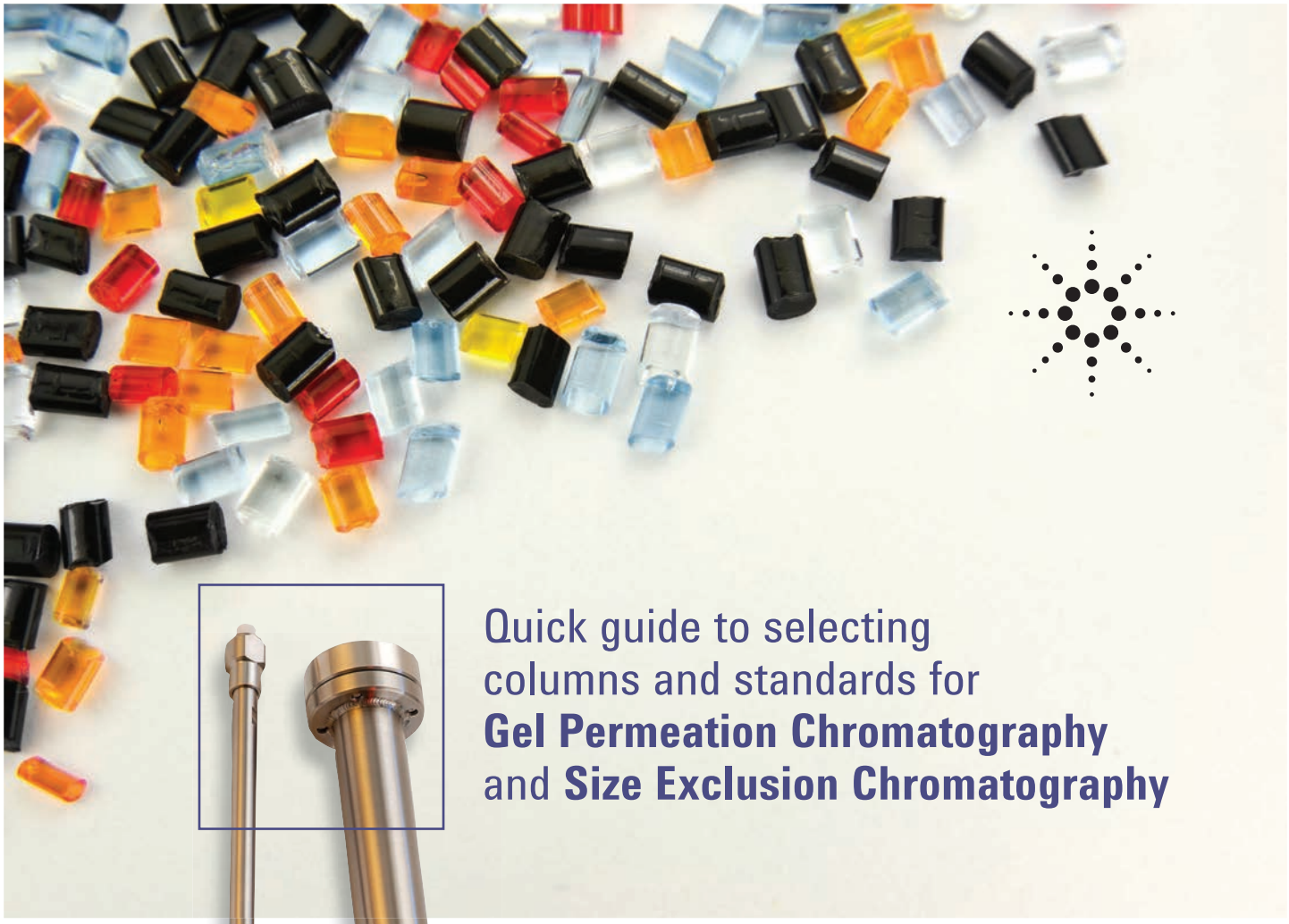


Quick guide to selecting columns and standards for **Gel Permeation Chromatography** and **Size Exclusion Chromatography**

SELECTION GUIDE

The Measure of Confidence

Agilent Technologies

Introduction

Gel permeation chromatography (GPC) and size exclusion chromatography (SEC) are techniques for measuring the molecular weight distribution of natural and synthetic polymers, a property that affects many of the physical parameters of materials such as strength, toughness and chemical resistance. GPC and SEC are liquid chromatographic techniques that separate individual polymer chains on the basis of their size in solution and not on their chemistry.

