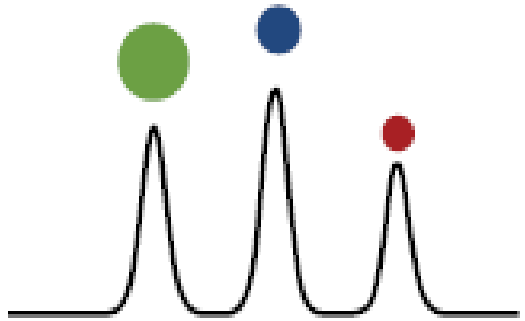
Aqueous Size Exclusion (SEC) of Water-Soluble Polymers

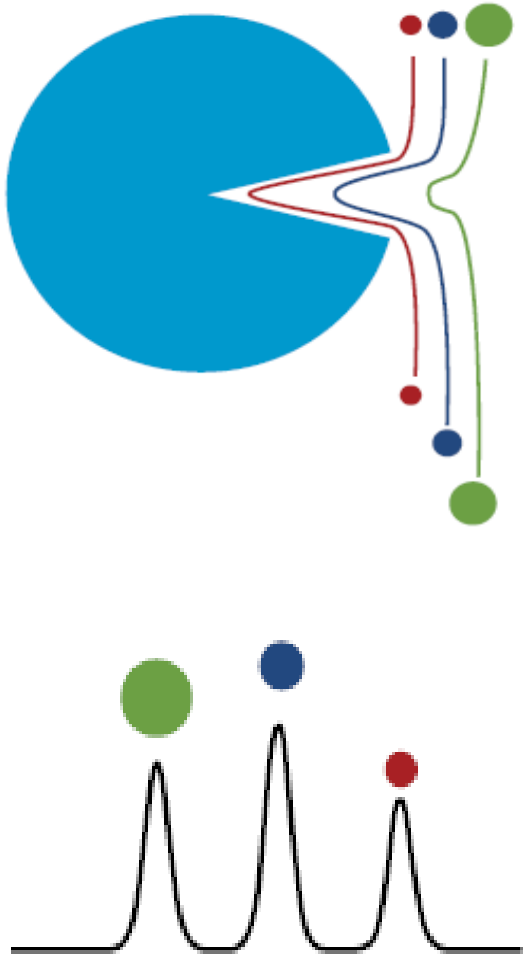
Why column selection and method conditions matter

Jean Lane
Technical Support
LC Columns and Consumables
November 30, 2023



SEC Separation Mechanism

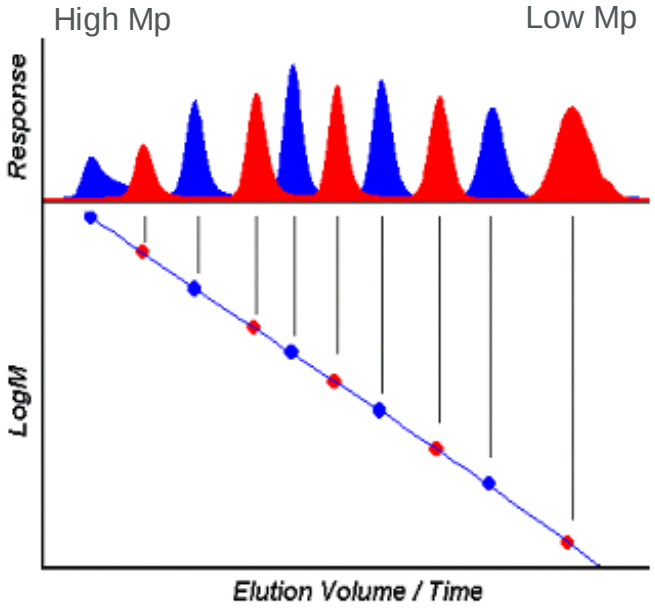
- An SEC column is packed with porous beads of controlled porosity and particle size
- The sample is prepared as a dilute solution in the eluent and injected into the system
- Large molecules are not able to permeate all pores and have a shorter residence time in the column
- Small molecules permeate 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



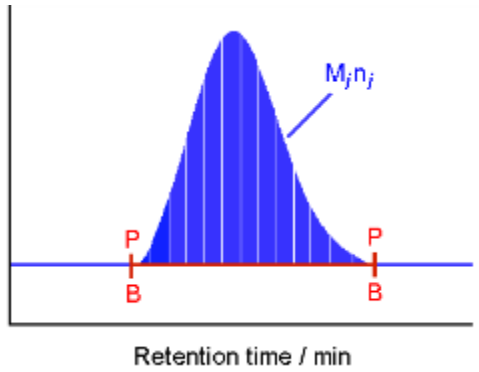
Conventional SEC Workflow

- Calibrate the SEC column with a set of narrow polymer standards
- Plot retention time (RT) versus peak log molecular weight (logM)
- Calibration is used to generate molecular weight (averages and distribution) of unknowns on the same system/column set
- Molecular weights are relative to the standards used

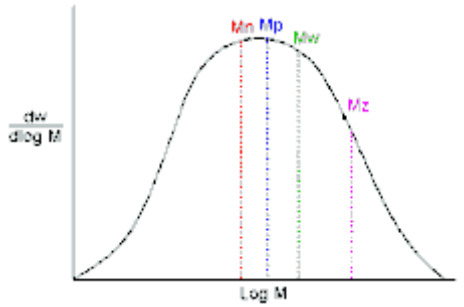
Chromatogram and plot of narrow standards



GPC sample chromatogram

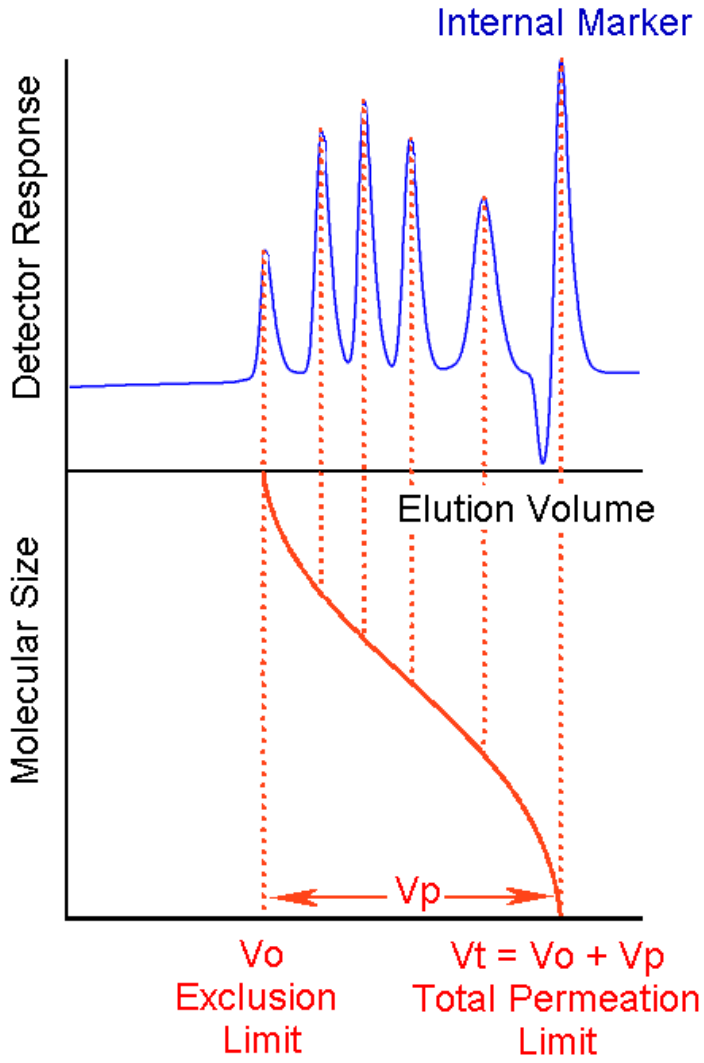


Molecular weight distribution



Elution Profiles

- As a result of the SEC separation mechanism, polymer molecules elute from the column in order of size in solution
- Largest elute first, smallest elute last
- The separation is purely a physical partitioning, there is no interaction or binding
- The separation is isocratic
- If polymer molecules have the same molecular dimensions, they will co-elute by SEC and may not be separated by this technique
- The calibration curve describes how different size molecules elute from the column



Column Selection

What do I need to know?

SEC column selection depends on:

- Molecular weight of sample
- Expected polydispersity of polymer
- Presence of additives
- Mobile phase conditions required
- Temperature required

Helpful to know the properties of the sample:

- Neutral, anionic, cationic
- Hydrophobic

Further Criteria for Column Selection

- The factors that govern which type of column is selected for an SEC experiment are the anticipated mol wt of the sample as well as the solvent the sample is soluble in
- Many polymers dissolve in only very limited numbers of solvents or conditions
- The columns used must be compatible with the solvent/mobile phase of choice
- The properties and range for the column must be considered when selecting them for an application
- Most importantly, the size exclusion mechanism must be maintained

Columns for Aqueous SEC

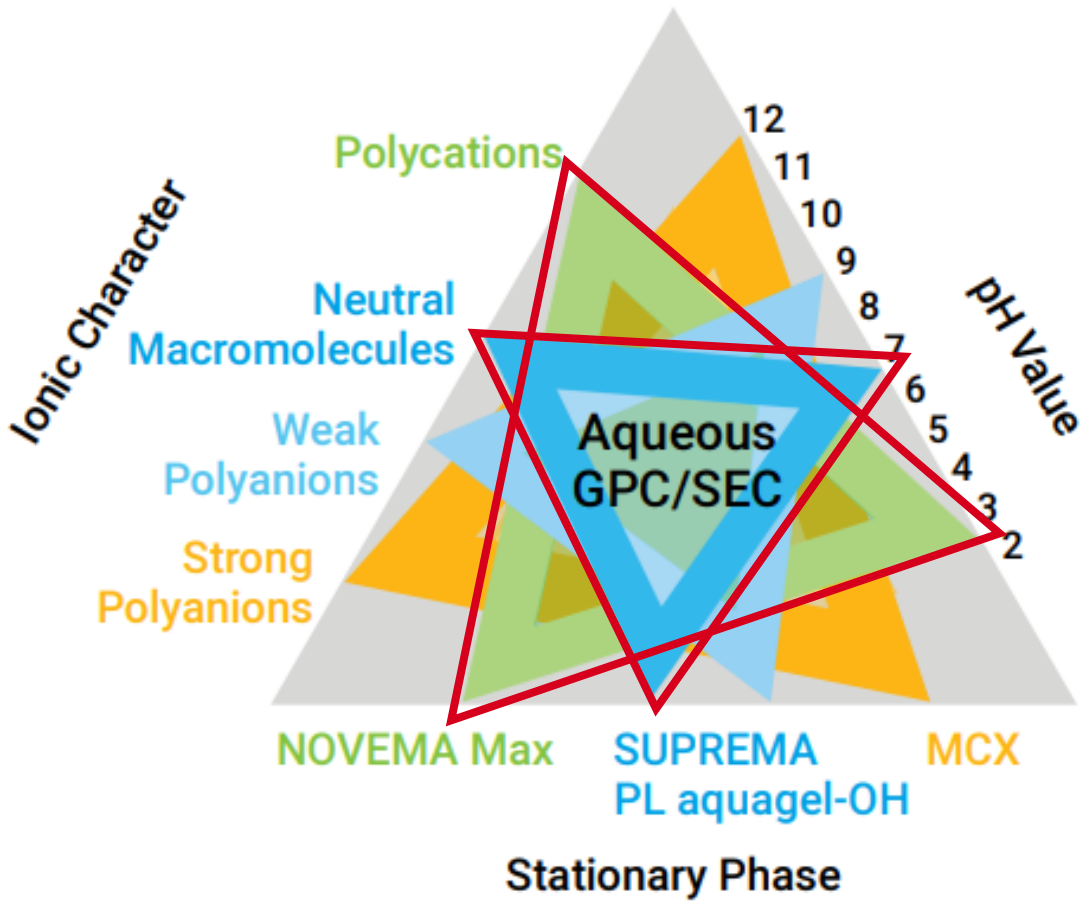
- Very hydrophilic surface required
- Minimal surface charge
- Good mechanical stability
- Wide range of pH compatibility
- Wide range for molecular weight

