From Proteins to Polymers-GPC/SEC

Understanding column selection and method considerations for your sample

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LC columns and consumables technical support applications engineer
August 15, 2019
• GPC/SEC
  – A quick review of terms and an overview of the separation mechanism

• Sample type
  – What type of sample do we have and how they differentiate
  – And why do we do GPC /SEC

• Solvent and mobile phase
  – Considerations for solvent selection and why solvent choice IS important

• Column selection
  – Important points to consider for making your column selection
  – How to maximize your column performance thru selection

• Detector and instrument considerations
  – Concentration detectors and advanced detection
  – Easy ‘fixes’ for ensuring optimal chromatography
Terminology

Same technique, but different acronyms:

- **GPC** – *Gel permeation chromatography*
  - Organic solvents like THF and methylene chloride

- **SEC** – *Size exclusion chromatography*
  - Primarily water and buffer mobile phases

- **GFC** – *Gel filtration chromatography*
  - Water and buffer mobile phases; common term for industrial purification step in the life sciences industry

GPC/SEC refers to the chromatographic technique that separates samples by their size.
Hydrodynamic Volume

- The size of a polymer/protein coil in solution
- Measure of molecular size in solution

Separation based on size in solution (hydrodynamic volume)

Identical Molecular weight Dissolved in TCB

Polyethylene

Polystyrene
Two different polymers will behave differently with solvent.

Column separates on basis of molecular size NOT molecular weight.

At any molecular weight, the two polymers will have different sizes in solution.
GPC/SEC Separation Mechanism

- A GPC/SEC column is packed with porous beads of controlled porosity and particle size
- Sample is prepared as a dilute solution in the eluent and injected into the system
- Large molecules are not able to permeate all of the pores and have a shorter residence time in the column
- Small molecules permeate deep into the porous matrix and have a long residence time in the column
- Sample molecules are separated according to molecular size, eluting largest first, smallest last
Sample Type – Polymer or BioMolecule

**Polymer**

**Questions that you need to ask**

What type of polymer do I have?

- Organic soluble
- Aqueous soluble

- GPC
- SEC

**BioMolecule**

**Questions that you need to ask**

What type of sample do I have?

- Peptides
- Proteins/globular proteins
- mAbs
- Protein conjugates
- Large BioMolecules

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Polymers are showing up in more and more pharmaceuticals

Polymers are found as part of:

- Gel coatings
  (For example, ethyl cellulose)

- Drug encapsulation
  (For example, PEG)

- Excipients (stabilization, fillers, and adsorption facilitation)
  (For example, polyvinyl pyrrolidone)
Sample Type

Why GPC/SEC is done?

- **Plastics**
  - Mol wt dictates polymer strength, flexibility, and physical properties

- **Sample cleanup**
  - Separates target molecules from large molecules that fragment in MS and cause interference

- **Water soluble polymers**
  - Mol wt impacts viscosity, surfactant effects, dissolution, and chemical characteristics

- **BioMolecules**
  - Mol tw is often known
  - Can be run on intact molecules
  - Aggregation can be dangerous

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Solvents

• Selecting a solvent system is one of the first steps in developing a GPC method.

• Some polymers or biomolecule samples are easy to dissolve, some are much harder.

• The solvent conditions must be appropriate for the sample to prevent any unwanted interactions between that of the sample with the packing particle. Interactions will give a false mol wt result.

• Agilent’s range GPC/SEC columns are available with phase chemistries that are optimized for all types of solvents that may be required: aqueous and organic, polar and nonpolar solvents.
Criteria for Solvent Selection

- Solvent must be able to fully solubilize the sample
- True sample solubility to avoid non-size exclusion effects
- Compatibility with column(s) and packing
- Permit adequate detection (for example, refractive index, UV cutoff)
- Safety (for example, toxicity, elevated temperature, and so on)
Question: What solvent is your sample soluble in?

