disks



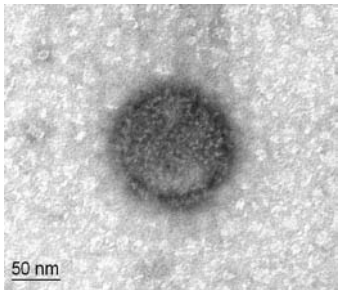
Large scale column

CIM Tubes



CIM Monoliths Applications Area

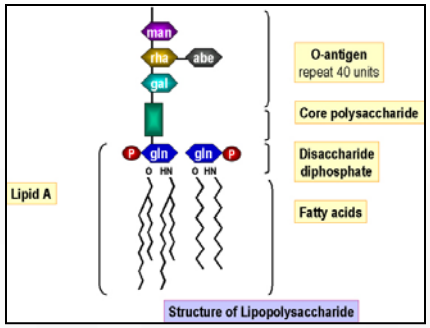
Virus



Plasmid DNA

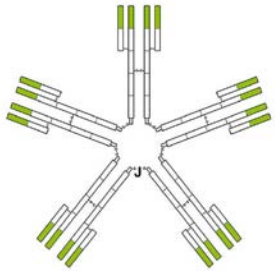
CIM

Endotoxin



DNA depletion

Large proteins

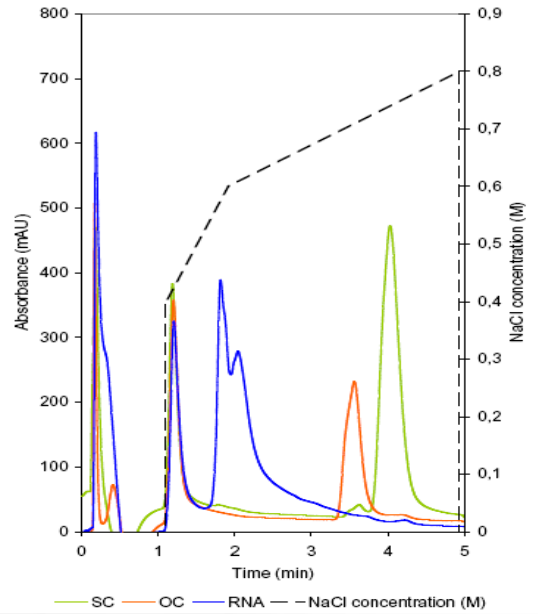


CIM DEAE for Plasmid DNA Purification

- High and flow independant dynamic binding capacity
  - 8 mg of pDNA per ml of CIM DEAE

- Separation of open circular and super coild plasmid DNA

  - RNA
  - OC pDNA
  - SC pDNA



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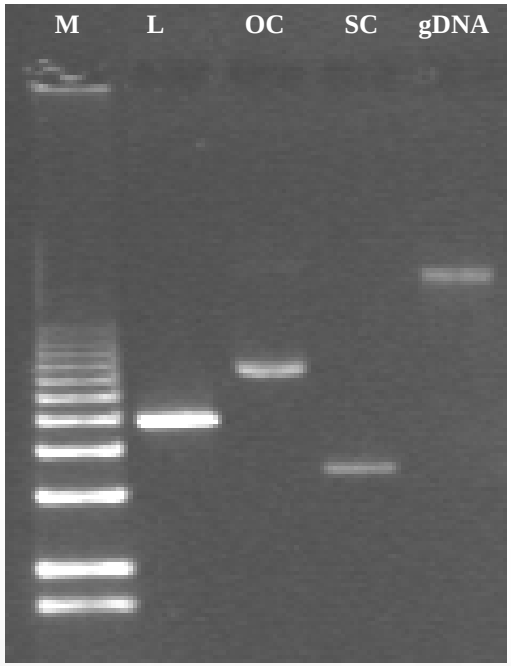
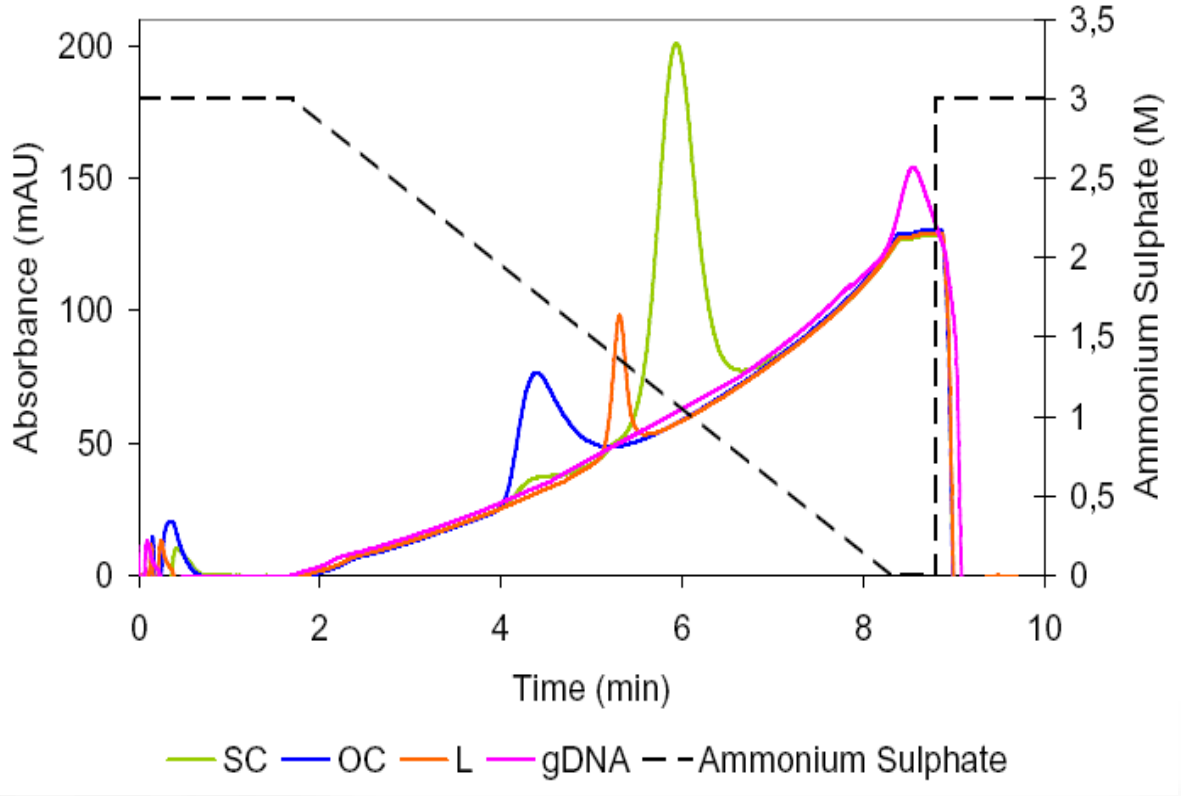
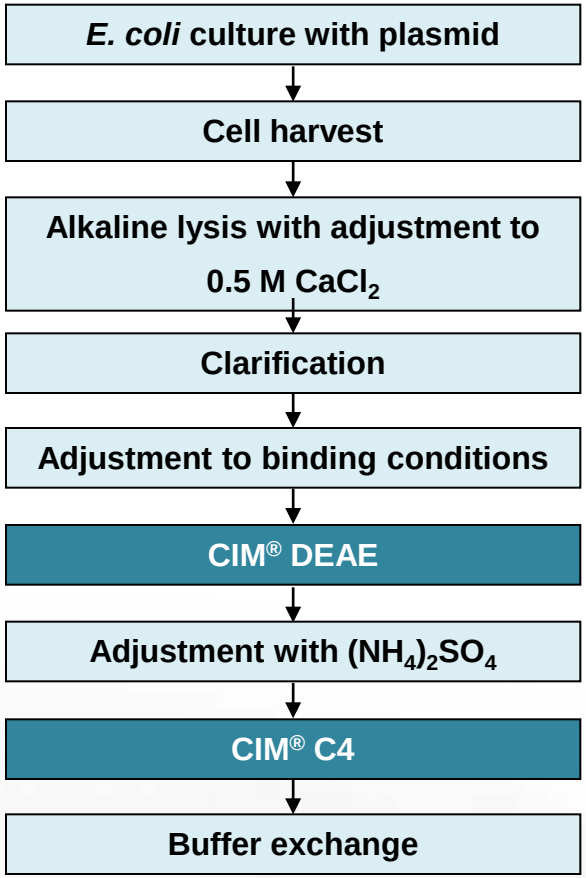
CIM C4 HLD for Plasmid DNA Purification