Aqueous GPC

Additional factors to be considered in aqueous SEC applications:

- pH effect
- Ionic strength effect
- Interaction with column packing
- Aggregation

Aqueous SEC
Column selection considerations



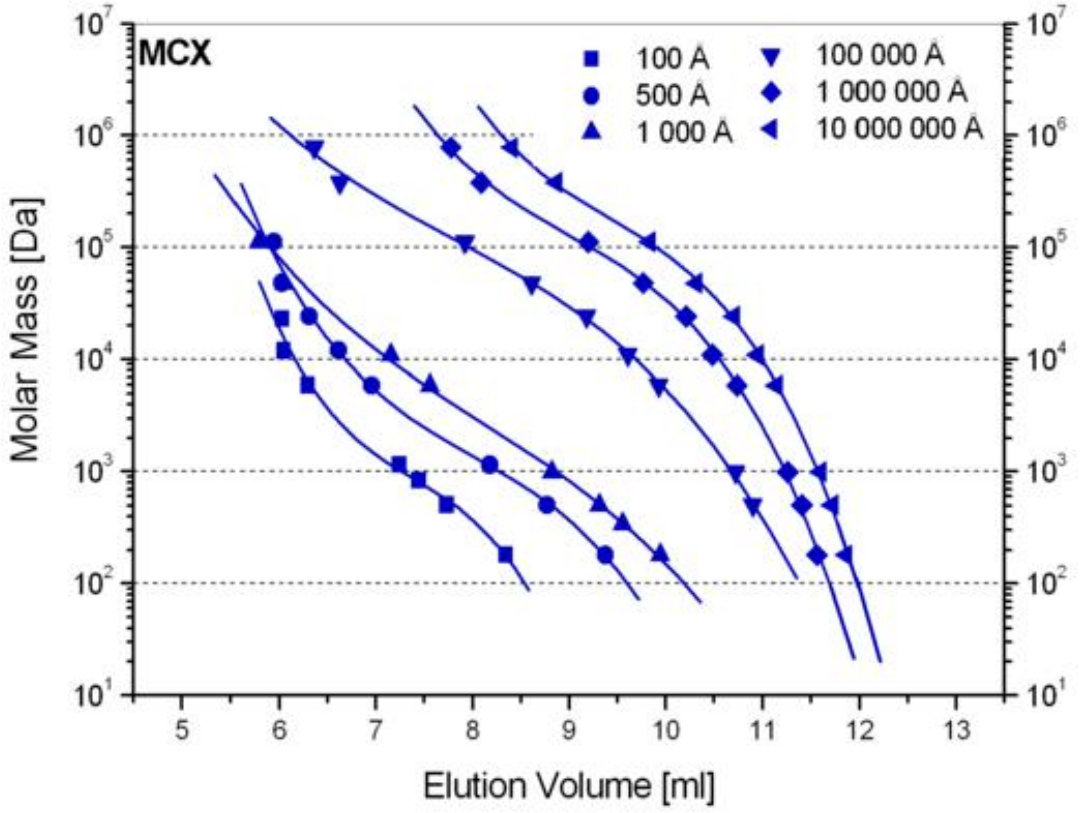
Water-soluble polymers

The main solvent is water, but your column choice should be made in correlation to the pH ranges at which the columns will be used.

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 a series, but you need to select carefully so you not to have a column ‘mismatch’

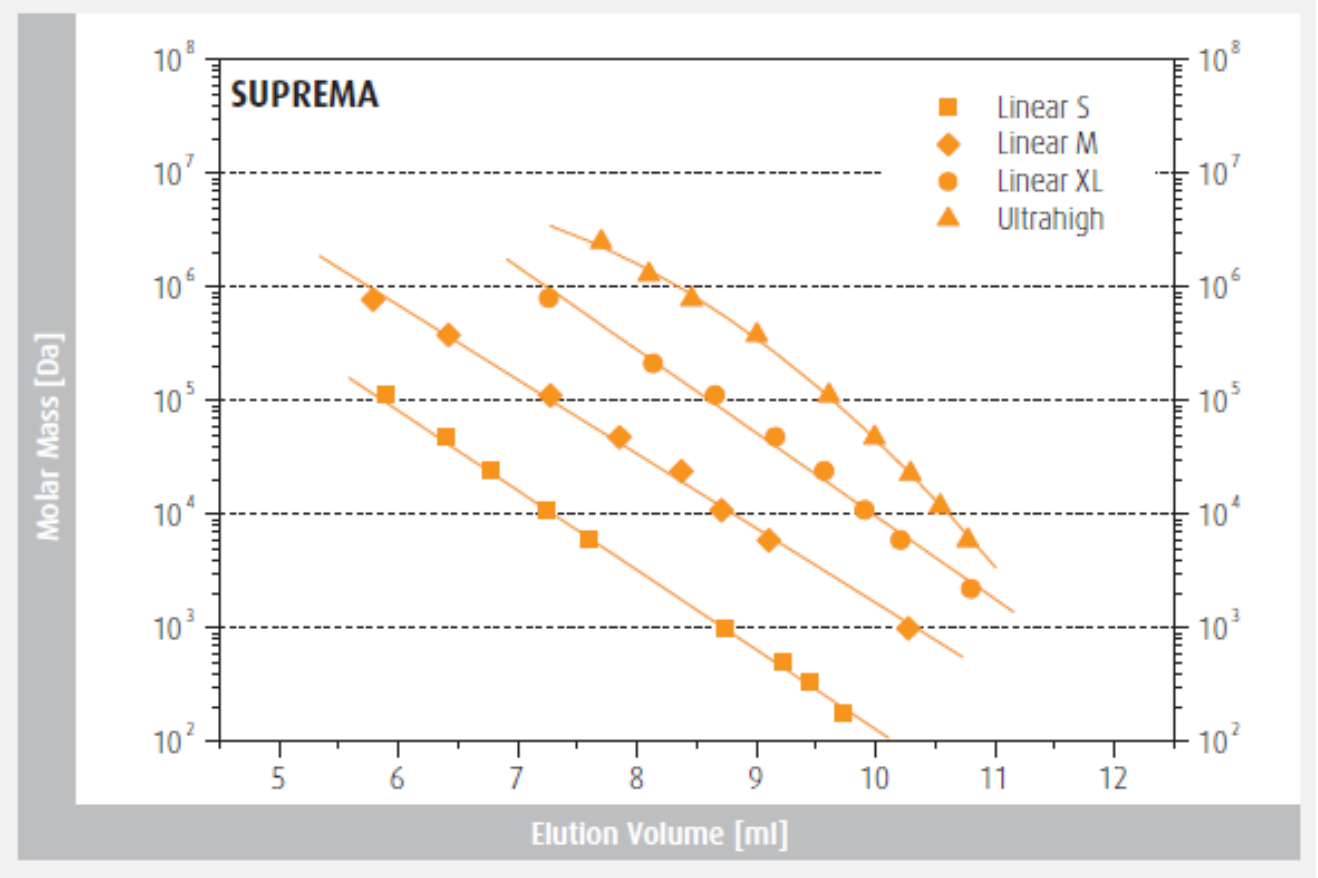
Calibration plots



MCX individual pore column calibration plots

Column Types: Linear/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



Example: SUPREMA Linear column calibration plots

Agilent Product Families for Aqueous SEC of Polymers

Product Family
PL aquagel OH
PL RAPIDE Aqua
SUPREMA
NOVEMA MAX
MCX

Agilent Columns for Polymer Applications Using Aqueous Solvents

PL aquagel-OH

Chemically and physically stable, wide pH range

Technical specifications:

- PL aquagel-OH – polymeric packing with a polyhydroxyl surface
- “Neutral” surface
- pH range 2 to 10
- Individual pore size and MIXED/linear columns
- Neutral, ionic and hydrophobic moieties

Typical application areas include PEG, PEO, polysorbate, celluloses, dextran, and acrylamide.

Available in 5, 8, and 15 µm particle size options and columns sizes ranging from narrow-bore to preparative.

Agilent Columns for Polymer Applications Using Aqueous Solvents

PL Rapide Aqua

Chemically and physically stable, wide pH range

8 µm particle size

Technical specifications for Rapide columns are equivalent to that of PL aquagel-OH columns

Typical application areas include PEG, PEO, celluloses, dextran, and acrylates

Designed for use for fast SEC and high throughput applications
Column dimensions: 7.5 x 150 mm and 10 x 100 mm column sizes

Two molecular weight range options available:

PL Rapide H – Molecular weight range: 6,000 to 10,000,000
PL Rapide L – Molecular weight range: 100 to 60,000

Agilent Columns for Polymer Applications Using Aqueous Solvents

SUPREMA

- Chemically and physically stable, wide pH range

Technical specifications:

- Suprema – modified acrylate copolymer
- “Neutral” surface
- pH range 2 to 12
- Individual pore size and linear columns
- SUPREMA LUX columns for light scattering also available

Typical application areas include PEO, PEG, pullulan, dextran, polyacrylamide, hyaluronic acid, polyacrylic acid, and carboxymethyl cellulose.

Available in 3, 5, and 10 µm particle size options and columns sizes ranging from narrow-bore to preparative.

Agilent Columns for Polymer Applications Using Aqueous Solvents

MCX series

- Well suited to charged **anionic** polymers
- Sulfonated styrene-divinylbenzene copolymer-based
- Robust at high pH, range pH 1 to 13
- Temperatures up to 80 °C
- Compatible with organic modifiers, up to 100% methanol or acetonitrile

Typical application areas include poly(styrene sulfonate), lignin sulfonate, modified starches, acids, alcohols, and pectins.

Flexible configurations available in two particle sizes, 5 µm and 10 µm, with a variety of porosities ranging from 100 Å to 10,000,000 Å.

Columns sizes ranging from analytical to preparative.

Agilent Columns for Polymer Applications Using Aqueous Solvents

NOVEMA Max series

- Well suited for charged **cationic** polymers
- NH-functionalized acrylate copolymer
- Robust at low pH
- Tolerate high buffer concentrations
- Temperatures up to 80 °C
- Compatible with organic modifiers, up to 100% methanol or acetonitrile
- NOVEMA LUX columns for light scattering also available

Typical application areas include polymeric quaternary ammonium compounds, poly(DADMAC), polyvinyl pyridine, chitosan, and poly(ethylene imine).