<table>
<thead>
<tr>
<th>Type</th>
<th>Typical Solvents</th>
</tr>
</thead>
</table>
| Organic                  | • THF  
|                          | • Chloroform  
|                          | • Toluene  
|                          | • TCB |
| Mixed or polar organic   | • THF/water  
|                          | • DMF  
|                          | • NMP |
| Aqueous                  | • Water  
|                          | • Buffer in water  
|                          | • Water/methanol (up to 50%)  
|                          | • Water/buffer, ACN |

Additives can be employed:
- Minimize nonsize exclusion interactions between the sample and the column
- Stabilize the solution of the polymer (ionic aggregation)
Solvent Considerations and Optimizing for Aqueous SEC

Water soluble polymer

Polymer chemistry:
- neutral
- anionic
- cationic

Suggested Eluent:
- pure water
- 0.2M NaNO₃, 0.01M NaH₂PO₄, pH 7-9
- 0.2M NaNO₃, 0.01M NaH₂PO₄, pH 2-7
- addition of up to 50% methanol

Guide to eluent selection for PL aquagel-OH applications
Solvent Considerations: Importance in Method Development

Biomolecules

- Phosphate buffer concentration
- pH
- Salt concentration

Mobile phase optimization

- Peak symmetry
- Dimer/monomer resolution
Recommended Starting Conditions

For AdvanceBio SEC columns, we recommend starting with 150 mM sodium phosphate, pH 7.0

Buffer concentration and ionic strength can impact retention time, peak shape, and resolution. Adjustments can be made depending on your sample requirements.
Buffers and SEC: Criteria for Optimal Mobile Phase

- Mobile phase should contain enough buffer/salt (to overcome ionic interactions).
- Mobile phase should not contain too much buffer/salt (to prevent hydrophobic interactions).
- Mobile phase should not alter the analyte (cause degradation/aggregation, and so on).
- Mobile phase should be made up fresh and used promptly (bacterial growth is rapid in dilute buffer stored at room temperature).
- Buffer shelf life <7 days unless refrigerated.
- Mobile phase should be filtered before use. Particulates may be present in water (less likely) or in buffer salts (more likely).
Selecting a GPC/SEC Column

Points to consider when making a column choice:

- Organic or aqueous eluents being used
- What is the expected mol wt range of your sample
- What type of column chemistry
- What are your key requirements for your GPC/SEC analysis?
  1. Resolution is important
  2. Reproducibility of sample chromatography and results
  3. Speed of analysis and sample throughput is something to improve on

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Key Requirement Might Be

- Resolution is too low
- Analysis time is too long
- Peak shapes are poor
- Results are not reproducible
Column Chemistries

**Polymer chemistries**
Common types:
- Polymethacrylate packings
- Polyester copolymers
- DVB, divinylbenzene
- PS-DVB, polystyrene divinylbenzene

**Silica chemistries**
Common types:
- Diol
- Surface modified hydroxyl
- Surface modified polymeric

AdvanceBio SEC 200Å 1.9 µm particles have a very narrow size distribution, which provides high efficiency.
Question: What is the expected molecular weight range of your polymer sample or your protein sample?

<table>
<thead>
<tr>
<th>Mol Wt</th>
<th>Mol Wt Range (g/mol or Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Up to several millions</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Up to hundreds of thousands</td>
</tr>
<tr>
<td>Low</td>
<td>Up to tens of thousands</td>
</tr>
<tr>
<td>Very low</td>
<td>A few thousand</td>
</tr>
</tbody>
</table>
Column Selection

Choose the right pore size

• Ensure you select a column with large enough pores to allow your molecule to permeate into the pore structure of the stationary phase and not be excluded.

• It should provide complete coverage for the mol wt range of your sample and for your calibration standards

• It is also essential to choose a pore size that is not too large

For example, for monoclonal antibodies the optimum pore size is around 300 Å.
Choose the Right Pore Size

- The example chromatogram and calibration curve illustrate how different size molecules elute from the column.
- Choose a pore size that allows you to work in the linear portion of the calibration curve.
Column Types: Individual Pore Size

- All particles have the same pore size
- Good separation, but narrow range of mol wt
- Very nonlinear curve; linear only over a narrow mol wt range
- Oldest technology, but still popular, and useful for separating very small and very large compounds
- Wider mol wt range possible by combining different columns in series, but need to select carefully so not to have column ‘mismatch’
Column Types: MIXED

- Individual pore size particles are mixed together/blended to make a linear curve
- Very wide ranges possible, but only a small amount of separation of each mol wt
- Linear curve makes chromatogram easy to read and analyze
- Most popular technology, well established and widely used
- Columns in series of same type are still linear

Column family: PLgel

PLgel MIXED calibration plots
Column Types: Multi-Pore Particle

- Newest, fastest growing technology
- Each particle has multiple pore sizes
- Increased pore volume
- Highest resolution and efficiency
- Best performance for most common mol wt ranges

Column family: PlusPore

PlusPore calibration plots
Column Selection
Effect of pore size

Main peak resolved
Low pore size
High pore size
Main peak excluded

* Samples run using PLgel individual pore size columns

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Importance of Pore Size Selection