- Separation of isoforms and genomic DNA





CIM Based Plasmid DNA Purification Process



	Results	Specs	
pDNA (µg/mL)	**300**	-	
pDNA (mg)	**34**	-	
Homogeneity (%SC)	**98**	>95	✓
Endotoxins (EU/mg pDNA)	**1.1**	<100	✓
Host cell proteins (µg/mL)	**1.1**	<10	✓
gDNA (µg/mg pDNA)	**3.4**	<50	✓
RNA (µg/mL)	**0**	<4%	✓
Yield (%)	**90%**	-	✓

Productivity of CIM Plasmid DNA Purification Process

Vaccine 28 (2010) 2039–2045

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Preparation of pharmaceutical-grade plasmid DNA using methacrylate monolithic columns

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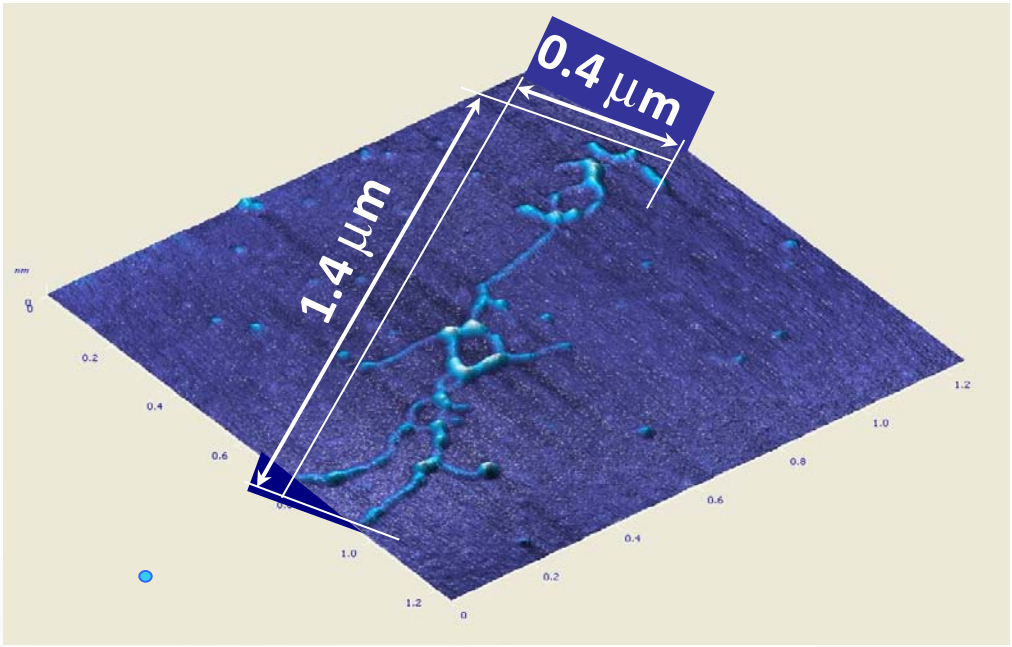
[b] University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

Process	SC (%)	Productivity
Monoliths	98	3.48 g l^{-1} h^{-1}
Particles	98	0.35 g l^{-1} h^{-1}