Flexible configurations available in two particle sizes, 5 µm and 10 µm, and four individual porosities, 30 Å, 100 Å, 1,000 Å and 3,000 Å, as well as a linear/mixed-bed version, providing a wide separation range.

Column dimensions range from analytical to preparative sizes.

SEC Column Selection

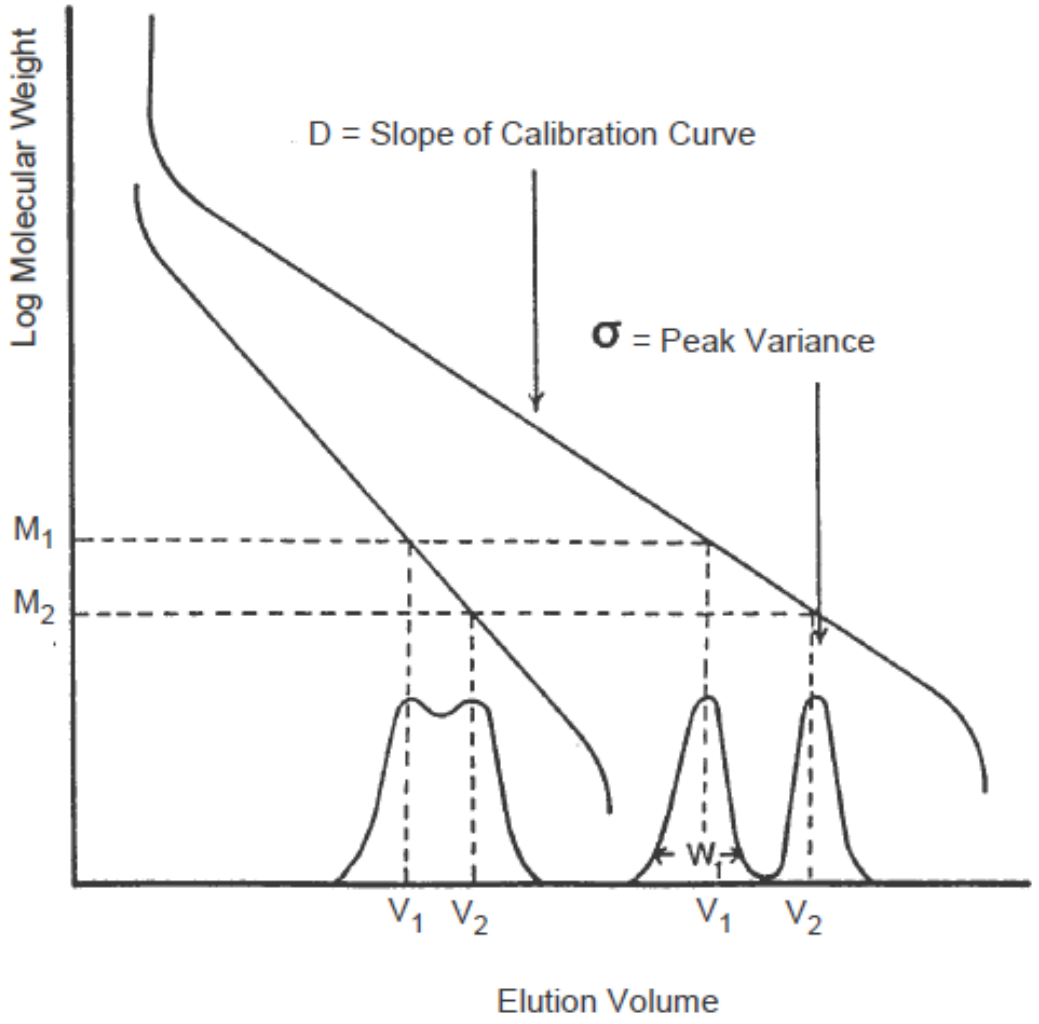
Choosing for molecular weight range

Example: PEO sample with expected mol wt range of 200K, resolution is important

Description	Particle size (µm)	MW range (g/mol) (PEG/PEO)	Guaranteed Efficiency (p/m)	Part No.
PL aquagel-OH 20	5	100 to 20,000	>55,000	PL1120-6520
PL aquagel-OH 30	8	100 to 60,000	>35,000	PL1120-6830
PL aquagel-OH 40	8	10,000 to 200,000	>35,000	PL1149-6840
PL aquagel-OH 40	~~15~~	10,000 to 200,000	>15,000	PL1149-6240
PL aquagel-OH 50	8	50,000 to 600,000	>35,000	PL1149-6850
PL aquagel-OH 50	~~15~~	50,000 to 600,000	>15,000	PL1149-6250
PL aquagel-OH 60	8	200,000 to 10,000,000	>35,000	PL1149-6860
PL aquagel-OH 60	~~15~~	200,000 to 10,000,000	>15,000	PL1149-6260
PL aquagel-OH MIXED-H	8	6,000 to 10,000,000	>35,000	PL1149-6800
PL aquagel-OH MIXED-M	8	1,000 to 500,000	>35,000	PL1149-6801

SEC Column Selection

Addition of columns in series



GPC Column Selection

Ways to improve resolution

Agilent InfinityLab

Running two columns in series using different pore sizes

- Extends the resolving range and enables analysis of multiple attributes in one run

Running two columns in series using the same pore size/same type

- Increasing pore volume increases the resolution

Use a packing with a smaller particle size

- Decreasing the particle size increases column efficiency

Changing flow rate

- Optimal flow rates can differ with the particle size of your column

SEC Column Selection

How many SEC columns to use

More than one column typically used
More columns = improved resolution

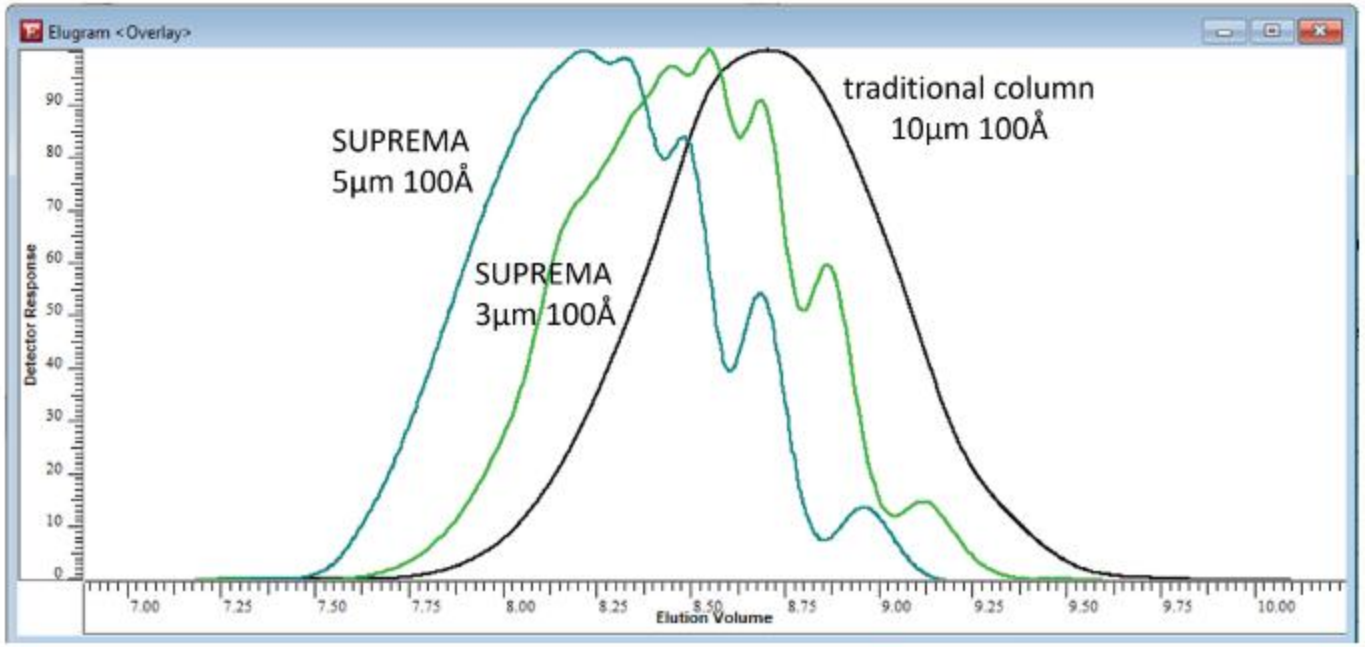
- The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution. More columns will be required to maintain the quality of the results.
- For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples.

Particle Size	Number of Columns
20 µm	4
15µm	3
10 µm	3
8 µm	3
5 µm	2
3 µm	2

Suggested guideline for number of columns to use

Column Selection

Reduce particle size to improve resolution



Normalized detector response, and elution volume (mL) of dextran (Mw: 1,260 Da) at a flow rate of 0.5 mL/min in H_2O, 0.05% NaN_3
(Red: typical 10 µm 100 Å column, dark green: Agilent SUPREMA 5 µm 100 Å, light green: Agilent SUPREMA 3 µm 100 Å)

Column: SUPREMA, 3 µm, 100 Å, 8 x 300 mm, p/n SUA0830031e2

SUPREMA, 5 µm, 100 Å, 8 x 300 mm, p/n SUA0830051e2

Eluent: Water, 0.05% sodium azide

Flow rate: 0.5 or 0.25 mL/min

Detector: RI

Sample: Dextran Mw 1200

Reduce particle size to improve efficiency
Improve resolution for low molecular weight oligomers

Publication number: 5994-5702EN

SEC Method Development

Criteria for mobile phase selection

The mobile phase needs to:

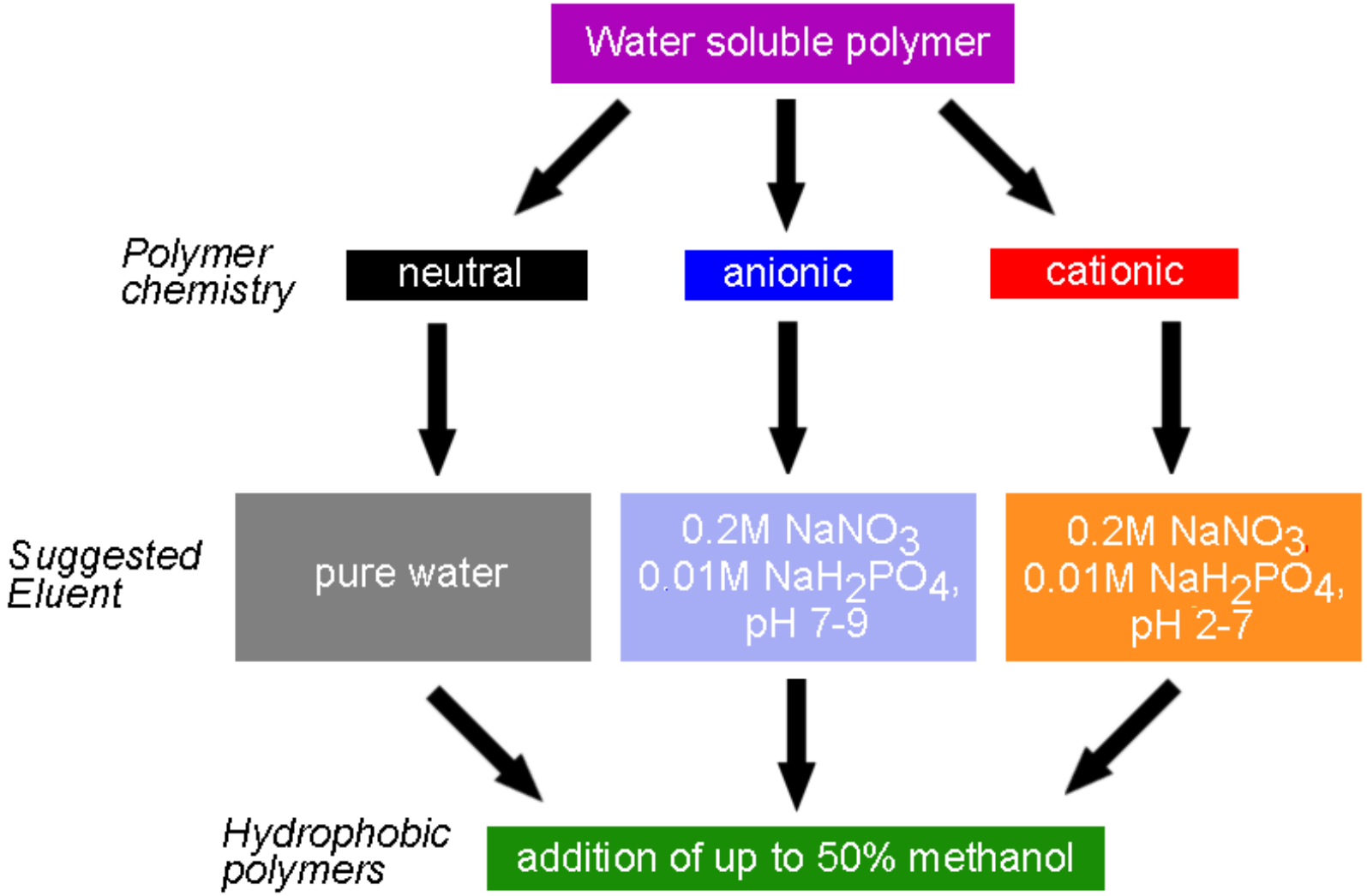
- Permit true sample solubility (polarity and time-dependent)
- Allow compatibility with columns
- Avoid non size exclusion effects
- Permit adequate detection (for example, refractive index, UV cut off)
- Account for safety (for example, toxicity, elevated temperature)

Challenges in aqueous GPC:

- Ion exchange
- Ion inclusion/exclusion
- (Intramolecular) electrostatic interactions
- Adsorption and repulsion
- Sample aggregation

Recommended Starting Conditions

PL aquagel OH and Rapide Aqua



Mobile Phase Conditions

Suggested parameters for SUPREMA, MCX, NOVEMA MAX

SUPREMA, MCX, NOVEMA MAX

Parameter	Range	Comment
Temperature	<80 °C	Optimum range 10-35 °C
Organic modifiers	<100% v/v	Ex MeOH, ACN, THF
Salts	<0.5 M	Ex NaN_3, NaCl, $NaNO_3$
Buffers		Compatible with most common buffers

Always consult the individual column user guide for specific technical specifications.

Mobile Phase Conditions

Suggested parameters continued

For your sample type:

Know what parameters are important for the column chemistry you are using.

SUPREMA

Parameter	Range	Comment
pH stability	2-12	Chemical stability
Optimum pH range	6-10	Due to slight residual anionic nature of the surface

MCX

Parameter	Range	Comment
pH stability	1-13	Chemical stability
Optimum pH range	7-13	Due to the anionic nature of the surface

For anionic polymers

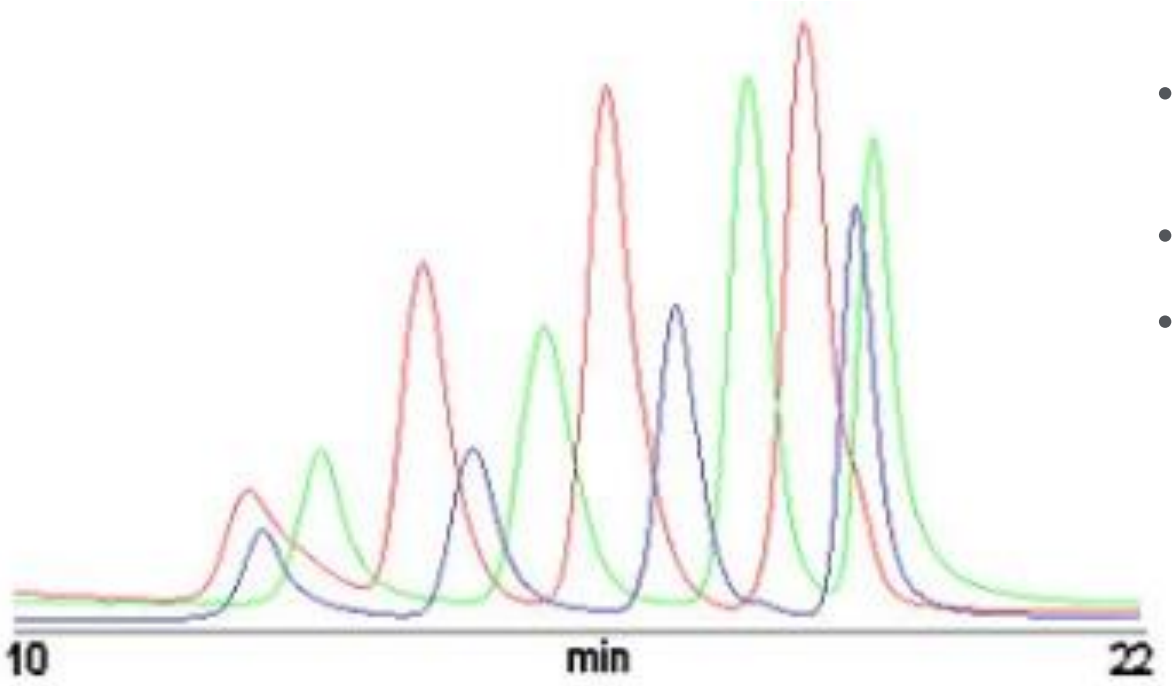
NOVEMA MAX

Parameter	Range	Comment
pH stability	1.5 -12	Chemical stability
Optimum pH range	1.5–7.0	Due to cationic nature of the surface

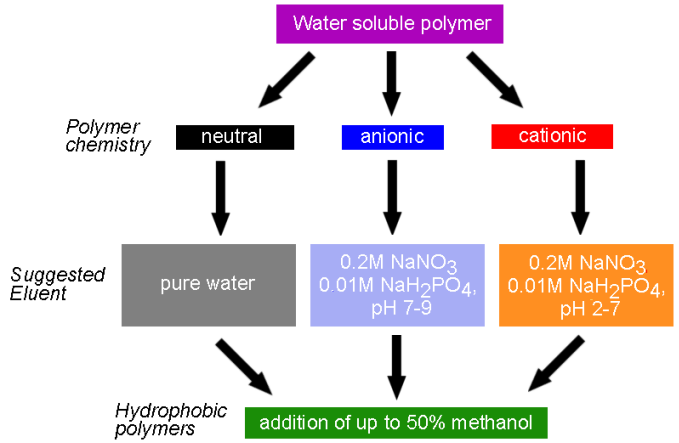
For cationic polymers

Solvent Selection

Aqueous SEC application example



- Column: 2 x PL aquagel-OH MIXED H, 8 µm 7.5 x 300 mm, p/n PL1149-6800
- Eluent: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7
- Flow rate: 1.0 mL/min
- Detector: RI