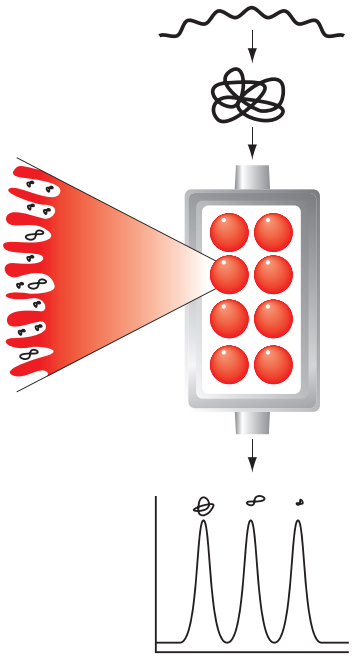
We use GPC to describe the analysis of polymers in organic solvents, such as tetrahydrofuran, and SEC to describe the analysis of polymers in water and water-based solvents, such as buffer solutions. GPC/SEC is the only established method for obtaining a comprehensive understanding of a polymer's molecular weight distribution.

How to use this selection guide

There are many columns available for the analysis of polymers by GPC/SEC. The purpose of this guide is to help you find a set of columns and conditions for the analysis of most common polymer types. A series of questions helps to narrow the choice down to the appropriate set. Some applications are not so easy to define and the required information may not be known, so consult your local expert in GPC/SEC for advice.

Mechanisms of GPC/SEC

- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- Polymer coils introduced to eluent flowing through a column
- Column packed with insoluble porous beads with well-defined pore structure
- Size of pores similar to that of polymer coils
- Polymer coils diffuse in and out of the pores
- Result is elution based on size – large coils first, smaller coils last
- Size separation converted to molecular weight separation by use of a calibration curve constructed by the use of polymer standards



Key

- Smaller coils can access many pores
- Larger coils can access few pores
- Very large coils access very few pores

Figure 1. *Mechanisms of GPC and SEC*

Polymer Laboratories was formed in 1976 to offer high quality columns, standards, instruments, and software for GPC/SEC. For over 30 years the company developed many market-leading products, including PLgel, PL aquagel-OH, PlusPore, PLgel Olexis, PolarGel columns, and EasiVial standards. Built on advanced in-house manufacturing technology, PL's products have the highest reputation for quality and performance, backed up by world-class technical and applications support.

With the acquisition of PL, Agilent offers an even wider range of GPC and SEC solutions for all types of polymer characterization of synthetic and bio-molecular polymers, with options for conventional GPC all the way up to complex determinations using multi-column and multi-detection methods.

Recommendations for setting up a GPC/SEC system

The following questions will help you find the recommended columns and standards for any given application, as well as system parameters such as injection volumes.

Choosing an eluent for GPC/SEC

Question	Answer	Recommendation	Comments
1. What is the sample soluble in? *Many polymers are only soluble in a small number of solvents. This is the key question when developing methods for analyzing polymers. The solvents mentioned here are all common eluents employed in GPC/SEC.*	Water or water buffer with up to 50% methanol	Agilent PL aquagel-OH	Best choice for water-based applications but cannot accommodate organics apart from methanol up to 50%
	Typical organic solvent such as THF, chloroform, toluene	Agilent PLgel or Agilent PlusPore	PLgel are the workhorse columns, PlusPore columns are an alternative
	Organic/water mixtures or polar organics such as, DMF, NMP	Agilent PolarGel	PolarGel is a smaller column range than PLgel or PL aquagel-OH columns but is suited to mixtures of organics and water