Plasmid DNA Purification: Large Plasmids



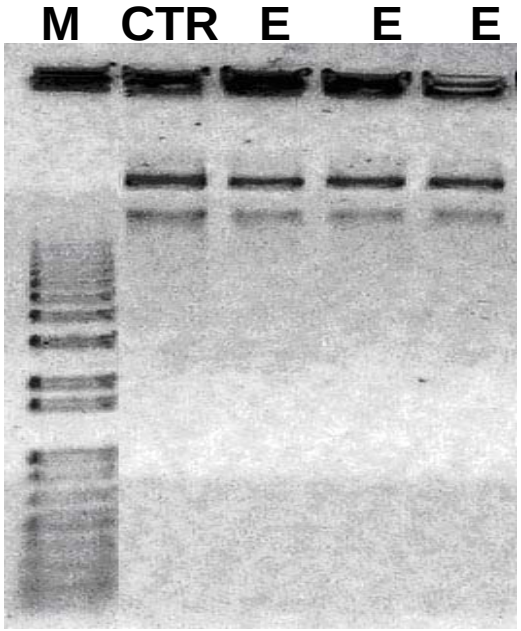
AFM picture of 39.4 kbp plasmid

Dynamic binding capacity of CIM DEAE for 39.4 kbp plasmid

Linear velocity	$q_{39.4kb\ 50\%}$
cm/h	mg/mL
119.4	13.5
238.7	12.4
334.2	12.8

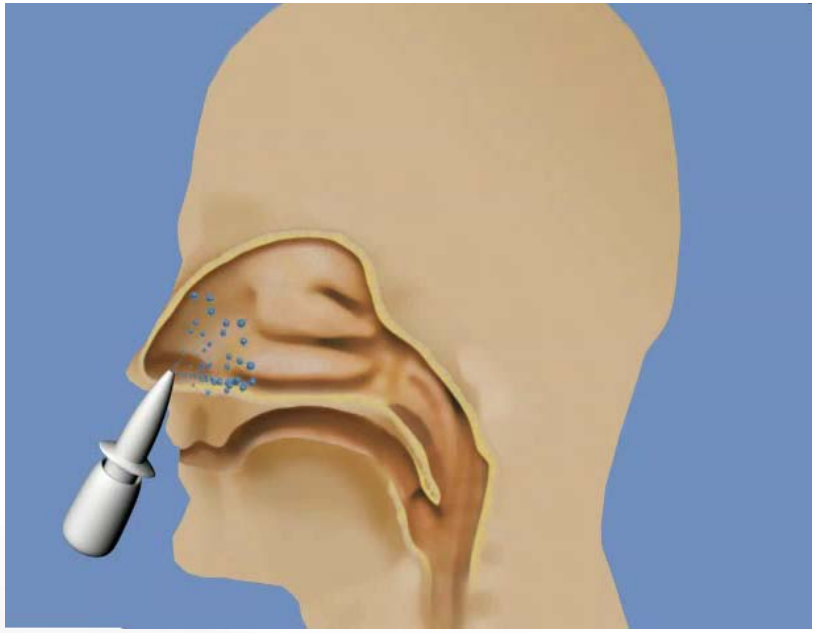
Plasmid DNA Purification: Large Plasmids