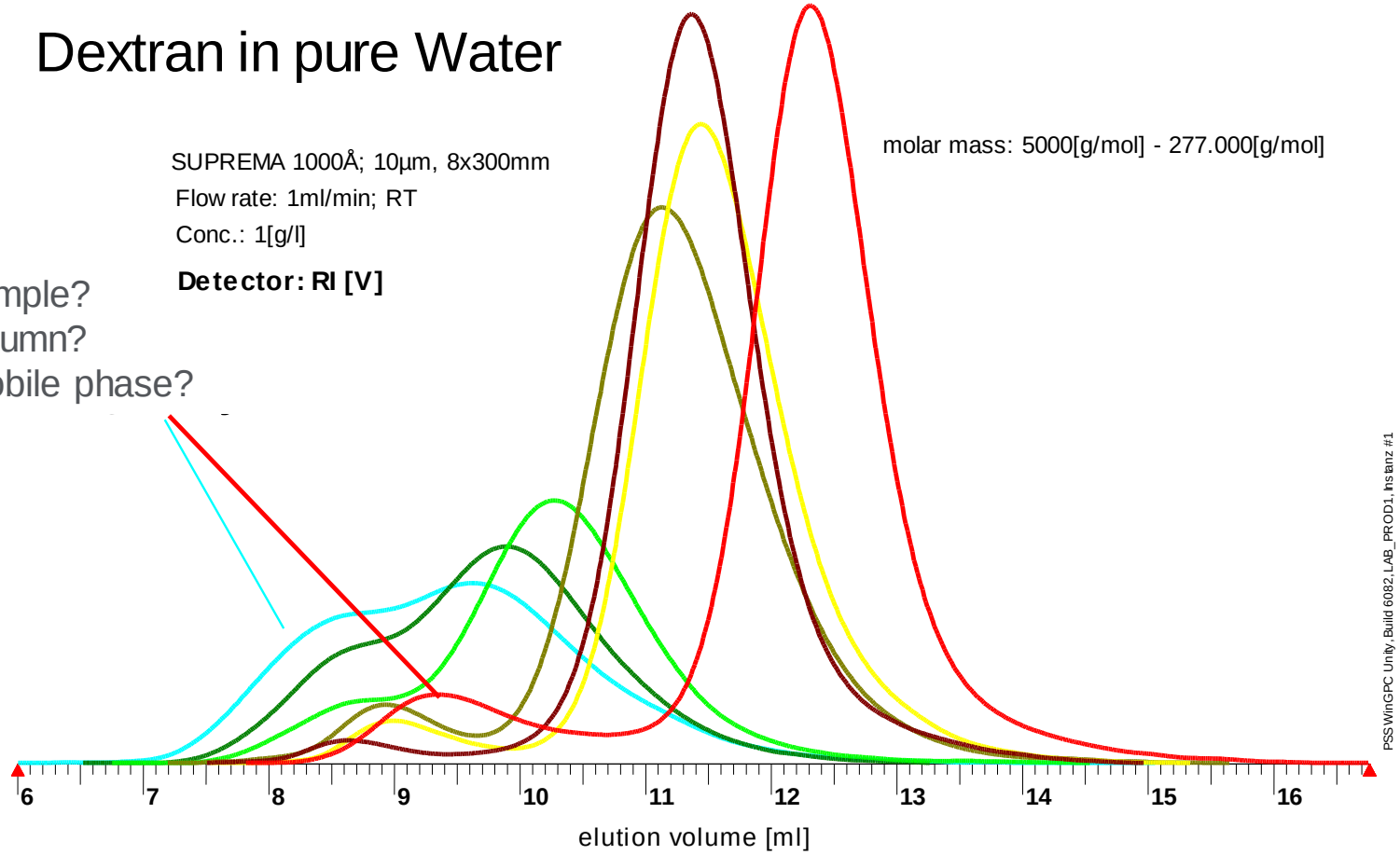


Sample: PEO, polyethylene oxide standards

Effect of Salt Concentration

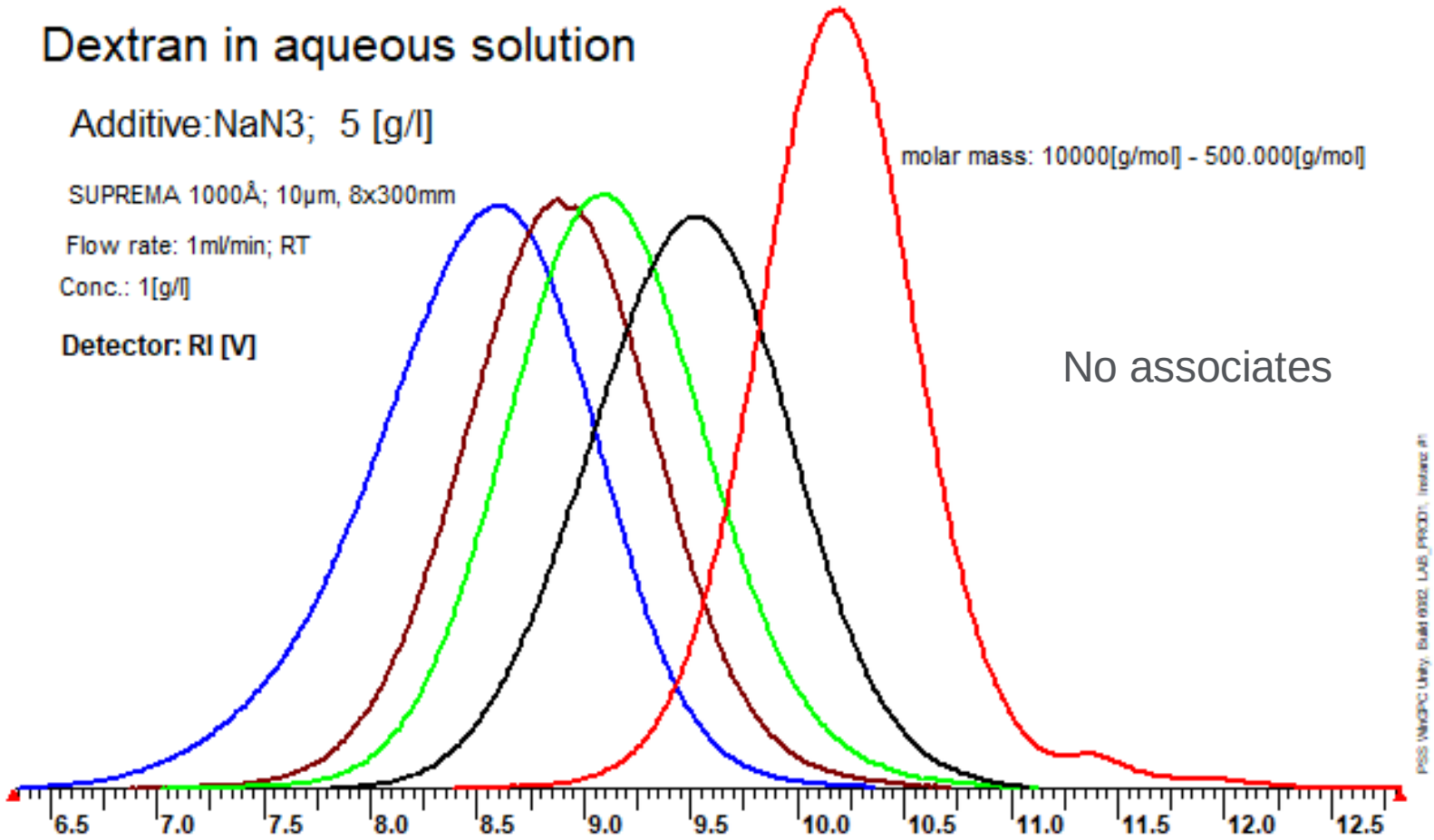
Not the distributions expected
Possible reasons for multimodal distributions

Problem with the sample?
Problem with the column?
Conditions of the mobile phase?



Effect of Salt Concentration

Salt effect in aqueous SEC



Bimodal distribution seen in the previous slide is not necessarily a quality issue of the column

What to Look For

Secondary interactions in aqueous SEC

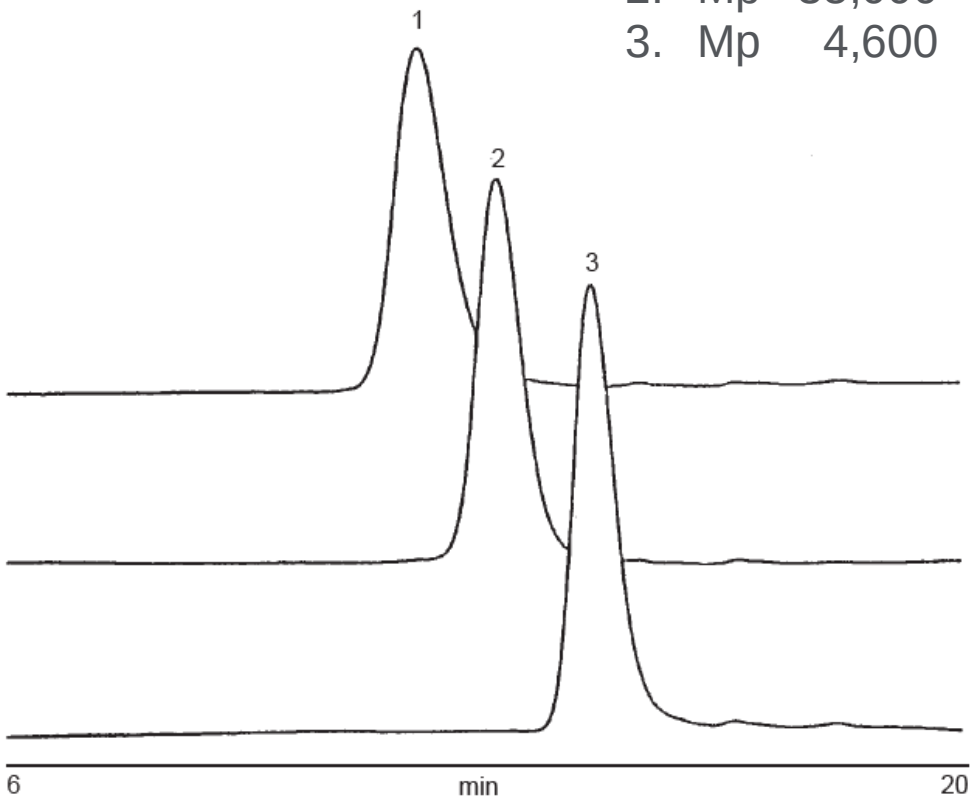
Type	Symptom	Action
Ion exclusion	Peaks elute early, maybe before the exclusion limit	Modify eluent, addition of salt, or pH adjustment
Ionic adsorption	Peaks elute late, maybe after SEC permeation limit. Peak tailing or no peaks detected	Modify eluent, addition of salt, or pH adjustment
Ionic inclusion	Peak at total permeation due to salt, even though sample prepared in the eluent	Recognize salt peak and do not include in the sample integration
Hydrophobic adsorption	Peaks elute late, maybe after SEC permeation limit. Peak tailing or no peaks detected	Modify eluent, addition of organic modifier. Check for compatibility with column and for mobile phase conditions

Solvent Selection

Aqueous SEC application example

Polystyrene sulfonate

1. Mp 100,000
2. Mp 35,000
3. Mp 4,600



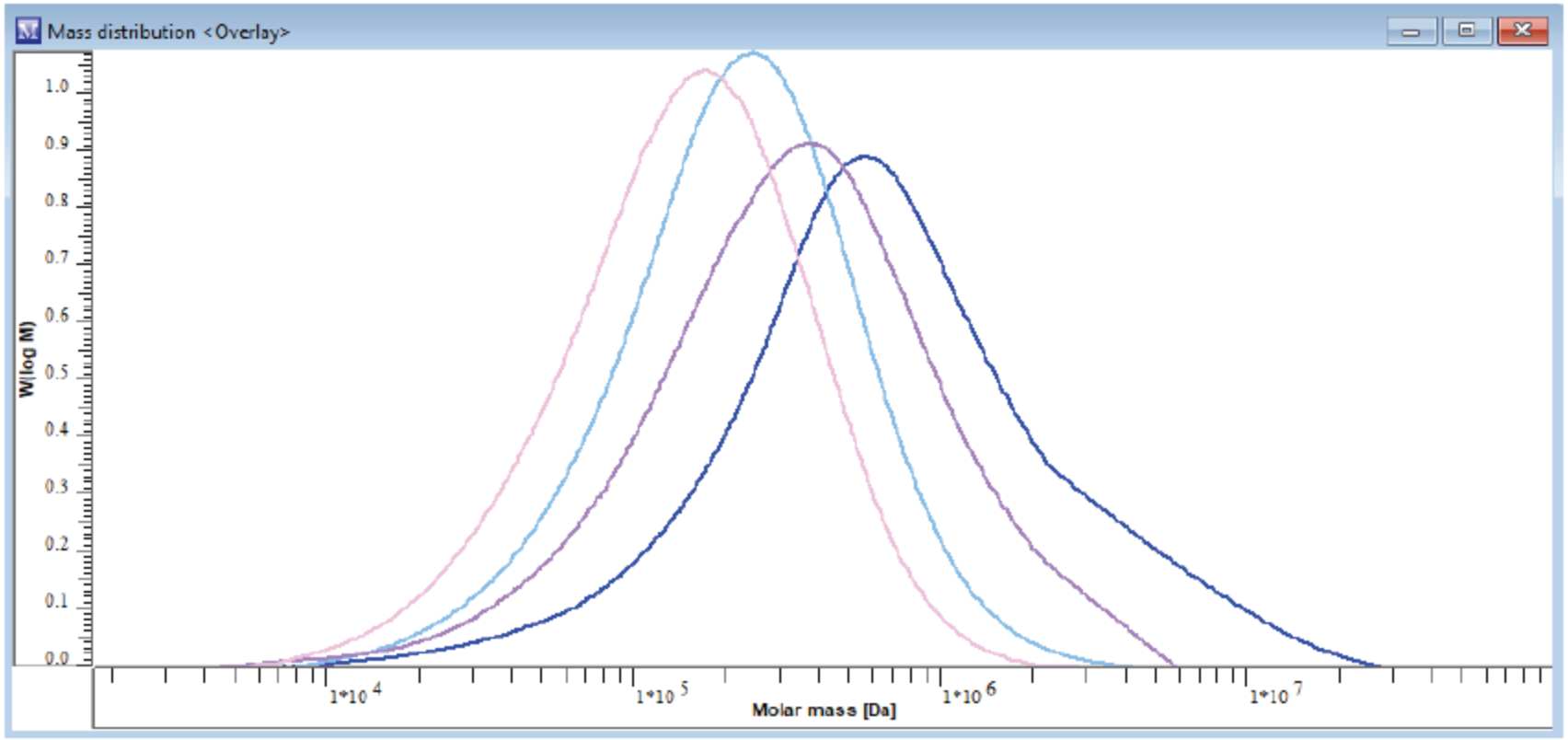
- Column: 2 x PL aquagel-OH 40, 8 µm
  7.5 x 300 mm, p/n PL1149-6840
- Eluent: 80% 0.3 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 9
  20% methanol
- Flow rate: 1.0 mL/min
- Detector: RI

These polymers are both ionic and relatively hydrophobic.