Calibrants

Instrument: Agilent 1290 Infinity Bio-inert Quaternary LC System
Mobile phase: 150 mM phosphate buffer, pH 7.0
Flow rate: 0.35 mL/min
Detector: UV, 220 nm
Sample: BioRad gel filtration standards mix

Column A: AdvanceBio SEC 300Å
4.6 x 300 mm, 2.7 μm (p/n PL1580-5301)

Column B: AdvanceBio SEC 130Å
4.6 x 300 mm, 2.7 μm (p/n PL1580-5350)

BioRad gel filtration standards mix

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>2</td>
<td>γ-globulin (bovine)</td>
</tr>
<tr>
<td>3</td>
<td>Ovalbumin (chicken)</td>
</tr>
<tr>
<td>4</td>
<td>Myoglobin (equine)</td>
</tr>
<tr>
<td>5</td>
<td>Vitamin B12</td>
</tr>
</tbody>
</table>
Importance of Pore Size Selection

Sample

[Diagram showing Polyclonal IgG separation]

**Column A:** AdvanceBio SEC 300Å
4.6 x 300 mm, 2.7 μm (p/n PL1580-5301)

**Column B:** AdvanceBio SEC 130Å
4.6 x 300 mm, 2.7 μm (p/n PL1580-5350)

**Instrument:** Agilent 1260 Infinity Bio-inert Quaternary LC System

**Mobile phase:** 150 mM phosphate buffer, pH 7.0

**Flow rate:** 0.35 mL/min

**Detector:** UV, 220 nm

**Sample:** Polyclonal IgG
# Agilent GPC Columns

Separation ranges and column choices for organic soluble polymers

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## Column families:
- PLgel
- PlusPore

### Polystyrene Molecular Weight

<table>
<thead>
<tr>
<th></th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
<th>$10^6$</th>
<th>$10^7$</th>
<th>$10^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultra high polymer distributions</td>
<td>20 μm MIXED-A</td>
<td>10 μm 500A</td>
<td>10 μm 10^4A</td>
<td>10 μm 10^5A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymer MW distributions</td>
<td>10 μm MIXED-B</td>
<td>5 μm MIXED-C &amp; PolyPore</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resins, condensation polymers</td>
<td>5 μm MIXED-D &amp; 3 μm ResiPore</td>
<td>5 μm 500A</td>
<td>5 μm 10^4A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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### Polystyrene Molecular Weight

<table>
<thead>
<tr>
<th></th>
<th>$10^2$</th>
<th>$10^3$</th>
<th>$10^4$</th>
<th>$10^5$</th>
<th>$10^6$</th>
<th>$10^7$</th>
<th>$10^8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low MW resins</td>
<td>5 μm 10^3A</td>
<td>3 μm MIXED-E Mesopore</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oils, oligomers</td>
<td>5 μm 100A</td>
<td>Oligopore</td>
<td>3 μm 100A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organics &lt;1000 MW</td>
<td>5 μm 50A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Agilent PL Aquagel-OH Columns

SEC analysis of water soluble polymers

<table>
<thead>
<tr>
<th>Molecular Weight</th>
<th>10^2</th>
<th>10^3</th>
<th>10^4</th>
<th>10^5</th>
<th>10^6</th>
<th>10^7</th>
<th>10^8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL aquagel-OH 60</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>1000</td>
</tr>
<tr>
<td>PL aquagel-OH 50</td>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>PL aquagel-OH 40</td>
<td></td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL aquagel-OH 30</td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL aquagel-OH 20</td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>1000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL aquagel-OH MIXED H &amp; M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

August 14, 2019

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## Agilent Size Exclusion Columns

SEC of biopolymers, proteins, mAbs

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.9 µm</td>
<td>2.7 µm</td>
<td>3 µm</td>
<td>5 µm</td>
<td>5 µm</td>
<td>4 µm, 6 µm</td>
</tr>
<tr>
<td>200 Å</td>
<td>130 Å, 300 Å</td>
<td>100 Å, 150 Å, 300 Å</td>
<td>100 Å, 150 Å, 300 Å, 500 Å, 1000 Å, 2000 Å</td>
<td>Nominal 300 Å (linear resolving range)</td>
<td>150 Å, 300 Å</td>
</tr>
<tr>
<td>Coated silica (USP L59)</td>
<td>Coated silica (USP L59)</td>
<td>Coated silica (USP L59)</td>
<td>Coated silica (USP L59)</td>
<td>Silica Diol (USP L20)</td>
<td>Zirconium stabilized silica diol (USP L35)</td>
</tr>
<tr>
<td>• mAb and ADC analysis</td>
<td>• mAb and ADC analysis</td>
<td>• Polypeptide to small proteins</td>
<td>• Broadest range of pore sizes for wide variety of biomolecules</td>
<td>• Unique linear resolving range</td>
<td>• Legacy product</td>
</tr>
<tr>
<td>• Dimer/monomer</td>
<td>• Higher-order aggregates</td>
<td>• MS capable separations</td>
<td></td>
<td>• Larger column dimensions</td>
<td>• Ideal for GF-450 and GF-250 in series</td>
</tr>
<tr>
<td>• LMW mAb fragments</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Increasing Resolution in GPC/SEC