Low shear forces: 39.4 kbp plasmid remain intact in sc form



Replication Defficient Influenza Virus Vaccine

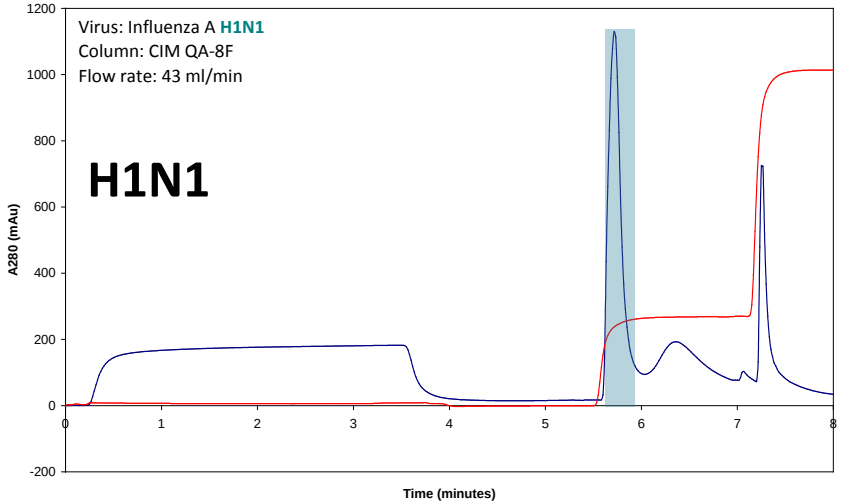
AVIR Green Hills Biotechnology

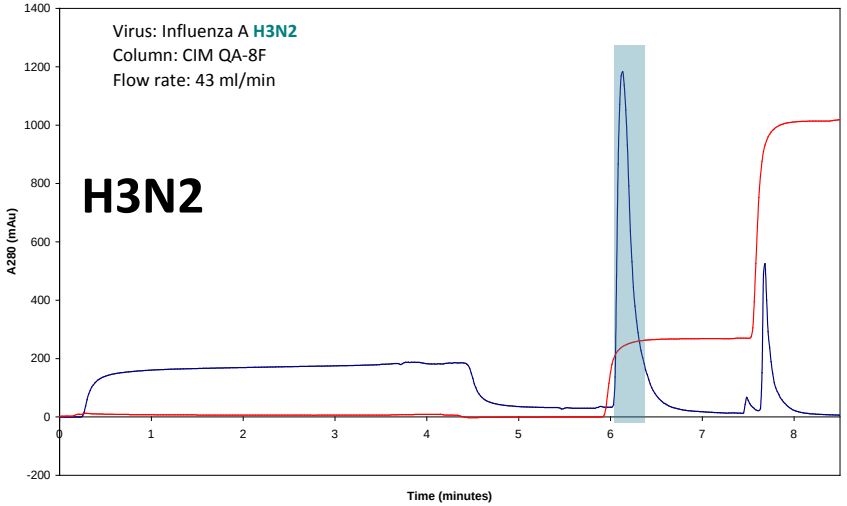


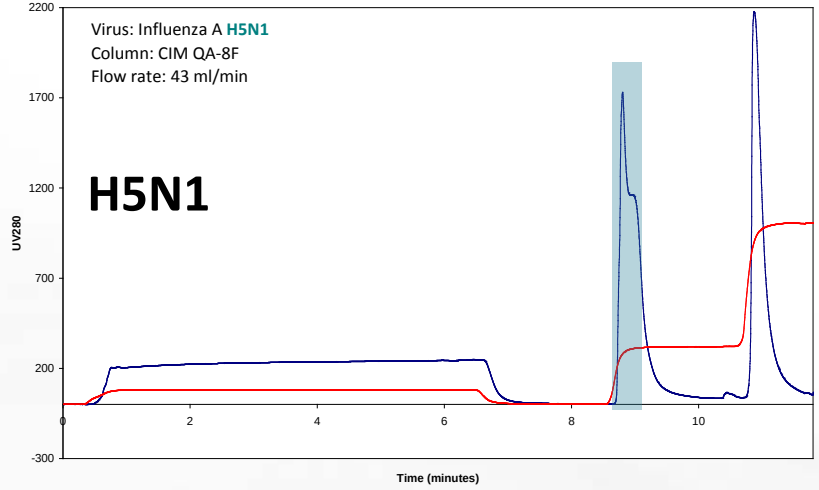
CIM QA vs CIM SO3

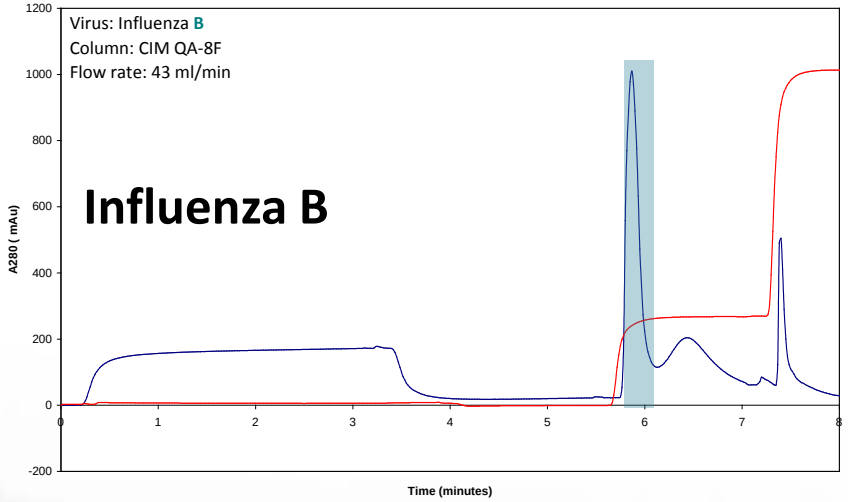