The eluent conditions are chosen to minimize sample-to-column interaction, which would otherwise result in late elution times.

SEC Application
Chitosan

- Column: 3 x NOVEMA Max 10 µm Ultrahigh, 8 x 300 mm, p/n NMA083010LUH
  NOVEMA Max 10 µm GUARD, 8 x 50 mm, p/n NMA080510
- Eluent: Water, 0.1 M NaCl, 0.3% vol trifluoracetic acid
- Flow rate: 1.0 mL/min
- Detector: RI



Overlay of the molecular weight distribution of Four Chitosan samples

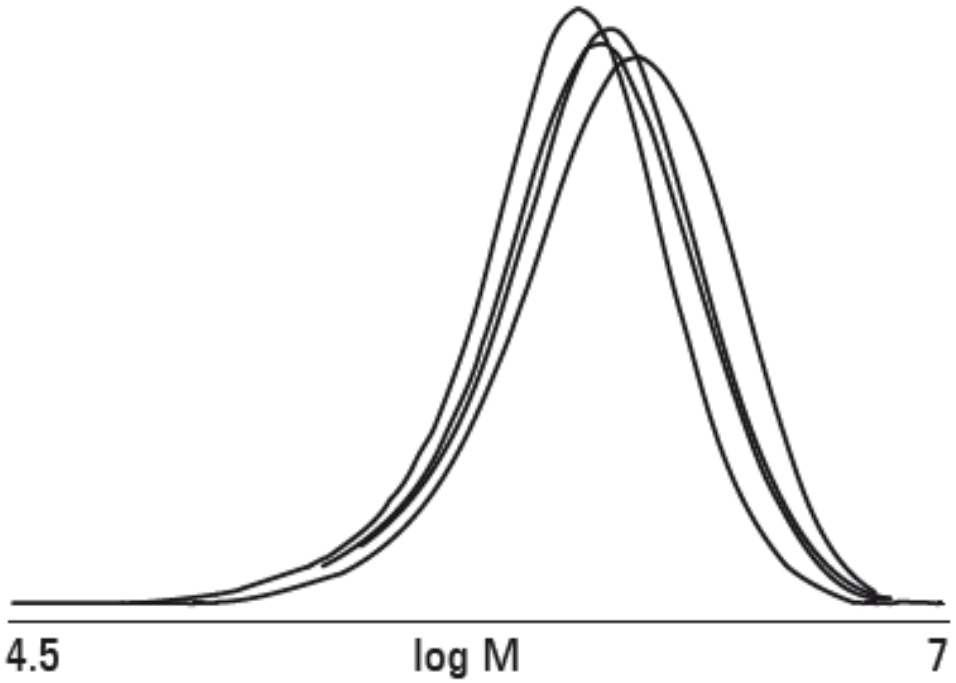
Using a conventional pullulan calibration, this would enable the analysis of the molecular weight distribution (MWD) as well as average molar mass values. Values calculated will be relative to the Pullulan standards used

High Molecular Weight Applications

- High molecular weight water-soluble polymers can exhibit extremely high viscosity in solution, even at quite low concentration
- Very low sample concentrations need to be employed (0.01 to 0.1% w/v is typical)
- Large particle size packing materials are required to avoid column pressure problems and polymer shear degradation
- Large pore size columns must be used to fully resolve all of the sample components
- Reduced flow rate may be required
- High molecular weight water soluble standards above ~ 1,000,000 mol wt are not readily available

SEC of High MW Polymers

Hyaluronic Acid



Column: 2 x PL aquagel-OH 40 and 60, 15 µm
7.5 x 300 mm
p/n PL1149-6240, PL1149-6260

Eluent: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow rate: 1.0 mL/min

Detector: RI

Sample	Mp	Mw	Mn	Polydispersity
1	1,677,000	1,680,050	1,084,400	1.5
2	1,444,400	1,465,700	932,200	1.6
3	1,351,400	1,398,500	882,500	1.6
4	1,201,100	1,210,800	826,800	1.5

High mol wt sample; to reduce the potential of shearing, use large particle size columns

Publication number: 5991-5787EN

Putting It Together

Polymer Calibration Standards for Aqueous SEC

Type	Individual	Calibration Kit	ReadyCal/EasiVial	LS/Visco Validation Kit
Dextran	X	X	X	X
Pullulan	X	X	X	
Polyethylene Glycol PEG	X	X	X / X	
Polyethylene Oxide PEO	X	X	X / X	
Poly methacrylic acid	X	x		
Poly(acrylic acid) sodium salt	X	X		
Poly(styrene sulfonate) sodium salt	X	X		
Poly(2-vinylpyridine)	X	X		

[GPC/SEC Standards | Agilent]

X – available in this format

Polymer Calibration Standards

Type of kits that are available

Agilent individual polymer calibration standards –small quantity and available in all MW ranges

Agilent EasiVial and ReadyCal calibration kits
Standard kits suggested for specific SEC columns

Agilent ReadyVLS GPC/SEC detector calibration kits
Required for additional detectors

Agilent EasiValid Validation Kit
For system suitability testing; IQ, OQ, PQ

Standard Format	Features
ReadyCal and EasiVial	Easy to use, preprepared vial format saves time
Conventional Calibration Kits	Preselected MW standards to generate an even distribution of points across the chosen MW range

Each EasiVial, ReadyCal, and conventional calibration kits cover a specific range of MW

Common Questions Around SEC Standards

What standards to use?

- *What is the sample type and eluent/mobile phase?*
- *What is the MW range for my Column set?*

[Publication number: 5991-2720EN]

Sample Type	Eluent	Recommendation
Synthetic polymers	Water or aqueous buffers	Polyethyene glycol/oxie (PEG/PEO)
Naturally occurring macromolecules	Water or aqueous buffers	Pullulan Polysaccharide (SAC) or dextran
Strong anionic macromolecules	Water or aqueous buffers*	Poly(2 vinylpyridine) or Pullulan Polysaccharide (SAC)
Cationic macromolecules	Water or aqueous buffers**	Poly(styrene sulfonate) sodium salt or Pullulan Polysaccharide (SAC)

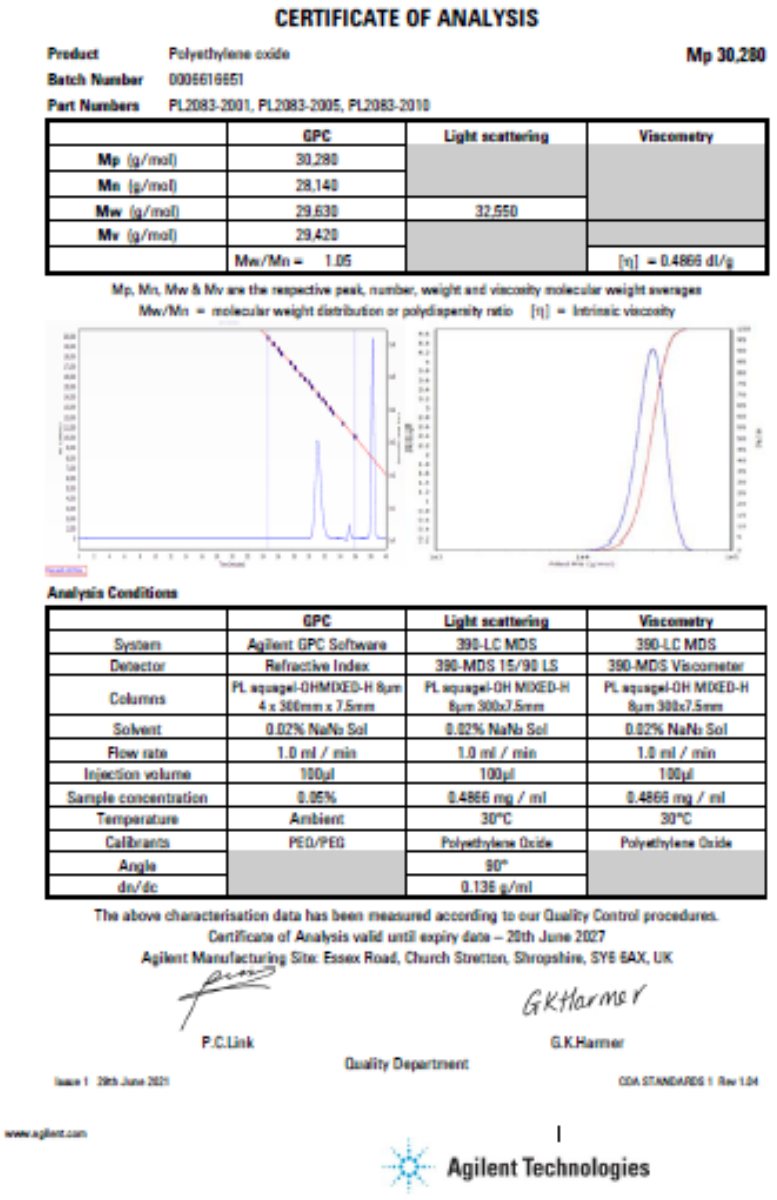
*Depending upon column selection, strong anion macromolecules are typically run in basic eluent conditions.

**Depending upon column selection, cationic macromolecules are typically run in acidic eluent conditions.

Well Characterized Polymer Standards

Example certificates of analysis

- Agilent standards are manufactured under an ISO 9001:2008 approved quality system.
- Each standard is fully traceable with a unique batch number and is provided with a complete certificate of analysis (CoA).
- Finally, all CoAs include details of the exact method and characterization results for maximum transparency and reproducibility.