Running two columns in series, same pore size
• Increase pore volume, increases resolution

Running two columns in series, different pore size
• Extends the resolving range and enables analysis of multiple attributes in one run

Use a packing with a smaller particle size
• Decrease particle size, increase column efficiency
Resolution in GPC
Add a column to improve resolution
Effect of Column Length on Resolution

Columns:
- 1xPLgel 10μm MIXED-B, 300x7.5mm (1110-6100)
- 3xPLgel 10μm MIXED-B, 300x7.5mm (1110-6100)

Eluent: THF
Flow Rate: 1.0ml/min
Detector: RI

Polystyrene Standards (EasiCal)
1. 3,040,000
2. 330,000
3. 66,000
4. 9,200
5. 580
Column in Series
Extend the mol wt resolving range

PL aquagel OH columns
Individual pore sizes

Conditions
Samples: Four samples of hyaluronic acid
Columns: 1 x PL aquagel-OH 60 15 µm,
300 x 7.5 mm (p/n PL1149-6280)
+ 1 x PL aquagel-OH 40 15 µm,
300 x 7.5 mm (p/n PL1149-6240)
Eluent: 0.2 M NaNO₃ + 0.01 M NaH₂PO₄ at pH 7
Flow Rate: 1.0 mL/min
Detection: RI

Figure 3. Overlay of the molecular weight distributions of four hyaluronic acid samples
Effect of Particle Size on Resolution

Column: PLgel 100 Å 300 x 7.5 mm  
Eluent: THF  
Flow rate: 1.0 mL/min  
Inj vol: 20 µL  
Detector: DRI

Polystyrene Mp 580 g/mol

Retention time / min

4.5 8.5

10µm 5µm 3µm
Comparison of 3 µm vs 5 µm Particle Size

Analysis of monoclonal antibody

Column: Bio SEC-3, 300Å
7.8 x 300 mm, 3 µm
(p/n 5190-2511)

Column: Bio SEC-5, 300Å
7.8 x 300 mm, 5 µm
(p/n 5190-2526)

Instrument: Agilent 1260 Infinity Bio-inert
Quaternary LC System

Mobile phase: 150 mM sodium phosphate, pH 7

Flow rate: 1 mL/min

Detector: UV, 220 nm

Sample: Humanized monoclonal antibody

1. Dimer
2. Monomer
3. Monomer fragment
Sub-2 µm Particle Size Comparison

Agilent AdvanceBio SEC 200 Å 1.9 µm

Competitor 1 SEC 200 Å 1.7 µm

Competitor 2 SEC 250 Å 2.0 µm

**LC Conditions**
- **Column dimension**: 4.6 x 300 mm
- **Mobile phase**: 50 mM sodium phosphate, 200 mM NaCl, pH 7.0
- **Temperature**: 25 °C
- **Sample**: Sigma mAb (spiked with its F(ab’)2 and Fc fragments)
- **Flow rate**: 0.35 mL/min
- **UV detection**: 220 nm

**Product Name** | **Particle** | **Column Hardware** | **Column Dimensions** | **Part Number**
--- | --- | --- | --- | ---
AdvanceBio SEC 200Å 1.9 µm (coated silica) | RRHD | 4.6 x 300 mm | 4.6 x 150 mm | PL1580-5201
| | | 4.6 x 30 mm guard | PL1580-3201
| | | | PL1580-1201
A Story Inside Every SEC Chromatogram

Interstitial volume

Pore volume or intraparticle volume

Silica particles

Exclusion limit/void volume

Pore volume

Interstitial volume

Total permeation

Column volume

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Effect of Increased Pore Volume