Influenza A (H1N1)	CIM QA	CIM SO3
TCID50 (%)	74.3	63.3
Proteins (%)	17.4	5.5
DNA (%)	0.53	2.8
DBC (TCID50/mL)	2.0E+10	9.0E+08

AIEX: Robustness









CIM QA for Influenza A and B

- H1N1, H5N1, H3N2, FluB

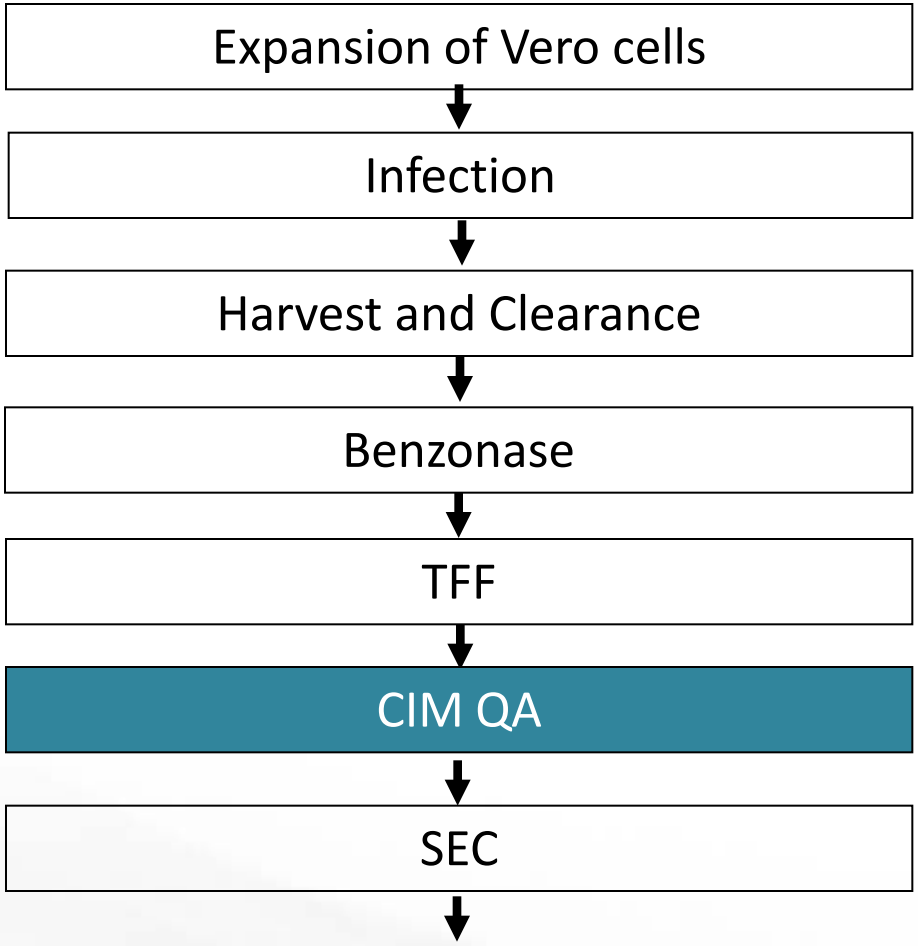
	%	
Virus yield	75	25
DNA depletion	72	10
Protein depletion	95	3