CERTIFICATE OF ANALYSIS

Product Polyethylene oxide **Mp 30,280**

Batch Number 0000616651

Part Numbers PL2083-2001, PL2083-2005, PL2083-2010

	GPC	Light scattering	Viscometry
Mp (g/mol)	30,280		
Mn (g/mol)	28,140		
Mw (g/mol)	29,630	32,550	
Mv (g/mol)	29,420		
	Mw/Mn = 1.05		[η] = 0.4866 dl/g

Mp, Mn, Mw & Mv are the respective peak, number, weight and viscosity molecular weight averages
Mw/Mn = molecular weight distribution or polydispersity ratio [η] = Intrinsic viscosity

Analysis Conditions

	GPC	Light scattering	Viscometry
System	Agilent GPC Software	390-LC MDS	390-LC MDS
Detector	Refractive Index	390-MDS 15/90 LS	390-MDS Viscometer
Columns	PL aquagel-OH MIXED-H 8µm 4 x 300mm x 7.5mm	PL aquagel-OH MIXED-H 8µm 300x7.5mm	PL aquagel-OH MIXED-H 8µm 300x7.5mm
Solvent	0.02% NaN_3 Sol	0.02% NaN_3 Sol	0.02% NaN_3 Sol
Flow rate	1.0 ml / min	1.0 ml / min	1.0 ml / min
Injection volume	100µl	100µl	100µl
Sample concentration	0.05%	0.4866 mg / ml	0.4866 mg / ml
Temperature	Ambient	30°C	30°C
Calibrants	PEO/PEG	Polyethylene Oxide	Polyethylene Oxide
Angle		90°	
dn/dc		0.136 g/ml	

The above characterisation data has been measured according to our Quality Control procedures.
Certificate of Analysis valid until expiry date – 20th June 2027
Agilent Manufacturing Site: Essex Road, Church Stretton, Shropshire, SY6 6AX, UK

P.C.Link G.K.Harmer
Quality Department

Issue 1 29th June 2021 COA STANDARDS 1 Rev 1.04

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Agilent Technologies

Example: Individual standard certificate

EasiVial Certificate of Analysis

CERTIFICATE OF ANALYSIS

Product Polyethylene oxide/glycol EasiVials (2ml)

Part Numbers PL2080-0201, PL2080-0202, PL2080-0700

Batch Number 0006731552

Vial Code	IV (dL/g)	Mw (g/mol) (Light Scattering)	Mn* (g/mol)	Mw* (g/mol)	Mw/Mn*	Mp* (g/mol)	Mass/vial (mg)
RED	7.8113	1,470,000	1,140,000	1,379,000	1.21	**1,511,000**	0.2
	1.5510	151,300	118,800	129,400	1.09	**141,700**	0.4
	0.2910	13,380	13,380	14,360	1.07	**15,190**	0.6
	0.0443	655	580	620	1.07	**610**	0.8
WHITE	6.3477	986,000	869,000	965,000	1.11	**1,044,000**	0.2
	0.9705	77,950	65,950	69,200	1.05	**71,050**	0.4
	0.1246	3,890	3,750	3,890	1.04	**3,880**	0.6
	-	-	-	-	1.00	**194**	0.8**
BLUE	3.6484	471,500	414,000	433,000	1.05	**427,500**	0.2
	0.4738	28,450	26,810	28,100	1.05	**28,480**	0.4
	0.0697	1,560	1,440	1,490	1.03	**1,470**	0.6
	-	-	-	-	1.00	**106**	0.8**

* Results of polymer characterisation by gel permeation chromatography using 0.02% Na N_3 in water as the eluent at a flow rate of 1.0ml/min and ambient temperature, PL aquagel-OH columns selected appropriate to the molecular weight of the polymer.

** Due to the volatile nature of this constituent weights may vary.

Storage:

The polymers in each vial are supplied under Argon, and the product should be stored in a refrigerator when not in use. After preparation, the polymer solutions should be stored in a cool, dark place and used within 48 hours.

Agilent Manufacturing Site:

Essex Road, Church Stretton, Shropshire, SY6 6AX, UK

The above characterisation data has been measured according to our Quality Control procedures.
Certificate of Analysis valid until expiry date: 23rd November 2026

M.Moore G.K.Harmer

Q.C. Department

Issue 1 28th February 2023 COA STANDARDS7-2 Rev 4.16

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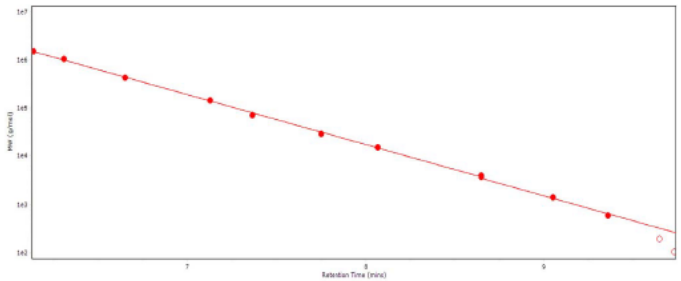
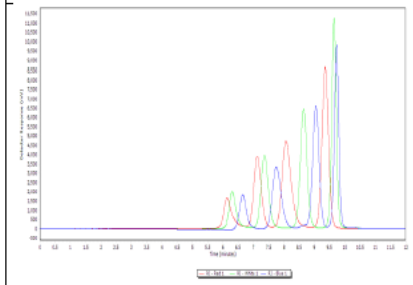
GPC/SEC Calibration Curve



GPC/SEC EasiVial Overlay



Calibration Details	
System	Agilent GPC Software
Detector	Refractive Index
Columns	1 x PL aquagel-OH MIXED-H 8µm 300mm x 7.5mm
Solvent	0.02% sodium azide
Flow rate	1.0 ml / min
Injection volume	20µl
Sample concentration	1ml solvent / vial
Temperature	Ambient

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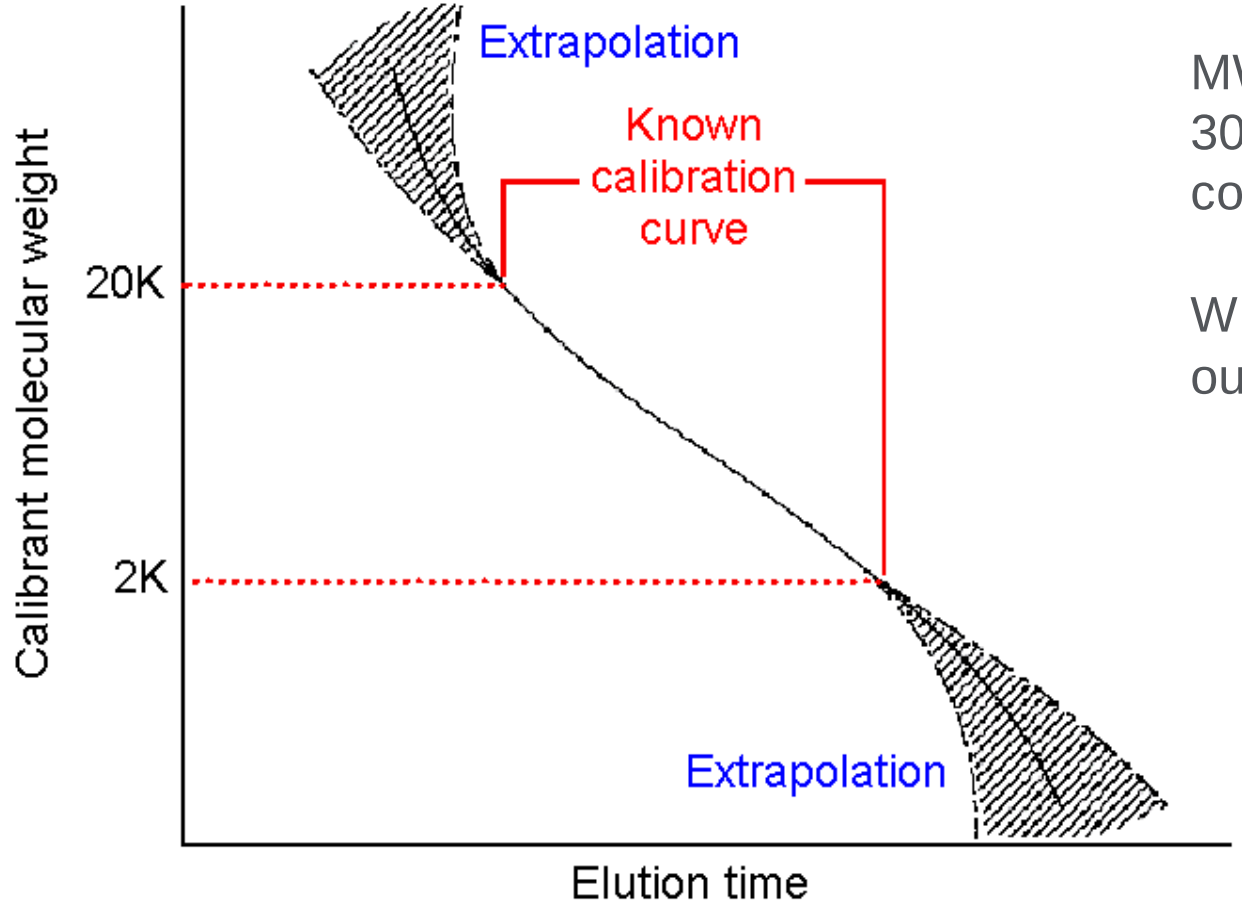
Calibrate for Full MW Range of Columns

Avoid errors due to limited calibration region



SUPREMA 5 µm, 100 Å, 8.0 x 300 mm, p/n SUA0830053e1

MW range for the column is 100 to 30,000, but we only calibrate the column from 2,000 to 20,000.

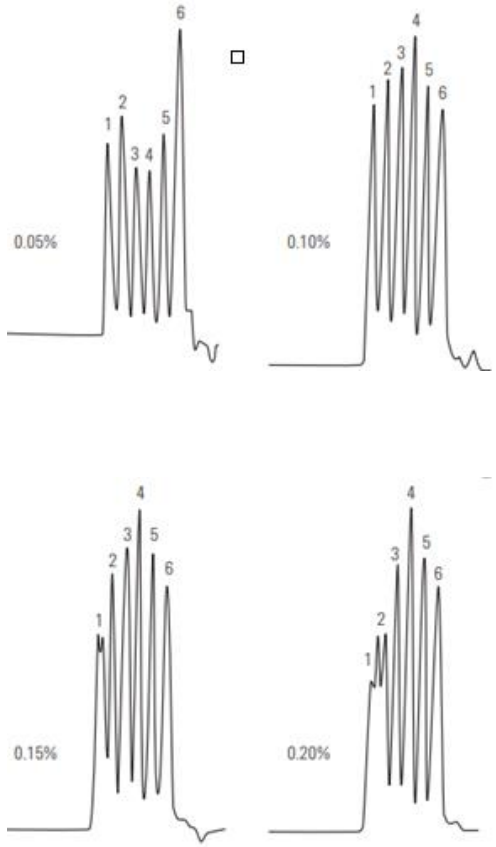
What if our sample elutes outside of our calibration region?

It's important that the column calibration should cover the full MR range of the column, and that for the sample to avoid errors due to extrapolation.

Common Questions Around SEC Standards

How are standards prepared?

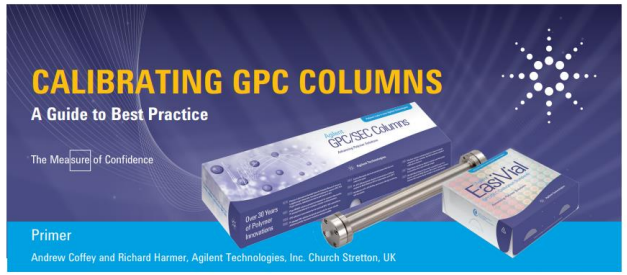
- Standard should always be dissolved with the same solvent being used as the mobile phase.
- Vortexing, sonicating, and shaking of standard solutions should also be avoided (these are high-shear activities will result in a change of peak shape, retention time and MW).
- Concentration is also of critical importance. Band broadening due to excessive sample viscosity can occur if the standard is too concentrated. Loss of resolution is also a factor if too concentrated.
- Signal-to-noise ratio will be too small to reproducibly integrate with too low a concentration.