Columns
2 x PLgel, 3 µm, 100 Å, 300 x 7.5 mm
2 x OligoPore, 300 x 7.5 mm

Eluent
THF

Flow rate
1.0 mL/min

Both columns have a similar exclusion limit but OligoPore has greater pore volume than PLgel 100 Å. Hence the slope of the curve is shallower leading to greater resolution for columns of the similar efficiency.
Column Pore Volume Analysis
(4.6 x 300 mm, 0.35 mL/min, pH 7.0 phosphate buffer, BioRad sample)

AdvanceBio SEC 200 Å 1.9 µm
BP = 334 bar

Total pore volume 5.811 min

Competitor 1 SEC, 200 Å 1.7 µm
BP = 423 bar

Total pore volume 5.689 min

Competitor 2 SEC, 250 Å 2.0 µm
BP = 254 bar

Total pore volume 5.674 min

BioRad #151-1901 | Molecular Weight
--- | ---
Thyroglobulin | 670,000
γ-globulin | 158,000
Ovalbumin | 44,000
Myoglobin | 17,000
Vitamin B12 | 1,350

<table>
<thead>
<tr>
<th>Column</th>
<th>Exclusion Limit (min)</th>
<th>Total Permeation (min)</th>
<th>Total Pore Volume (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdvanceBio SEC 200 Å 1.9 µm</td>
<td>5.102</td>
<td>10.913</td>
<td>5.811</td>
</tr>
<tr>
<td>Competitor 1 SEC, 200 Å 1.7 µm</td>
<td>4.953</td>
<td>10.642</td>
<td>5.689</td>
</tr>
<tr>
<td>Competitor 2 SEC, 250 Å 2.0 µm</td>
<td>5.238</td>
<td>10.912</td>
<td>5.674</td>
</tr>
</tbody>
</table>

AdvanceBio SEC 200 Å 1.9 µm columns provide widest separation window.
Improving Speed for Analysis Without Sacrificing Resolution

The diagrams show different flow rates overlaid to show that faster doesn’t sacrifice resolution. The chromatograms have been normalized to better illustrate the differences.

Sample: Polystyrene mol wt 580
Column: OligoPore 250 x 4.6 mm

MW Range: up to 3,300 (g/mol)
Nominal Particle Size: 6 µm
Typical Efficiency: >55,000 p/m
Fast GPC
Example with MesoPore columns

**Conditions**
- **Column**: 2 x MesoPore, 4.6 x 250 mm (PL1513-5325)
- **Sample**: Epoxy resin
- **Eluent**: THF
- **Flow rate**: 0.35 and 1.2 mL/min
- **Inj vol**: 4 µL
- **System**: 1260 Infinity GPC/SEC System, UV, 254 nm

Easy method transfer from standard to rapid GPC on MesoPore 250 x 4.6 mm GPC columns

**MW Range**: up to 25,000 (g/mol)
- **Nominal Particle Size**: 3 µm
- **Typical Efficiency**: >80,000 p/m
Fast SEC AdvanceBio SEC 200 Å 1.9 µm

**LC Conditions**
- **System**: 1260 Infinity II Bioinert LC
- **Column used**: Agilent AdvanceBio SEC 200 Å, 1.9 µm, 4.6 x 150 mm
- **Mobile phase**: 50 mM sodium phosphate, 200 mM NaCl, pH 7.0
- **Temperature**: 25 °C
- **Sample**: Sigma mAb
- **UV detection**: 220 nm

**Flow Rate (mL/min)**
- 0.3: 6.8 min
- 0.4: 5.2 min
- 0.5: 4.2 min
- 0.6: 3.6 min
- 0.7: 3 min

**Retention time [min]**
- 0.3 mL/min: Rs=1.81 164 bar
- 0.4 mL/min: Rs=1.79 218 bar
- 0.5 mL/min: Rs=1.78 272 bar
- 0.6 mL/min: Rs=1.77 324 bar
- 0.7 mL/min: Rs=1.58 380 bar

**Fast Analysis**
- 150 mm 0.3 mL/min: 2.3 times faster
- 300 mm 0.3 mL/min: 4.6 times faster

<table>
<thead>
<tr>
<th>Flow Rate (mL/min)</th>
<th>Dimer Area (%)</th>
<th>Samples Per Hour</th>
<th>Samples Per Day (24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>2.33</td>
<td>8-9</td>
<td>211</td>
</tr>
<tr>
<td>0.4</td>
<td>2.35</td>
<td>11-12</td>
<td>276</td>
</tr>
<tr>
<td>0.5</td>
<td>2.35</td>
<td>14</td>
<td>342</td>
</tr>
<tr>
<td>0.6</td>
<td>2.39</td>
<td>16-17</td>
<td>400</td>
</tr>
<tr>
<td>0.7</td>
<td>2.30</td>
<td>20</td>
<td>480</td>
</tr>
</tbody>
</table>
Detectors and Instrument Considerations