Host cell DNA Removal

Exp	DNA conc.(ng/ml)	Load volume (ml)	DNA conc. (ng/ml)	Elution volume (ml)	DNA removal (%)
LC1-1	8527	1300	25,4	120	**99,97**
LC1-2	10153	1300	110,5	120	**99,90**
R&D3	1510	1350	1,5	115	**99,99**
LPC1	1273	1400	4,4	120	**99,97**
LPC2	1843	1300	8,73	120	**99,96**

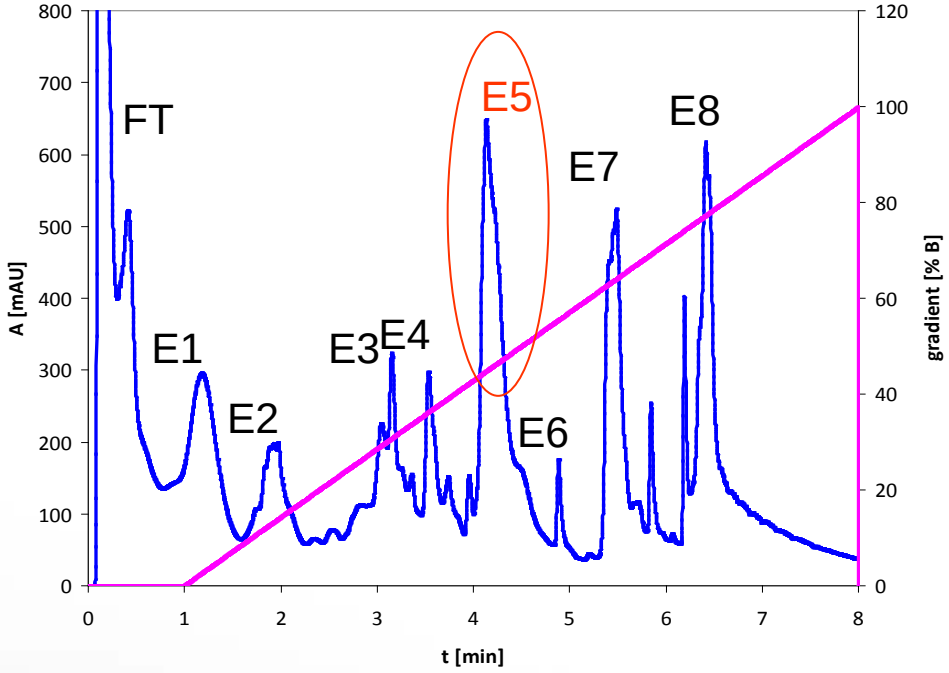
- CIM Monoliths have high ligand density

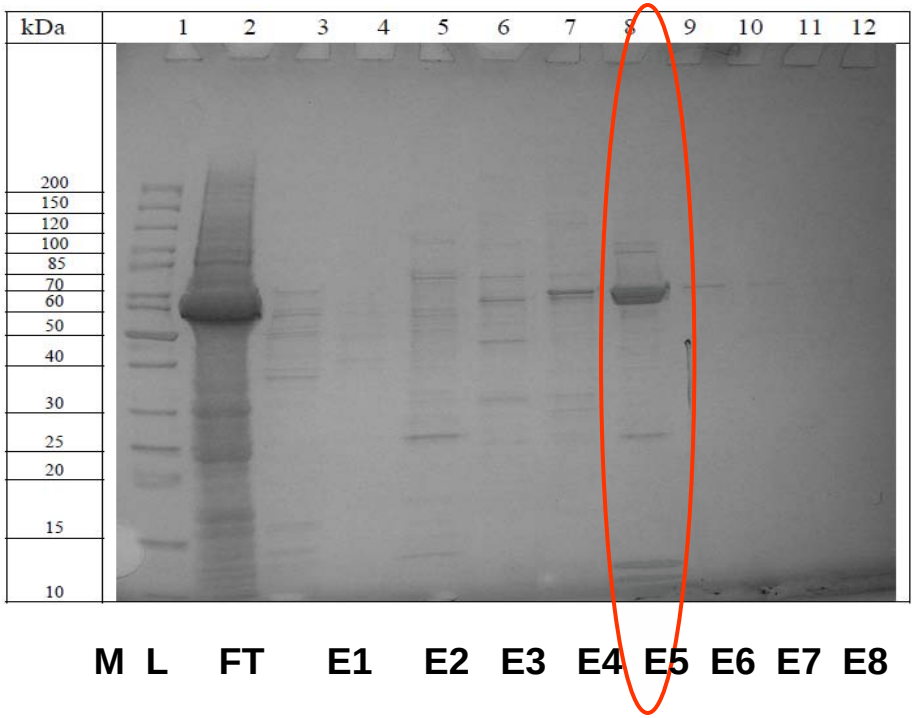
Purification of Replication Defficient Influenza Vaccine: The Process



Poster 36

Purification of Ad3 Dodecahedron Particles (VLP)





CIMac QA

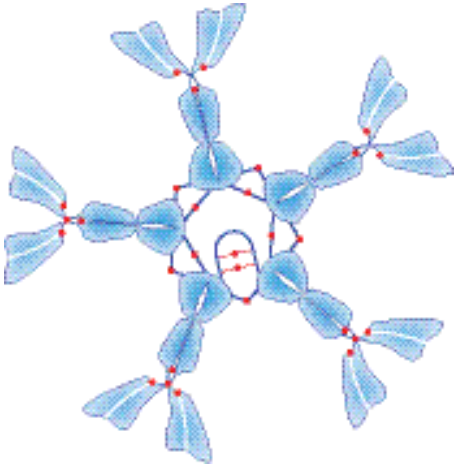
20 mM Tris + 1 mM EDTA + 5% glicerol, pH 7.5;