How frequently should new standards be prepared?

- Standards are typically stable in the form they are supplied in, but once made up can degrade, expedited by UV and heat. ***It is good practice to make up new standards on a weekly basis.***
- PEOs and PEGs should be refrigerated for storage, but brought to ambient temperature before use.

Common Questions Around SEC Standards



Publication number: 5991-2720EN

How often should you calibrate?

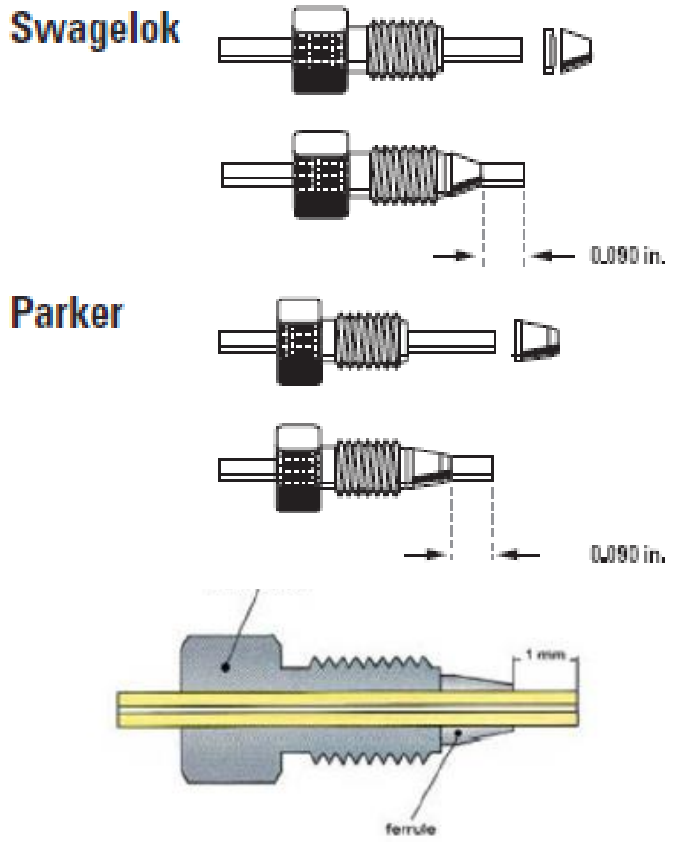
- Frequency of calibration is subjective. For continuous work, it is advisable to calibrate daily, but with the use of internal verification, a weekly calibration should be performed at minimum. *Once a week is suggested,* if no major changes occur with system/columns.

- It is essential to recalibrate *whenever a component of the system is altered or if there is an eluent change*

Calibrating frequently can also help to identify potential issues and can allow you to quickly to put into place corrective/preventative steps.

Other Considerations

Importance of proper fittings



If stainless steel nuts and ferrules are used, it is important to to be sure to use the proper fittings.

For the Aquagel OH columns, these are compatible with both Swagelok and Parker type fittings.
Note the placement for the ferrule on the tubing – 0.090 in.

For the Suprema, MCX, and NOVEMA MAX columns, it is recommended to use standard SS fittings and a one piece ferrule, for example, Parker type.
Note the placement for the ferrule on the tubing is different to that for the Aquagel OH columns – 1 mm.

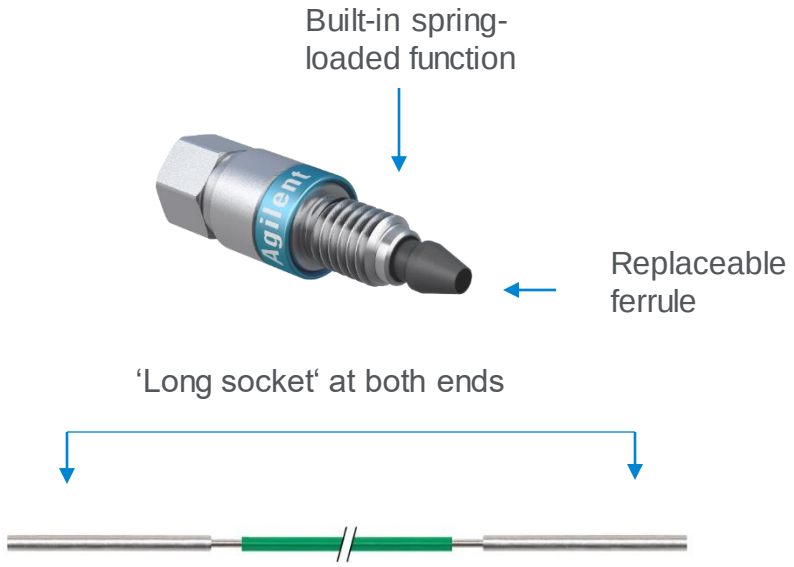
Result of using improper fittings/ferrules:

- Leaks
- Peak shape issues

Parker fittings:
p/n PL1310-0007, column connecting nuts
p/n PL1310-0008, tubing ferrules, 5/pk

InfinityLab Quick Connect Fittings

Correct connection to the column every time



Quick Turn Fitting
• Spring-loaded function for dead volume-free connections • Replaceable ferrule and capillary • Capillaries in various lengths and ids are available
• 300 to 400 bar (finger tight, user-dependent) • 1300 bar (with mounting tool, p/n 5043-0915)

It is important that Quick Connect fittings are only used with capillaries specially designed for them, to ensure proper function.

Infinity Lab Quick Turn Quick Connect 5991-5164EN

Importance of Filtering SEC Mobile Phases
Instrument and buffers can be a source of particulates

InfinityLab solvent filtration assembly, p/n 3150-0577

Instrument and buffers can be a source of particulates

- Filter buffered LC solvents with Agilent solvent filtration equipment to remove precipitated/undissolved salts
- Use Agilent inline filters in the pump to remove pump seal wear

Filter membrane types

- Regenerated cellulose 47 mm, 0.45 µm
- Nylon 47 mm, 0.45 µm
- PTFE 47 mm, 0.45 µm

Consumable parts for assembly are sold separately

Solvent inlet filters

Glass filter, 20 µm pore size, p/n 5041-2168
Stainless steel filter, 10 µm pore size, p/n 01018-60025

The Value of Guard Columns

Agilent InfinityLab

Use Guards to protect your column in many ways

What They Do

- Provide protection for the analytical column
  - Filter particulates
  - Prevent unwanted chemical contamination
  - Protect against pressure spikes
- Increase total “column length”
  - Increase resolution

What They Don’t Do

- Replace good sample cleanup
- Replace column hygiene

The Guard is not a *“magic device”*

SEC guard column

Before Your Analysis

Do a performance test of your column

Every new column should be tested on your instrument

LC Column Performance Report

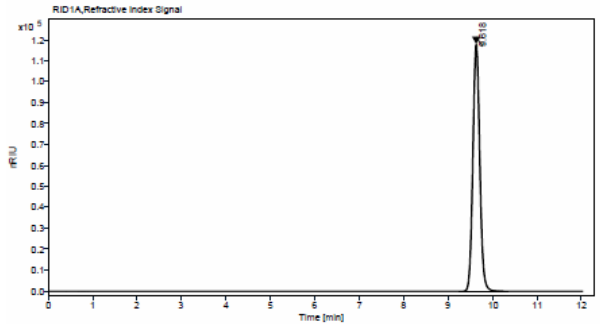
Agilent Technologies

Serial number	0006742315-197
Part number	PL1149-6800
Description	PL aquagel-OH MIXED-H 8um 300 x 7.5mm
Batch number	0006742315

Test Conditions

Mobile phase	Water containing 0.02% sodium Azide
Column pressure	19 Bar (includes system pressure)
Flow rate	1.00mL/min
Temperature	Ambient
Injection volume	2.4µl
Test mixture	Glycerol (5% in water containing 0.02% sodium azide)
LC system	Agilent LC Test system with OpenLab CDS 2

	TEST VALUES	SPECIFICATIONS
Theoretical plates 1/2 height per metre	62,992	>56,000
Theoretical plates 5 sigma per metre	55,723	>47,000
Peak asymmetry 10%	1.11	0.80 - 1.35



Operator MB

This column is shipped in water containing 0.02% sodium azide Agilent Test LC systems are optimized to minimize extra-column volume, so performance results may vary from those on systems in your lab Peak widths are dependent on integration settings, so results may also vary between data systems

Performance verification based on Agilent checkout

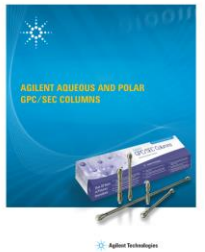
- Run Agilent checkout before use
  - Record the difference between your instrument and performance report (use as a base value)
- Perform again if the column seems to lose performance
  - Compare with results from your first run
- Running a set of polymer standards for your column at its first use
- Perform again if column seems to lose performance
  - Compare with results from first run

Agilent SEC Column Product Families For Water-soluble Polymers

	Dextran Saccharides Hyaluronic Acid Acrylates Acrylamides Heparin Gum	PL aquagel-OH PL Multisolvent PL Rapide Aqua SUPREMA SUPREMA Lux*	– Individual pore sizes and mixed-bed columns – SUPREMA compatible with 100% organic modifiers
	Sulfonated Polyanions Lignins	MCX	– Charged anionic polymers – Robust at high pH – Compatible with organic modifiers – Available in 5 µm particle sizes
	Chitosan Food Ingredients Cationic Polymers	NOVEMA Max NOVEMA Max Lux*	– Charged cationic polymers – Robust at low pH – Compatible with organic modifiers – Available in 5 µm particle sizes

SEC Columns, Supplies, and Application Resources

- Agilent webpage GPC/SEC: GPC/SEC Columns & Standards | Agilent
- **Expanded** portfolio GPC/SEC columns and standards: Agilent GPC/SEC Columns and Standards Brochure
- Aqueous SEC Columns: Aqueous SEC Columns | Agilent
- Organic GPC Columns: Organic GPC Columns | Agilent
- GPC/SEC Polymer Standards: GPC/SEC Standards | Agilent
- GPC/SEC User Guide: GPC/SEC column user guide
- GPC/SEC Column Selector Tools: GPC/SEC Column Selector Tools
- Polymer to Solvent Reference Table: Polymer to Solvent Reference Table
- GPC Troubleshooting poster: GPC Troubleshooting Guide
- InfinityLab Supplies catalog: InfinityLab LC Supplies Catalog
- Consumables Community: Agilent Collection of Columns, Supplies, and Standards Resources - Consumables - Agilent Community
- App finder: Application Finder | Agilent
- Agilent GPC/SEC solutions: GPC/SEC Solutions for Macromolecular Characterization | Agilent
- Your local product specialists
- Webinars, upcoming and recorded: LC and LC/MS Column Webinars | Agilent



Contact Agilent Chemistries and Supplies Technical Support

Available in the U.S. and Canada, 8-5 all time zones

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

Option 6 for Prozyme products

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

pzi.info@agilent.com

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Trusted Answers