CIM Monolith Technology: Enabling Economic Vaccines Production

Matjaž Peterka
Vaccine Technology III, June 2010
Nuevo Vallarta, Mexico
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 accessibility
- Low pressure drop
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 induced by external force
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

<table>
<thead>
<tr>
<th>molecule</th>
<th>MW</th>
<th>D (cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺</td>
<td>1 Da</td>
<td>1 x 10⁻⁴</td>
</tr>
<tr>
<td>NaCl</td>
<td>58 Da</td>
<td>1.4 x 10⁻⁵</td>
</tr>
<tr>
<td>BSA</td>
<td>66 kDa</td>
<td>6.1 x 10⁻⁷</td>
</tr>
<tr>
<td>IgG</td>
<td>150 kDa</td>
<td>4.2 x 10⁻⁷</td>
</tr>
<tr>
<td>TMV</td>
<td>40 000 kDa</td>
<td>5 x 10⁻⁸</td>
</tr>
<tr>
<td>DNA</td>
<td>4.4 kbp</td>
<td>1.9 x 10⁻⁸</td>
</tr>
<tr>
<td>DNA</td>
<td>33 kbp</td>
<td>4 x 10⁻⁹</td>
</tr>
</tbody>
</table>

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

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 Accessibility

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.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteins</td>
<td>1-3</td>
</tr>
<tr>
<td>IgM</td>
<td>25</td>
</tr>
<tr>
<td>Plasmids</td>
<td>150-250</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>130</td>
</tr>
<tr>
<td>Poxvirus</td>
<td>200 x 500</td>
</tr>
<tr>
<td>T4</td>
<td>220 x 85</td>
</tr>
</tbody>
</table>

Courtesy P. Gagnon www.validated.com
Surface accessibility for CIM Monoliths

- High capacity for viruses and DNA

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Column</th>
<th>Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid DNA</td>
<td>CIM DEAE</td>
<td>8 mg/mL</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>CIM DEAE</td>
<td>15 mg/mL</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>CIM QA</td>
<td>&gt;115 mg/mL</td>
</tr>
<tr>
<td>ToMV</td>
<td>CIM QA</td>
<td>2.0E+14 vp/mL</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>CIM QA</td>
<td>2.0E+10 vp/mL</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>CIM QA</td>
<td>3.0E+12 vp/mL</td>
</tr>
<tr>
<td>Ad3 VLPs</td>
<td>CIM QA</td>
<td>7.3E+16 VLP/mL</td>
</tr>
</tbody>
</table>
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

Endotoxin

Plasmid DNA

DNA depletion

Large proteins
CIM DEAE for Plasmid DNA Purification

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

- Separation of open circular and super coiled plasmid DNA
  - RNA
  - OC pDNA
  - SC pDNA
CIM C4 HLD for Plasmid DNA Purification

- Separation of isoforms and genomic DNA
E. coli culture with plasmid

Cell harvest

Alkaline lysis with adjustment to 0.5 M CaCl₂

Clarification

Adjustment to binding conditions

CIM® DEAE

Adjustment with (NH₄)₂SO₄

CIM® C4

Buffer exchange

<table>
<thead>
<tr>
<th>Results</th>
<th>Specs</th>
</tr>
</thead>
<tbody>
<tr>
<td>pDNA (μg/mL)</td>
<td>300</td>
</tr>
<tr>
<td>pDNA (mg)</td>
<td>34</td>
</tr>
<tr>
<td>Homogeneity (%SC)</td>
<td>98</td>
</tr>
<tr>
<td>Endotoxins (EU/mg pDNA)</td>
<td>1.1</td>
</tr>
<tr>
<td>Host cell proteins (μg/mL)</td>
<td>1.1</td>
</tr>
<tr>
<td>gDNA (μg/mg pDNA)</td>
<td>3.4</td>
</tr>
<tr>
<td>RNA (μg/mL)</td>
<td>0</td>
</tr>
<tr>
<td>Yield (%)</td>
<td>90%</td>
</tr>
</tbody>
</table>
Preparation of pharmaceutical-grade plasmid DNA using methacrylate monolithic columns

Franc Smrekar, Aleš Podgornik, Mateja Ciringer, Sandra Kontrec, Peter Raspor, Aleš Štrancar, Matjaž Peterka