20 mM Tris + 1 mM EDTA + 5% glicerol + 1 M NaCl, pH 7.5

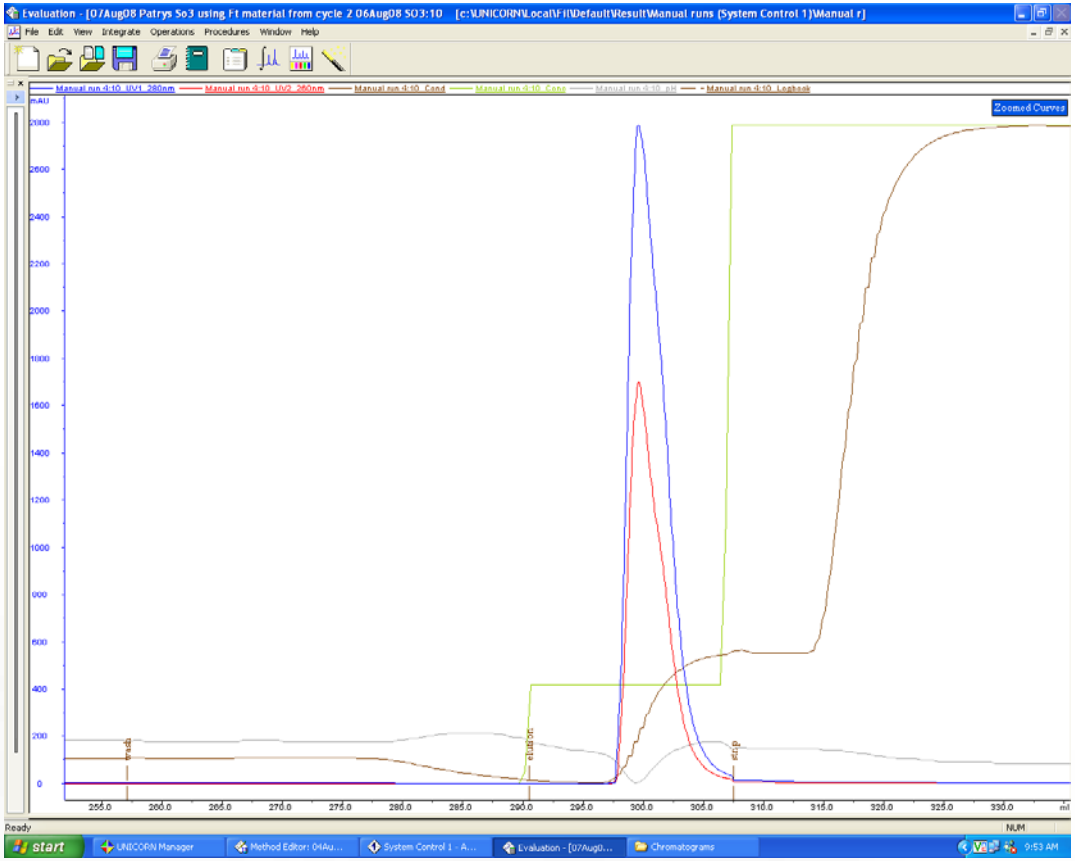
Flow: 1 mL/min

V inj. = 60 µL

IgM Purification



- Polishing step on CIM SO3



Load: 43 mL QA eluate pool

Column: 8 mL SO_3 monolith

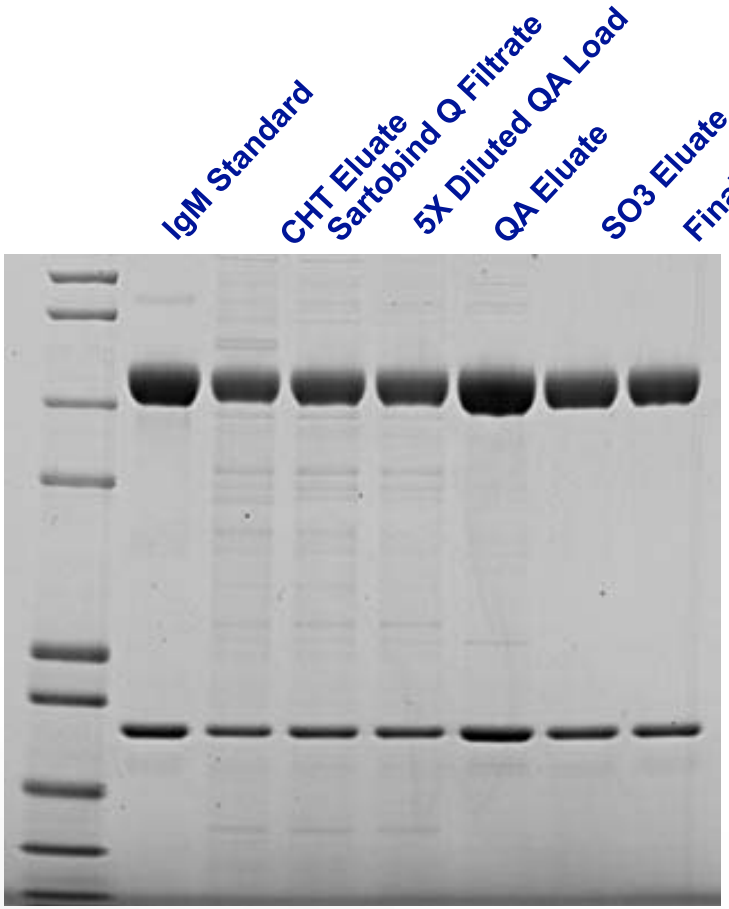
Flow rate: 30 mL/min; 3.75 CV/min

B. A: 10 mM Na phosphate, 2 M Urea, pH 7.0

B. B: 500 mM Na phosphate, pH 7.0

Courtesy P. Gagnon www.validated.com

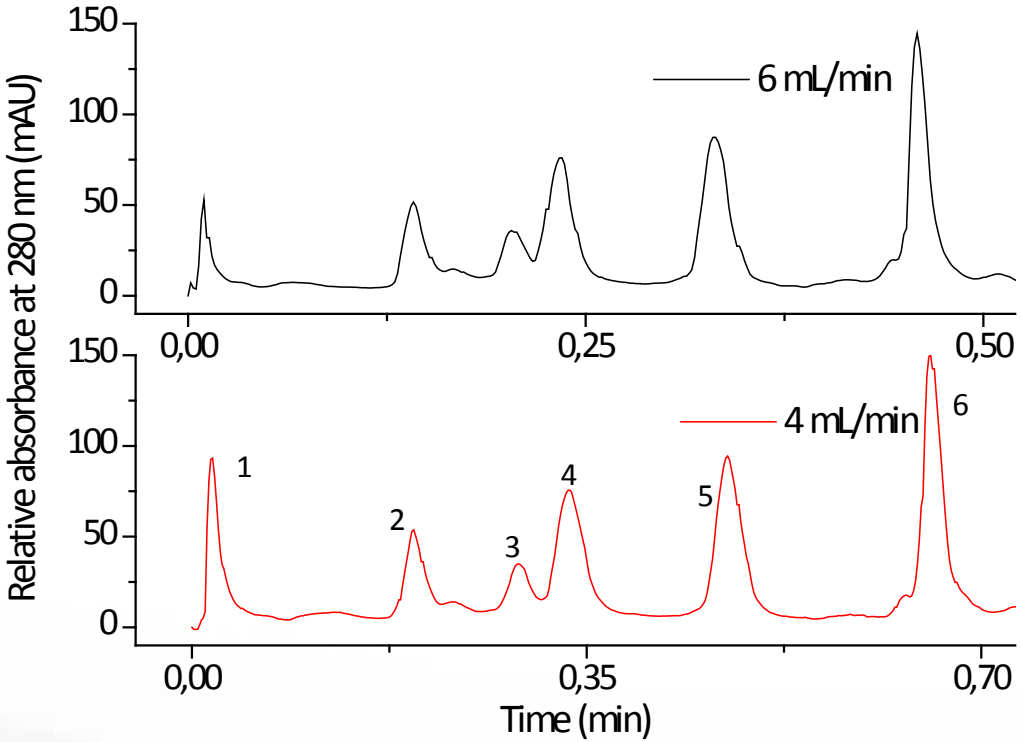
IgM Purification



Courtesy P. Gagnon www.validated.com

CIM Monoliths for Analytics and IPC

CIMac SO_3 Analytical Column™ (5.2 mm I.D. x 5.0 mm)
Buffer A: 20 mM phosphate, pH 6.0
Buffer B: 20 mM phosphate + 1.0 M NaCl, pH 6.0
Linear gradient from 0 to 25 % buffer B in 20 CV
Injection volume: 10 µl
(1)myoglobin,
(2)trypsinogen,
(3)ribonuclease A,
(4)alpha-chymotrypsinogen A,
(5)cytochrome C,
(6)lysozyme