Choosing a column for GPC/SEC

Columns shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
2. What is the expected molecular weight? *It may seem strange to ask this question, but in GPC/SEC the resolution of a column is related to the resolving range. Knowing something of the expected molecular weight of a sample helps to choose the best column that will give optimum results.*	High (up to several millions)	*Aqueous solvents* **PL aquagel-OH MIXED-H 8 µm** or combination of PL aquagel-OH 40 and 60 15 µm	The 15 µm column combination is best only where sample viscosity is very high, otherwise 8 µm columns give greater resolution
		Organic solvents **PLgel 10 µm MIXED-B** or PLgel 20 µm MIXED-A	The PLgel MIXED-A column resolves higher than the PLgel MIXED-B but at lower efficiency due to larger particle size
		Mixed solvents **PolarGel**	No PolarGel column available for this molecular weight range. Contact your local GPC/SEC expert for advice
	Intermediate (up to hundreds of thousands)	*Aqueous solvents* **PL aquagel-OH MIXED-M 8 µm**	A wide-ranging column that covers most water-soluble polymers
		Organic solvents **PLgel 5 µm MIXED-C** or PLgel 5 µm MIXED-D, PolyPore or ResiPore	The PLgel columns are the most widely applicable for the majority of applications; PolyPore and ResiPore columns are alternatives
		Mixed solvents **PolarGel-M**	Covers most applications
	Low (up to tens of thousands)	*Aqueous solvents* **Combination of PL aquagel-OH 40 and PL aquagel-OH 30 8 µm**	These two columns in a combined set cover the low end of the molecular weight range
		Organic solvents **PLgel 3 µm MIXED-E** or MesoPore	The PLgel column provides high resolution and is designed for low molecular weight applications; the MesoPore column is an alternative
		Mixed solvents **PolarGel-L**	For low molecular weight applications
	Very low (a few thousand)	*Aqueous solvents* **PL aquagel-OH 20 5 µm**	This high-performance column gives high resolution at low molecular weight
		Organic solvents **OligoPore** or PLgel 3 µm 100Å	The OligoPore column offers the best possible oligomer separation, PLgel also works well
		Mixed solvents **PLgel**	No PolarGel column covers this range so use PLgel columns as alternatives
	Unknown	*Aqueous solvents* **PL aquagel-OH MIXED-M 8 µm**	Covers the molecular weight ranges of most polymer samples
		Organic solvents **PLgel 5 µm MIXED-C** or PolyPore	This PLgel column is the most widely applicable for the majority of applications
		Mixed solvents **PolarGel-M**	Covers the majority of applications

Setting up the GPC/SEC system

Question	Answer	Recommendation	Comments
3. How many columns to use? *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 and the more columns are 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 during analysis.*	Depends on the particle size of the columns	Particle size 20 µm use 4 columns	Increased number of columns required for large particle sizes to make up for low efficiencies
		Particle size 13 µm use 3 columns	
		Particle size 10 µm use 3 columns	
		Particle size 8 µm use 2 columns	
		Particle size 5 µm use 2 columns	
		Particle size 3 µm use 2 columns	

Question	Answer	Recommendation	Comments
4. What size injection volume? *The injection volume required is dependent on the particle size of the column – smaller particles need lower injection volumes to minimize dead volume. Larger injection volumes allow the introduction of high molecular weight samples at lower concentrations, reducing viscosity and ensuring a quality chromatogram is obtained. Injection volume also varies dependent on column id. 4.6 mm id columns will require a lower injection volume than 7.5 mm id columns, and 25 mm id columns will require a higher injection volume.*	Depends on the particle size of the columns	Particle size 20 µm use 200 µL injection	Smaller particle sizes require smaller loops to minimize band broadening
		Particle size 13 µm use 200 µL injection	
		Particle size 10 µm use 200 µL injection	
		Particle size 5 µm use 100 to 200 µL injection	
		Particle size 3 µm use 20 µL injection	

What standards should I use?