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b University of Ljubljana, Biotechnical Faculty, Department of Food Science and Technology, Jammikarjeva 161, SI-1000 Ljubljana, Slovenia

<table>
<thead>
<tr>
<th>Process</th>
<th>SC (%)</th>
<th>Productivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoliths</td>
<td>98</td>
<td>3.48 g l⁻¹ h⁻¹</td>
</tr>
<tr>
<td>Particles</td>
<td>98</td>
<td>0.35 g l⁻¹ h⁻¹</td>
</tr>
</tbody>
</table>
Plasmid DNA Purification: Large Plasmids

Dynamic binding capacity of CIM DEAE for 39.4 kbp plasmid

<table>
<thead>
<tr>
<th>Linear velocity (cm/h)</th>
<th>$q_{39.4\text{kb} \ 50%}$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>119.4</td>
<td>13.5</td>
</tr>
<tr>
<td>238.7</td>
<td>12.4</td>
</tr>
<tr>
<td>334.2</td>
<td>12.8</td>
</tr>
</tbody>
</table>

AFM picture of 39.4 kbp plasmid
Plasmid DNA Purification: Large Plasmids

Low shear forces: 39.4 kbp plasmid remain intact in sc form
Replication Deficient Influenza Virus Vaccine
## CIM QA vs CIM SO3

<table>
<thead>
<tr>
<th>Influenza A (H1N1)</th>
<th>CIM QA</th>
<th>CIM SO3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCID50 (%)</td>
<td>74.3</td>
<td>63.3</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>17.4</td>
<td>5.5</td>
</tr>
<tr>
<td>DNA (%)</td>
<td>0.53</td>
<td>2.8</td>
</tr>
<tr>
<td>DBC (TCID50/mL)</td>
<td>2.0E+10</td>
<td>9.0E+08</td>
</tr>
</tbody>
</table>
AIXE: Robustness

H1N1

Virus: Influenza A H1N1
Column: CIM QA-8F
Flow rate: 43 ml/min

H3N2

Virus: Influenza A H3N2
Column: CIM QA-8F
Flow rate: 43 ml/min

H5N1

Virus: Influenza A H5N1
Column: CIM QA-8F
Flow rate: 43 ml/min

Influenza B

Virus: Influenza B
Column: CIM QA-8F
Flow rate: 43 ml/min
CIM QA for Influenza A and B

- H1N1, H5N1, H3N2, FluB

<table>
<thead>
<tr>
<th></th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus yield</td>
<td>75</td>
</tr>
<tr>
<td>DNA depletion</td>
<td>72</td>
</tr>
<tr>
<td>Protein depletion</td>
<td>95</td>
</tr>
</tbody>
</table>
Host cell DNA Removal

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

- CIM Monoliths have high ligand density
Purification of Replication Defficient Influenza Vaccine: The Process

- Expansion of Vero cells
  - Infection
  - Harvest and Clearance
  - Benzonase
  - TFF
  - CIM QA
  - SEC
  - Purified Vaccine Bulk
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 SO₃ 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

Relative absorbance at 280 nm (mAU)

Time (min)
CIMac Based Analytics of Plasmid DNA

- **E. coli culture with plasmid**
- **Cell harvest**
- **Alkaline lysis with adjustment to 0.5 M CaCl₂**
- **Clarification**
- **Adjustment to binding conditions**
- **CIM® DEAE**
- **Adjustment with (NH₄)₂SO₄**
- **CIM® C4**
- **Buffer exchange**
Rapid high-performance liquid chromatographic analysis of adenovirus type 5 particles with a prototype anion-exchange analytical monolith column

Robert J. Whitfield, Suzanne E. Battom, Miloš Barut, David E. Gilham, Philip D. Ball

Eden Biodesign Ltd., National Biomanufacturing Centre, Estuary Business Park, Liverpool, UK
BIA Separations d.o.o., Teslova 30, SI-1000 Ljubljana, Slovenia
Gene and Immunotherapy Group, Cancer Research UK Department of Medical Oncology, Paterson Institute for Cancer Research, University of Manchester, Manchester, UK
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 accessibility
  – Low pressure drop
  – Chemical resistant

• Designed for large proteins, DNA and viruses
Acknowledgments

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Thank you

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