CIMac Based Analytics of Plasmid DNA

E. coli culture with plasmid

↓

Cell harvest

↓

Alkaline lysis with adjustment to 0.5 M $CaCl_2$

↓

Clarification

↓

Adjustment to binding conditions

↓

CIM® DEAE

↓

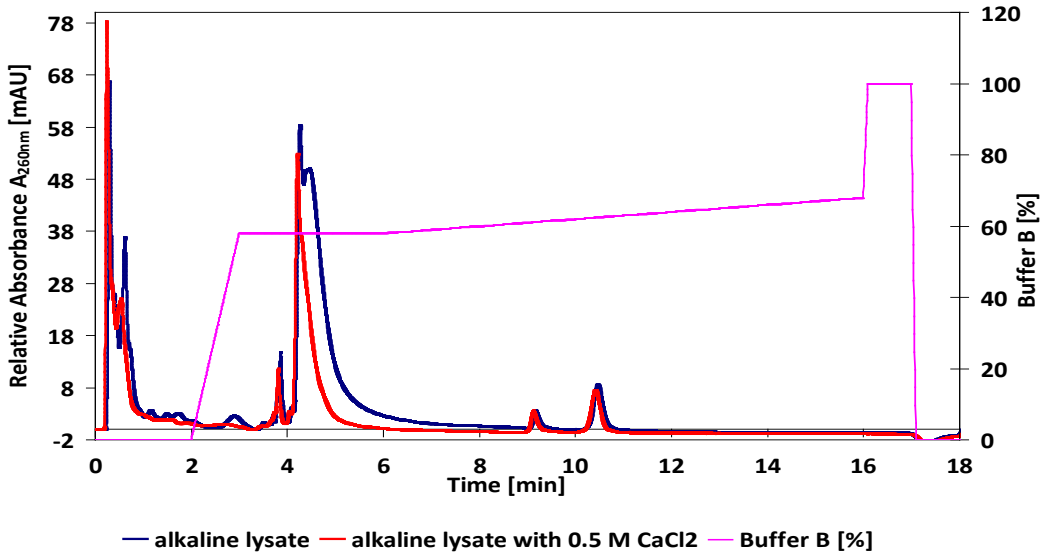
Adjustment with $(NH_4)_2SO_4$

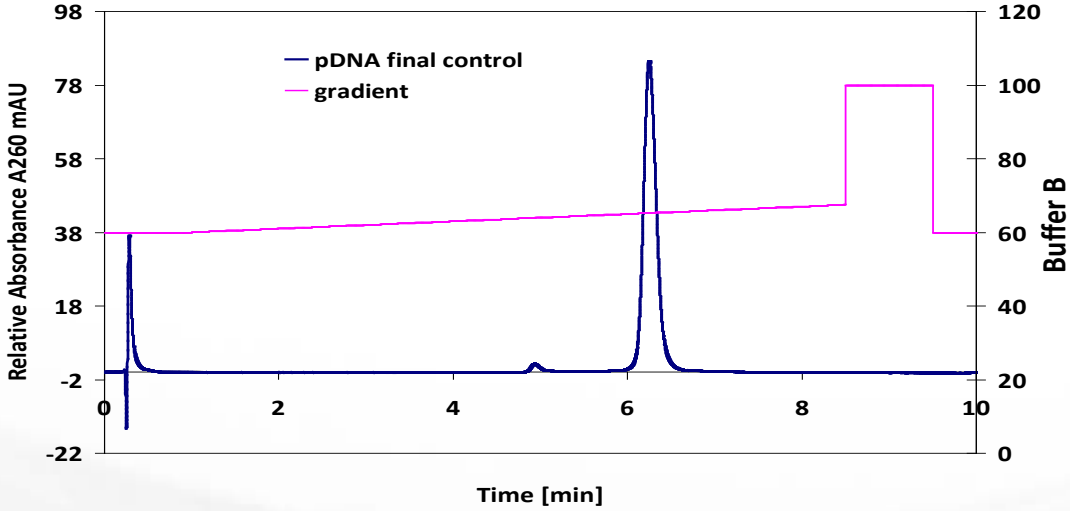
↓

CIM® C4

↓

Buffer exchange





Virus Quantification by Monolith Chromatography

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journal homepage: www.elsevier.com/locate/chroma

Rapid high-performance liquid chromatographic analysis of adenovirus type 5 particles with a prototype anion-exchange analytical monolith column

Robert J. Whitfield[a], Suzanne E. Battom[a], Miloš Barut[b], David E. Gilham[c], Philip D. Ball[a,*]

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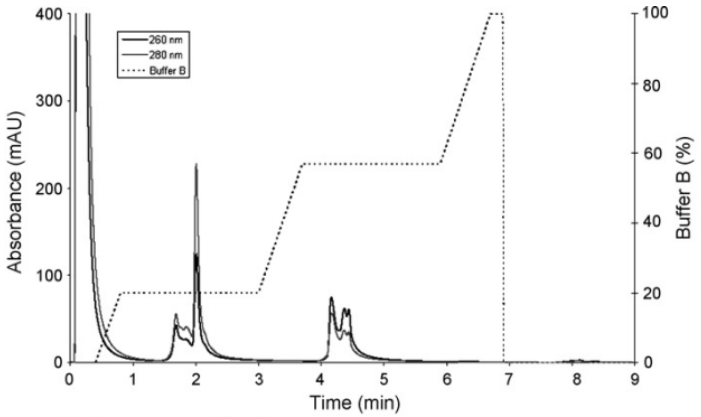
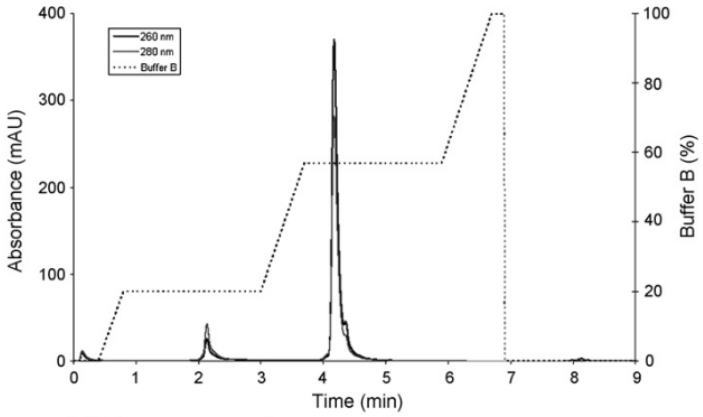
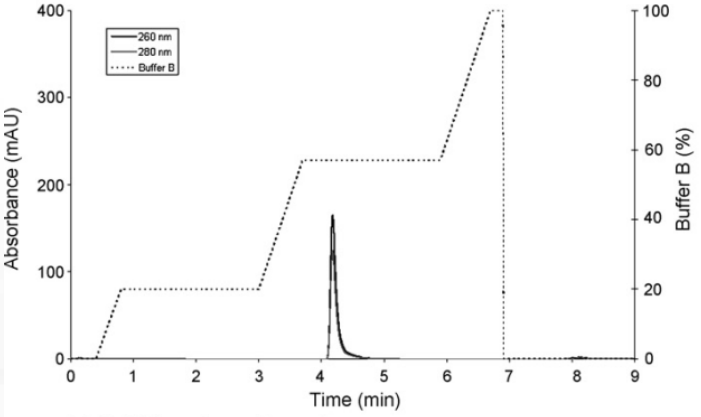
Virus Quantification by Monolith Chromatography



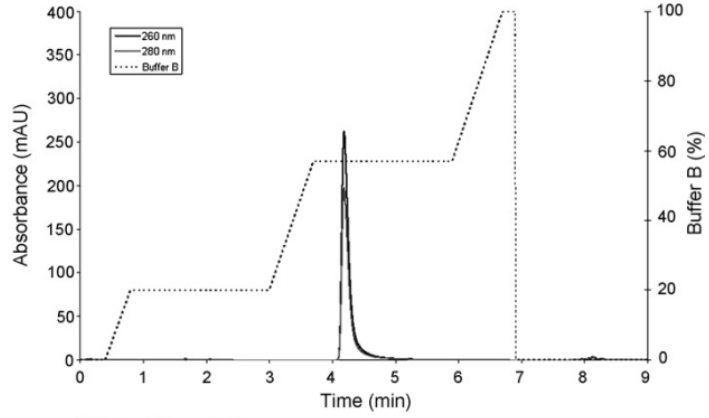
(a) Benzonase / filtration



(b) Primary capture chromatography



(c) Polishing chromatography



(d) Final formulation

Conclusions

- CIM Monoliths
  - Large channels (1.5 um)
  - Convective mass transport
  - High surface accesibility
  - Low pressure drop
  - Chemical resistant

- Designed for large proteins, DNA and viruses

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Thank you

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