Concentration detectors

• Most common detectors for GPC/SEC are concentration detectors:

RID  UV/DAD  ELSD

• These provide information on the amount of polymer or sample eluting from the column at any given time.
Detector Selection
Refractive Index vs ELSD

RI:
- Low response for sample
- Unable to detect additives
- System interference peaks present

ELSD:
- Improved response
- Additives detected
- No system interference peaks
Expanding Conventional GPC/SEC
Viscometer and light scattering detectors

Advanced detectors give a greater understanding of the analyte as well as overcoming the limitations of conventional GPC.

<table>
<thead>
<tr>
<th>GPC/SEC Technique</th>
<th>Molecular Weight</th>
<th>Molecular Size</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional (RI or UV)</td>
<td>Relative to standards used for calibration</td>
<td>No</td>
<td>Molecular weight distribution, concentration</td>
</tr>
<tr>
<td>Viscometry</td>
<td>More accurate from universal calibration</td>
<td>Yes, hydrodynamic radius (Rh).</td>
<td>Conformation, branching. Works with copolymers</td>
</tr>
<tr>
<td>Light scattering</td>
<td>Absolute determination</td>
<td>Yes, radius of gyration (Rg) directly.</td>
<td>Conformation, branching</td>
</tr>
<tr>
<td>Triple</td>
<td>Absolute determination</td>
<td>Yes, Rg and Rh, directly.</td>
<td>The ultimate configuration for comprehensive polymer characterization</td>
</tr>
</tbody>
</table>

Agilent InfinityLab II 1260 with MDS
Advanced Detection for Proteins and Biomolecules

- Combined static and dynamic light scattering detector simultaneously determines the absolute molecular weight (mol wt), as well as the size of the molecule (Rh).
- A solution for sizing and aggregation studies of proteins and other large biomolecules using size exclusion chromatography.

Agilent 1260 Infinity with Multi-Detector suite
Best Practice

AVOID BAD CONNECTIONS!
Ensure column connections do not leave dead spots/voids.
Use proper fittings/ferrules to ensure correct connections

Tailing Factor 0.901
Tailing Factor 1.093

Agilent quick Connect & Quick Turn fittings
Instrument considerations
Best practices

Low dispersion LC
Use optimal tubing ID and minimize length to reduce extra-column volume and band broadening

Use Correct Data Collection Rates
Data collection rates of 10 – 20 Hz could result in 4 – 5% reduction in column efficiency compared to 40 or 80 Hz.*

* - if working with a sub 2um SEC column
### In Summary

<table>
<thead>
<tr>
<th>Sample type and solvent selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consider your choice of solvent carefully for the type of sample, conditions, and columns required for analysis.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic or aqueous. Polymer or protein. Look to make the appropriate selection based on expected mol wt range, but also be sure to ask ‘what is it that I want or need for my analysis’?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Column selection and key requirement of analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choose the right pore size packing for your sample. Multiporous, MIXED, or individual. Keeping in mind your key requirement, be sure to also consider particle size, pore volume, and # of columns needed.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Detectors and instrument considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration type detectors for conventional GPC/SEC or look to Multi-Detector SEC to get additional information for your polymer or protein sample.</td>
</tr>
</tbody>
</table>
Resources for Support

- GPC/SEC Columns and Standards Product Guides: 5990-7994EN, 5990-7995EN, 5990-7996EN
- Agilent Community for Liquid Chromatography: https://community.agilent.com/community/technical/lc
  - LC Documents
  - LC Helpful Links
  - LC Videos
  - Quick reference guides
  - Catalogs, consumables supplies guide, column user guides
  - Online selection tools, how-to videos

Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:
Option 1 for GC and GC/MS columns and supplies
Option 2 for LC and LC/MS columns and supplies
Option 3 for sample preparation, filtration and QuEChERS
Option 4 for spectroscopy supplies
Option 5 for chemical standards

Available in the USA and Canada 8-5 all time zones

gc-column-support@agilent.com
lc-column-support@agilent.com
spp-support@agilent.com
spectro-supplies-support@agilent.com
chem-standards-support@agilent.com

For Research Use Only. Not for use in diagnostic procedures.
Thank you for attending

Any questions?