Standards shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
5. What is the eluent? *Standards are polymers, so the choice of standard mainly reflects solubility in the chosen eluents.*	Water or water buffer with up to 50% methanol	**Polyethylene glycol (PEG)/oxide (PEO)** or polysaccharides (SAC)	These standards perform in all water-based systems in convenient Agilent EasiVial format
	Typical organic solvent such as THF, chloroform, toluene	**Polystyrene (PS)** or polymethylmethacrylate (PMMA)	Polystyrene is the most commonly used standard in convenient EasiVial format
	Organic/water mixtures or polar organics such as DMF, NMP	**Polyethylene glycol/oxide** or polymethylmethacrylate	Polar standards perform well

Question	Answer	Recommendation	Comments
6. What format of standards are recommended? *Different formats of standards are available depending on customer preference.*	For the quickest and simplest approach where accurate concentrations are not required	Easiest option – **EasiVial** or Agilent EasiCal	Simple to use, EasiVial preferred before EasiCal because of the wider choice of polymer types
	If accurate concentrations are required	Accurate concentrations required – **EasiVial** or individual standards	Both formats allow accurate sample concentrations, EasiVials are simpler to use

Typical polymer molecular weights

If you are unsure of the molecular weight of your sample, the table below shows some approximate molecular weight ranges for common polymers, which will help you select the right column for your application.

Polymer Type	Typical molecular weight of polymer	Typical polydispersity[1] of polymer
Polymers from free radical synthesis	High (up to several millions)	~ 2
	Intermediate (up to hundreds of thousands)	
Polymers from ionic synthesis	Intermediate (up to hundreds of thousands)	~ 1.01
	Low (up to tens of thousands)	
Polymers from addition synthesis	Intermediate (up to hundreds of thousands)	~ 2
	Low (up to tens of thousands)	
Polymers from controlled radical polymerization	Low (up to tens of thousands)	~ 1.1 to 1.5
	Very low (a few thousand)	
Polyolefins	Intermediate (up to hundreds of thousands)	~ 2 to 200
	High (up to several millions)	
Acrylates	Intermediate (up to hundreds of thousands)	~ 2
	High (up to several millions)	
Small molecule additives	Very low (a few thousand)	1
Pre-polymers	Low (up to tens of thousands)	~ 2 to 10
	Very low (a few thousand)	
Resins	Low (up to tens of thousands)	~ 2 to 10
	Very low (a few thousand)	
Natural biopolymers such as polysaccharides	Intermediate (up to hundreds of thousands)	~ 2 to 10
	High (up to several millions)	
Rubbers	Intermediate (up to hundreds of thousands)	~ 2 to 10
	High (up to several millions)	
Biodegradable polymers	Intermediate (up to hundreds of thousands)	~ 1.1 to 2
	Low (up to tens of thousands)	

[1] *Polydispersity is a measure of the distribution of molecular mass of a polymer*

Ordering Information

Table 1. Ordering information for columns for organic solvents

Organic GPC columns		
Description	**MW Range (g/mol)**	**Part Number**
PLgel 20 µm MIXED-A, 300 x 7.5 mm	2,000 to 40,000,000	PL1110-6200
PLgel 20 µm MIXED-A LS, 300 x 7.5 mm	2,000 to 10,000,000	PL1110-6200LS*
PLgel 10 µm MIXED-B, 300 x 7.5 mm	500 to 10,000,000	PL1110-6100
PLgel 10 µm MIXED-B LS, 300 x 7.5 mm	500 to 10,000,000	PL1110-6100LS*
PLgel 5 µm MIXED-C, 300 x 7.5 mm	200 to 2,000,000	PL1110-6500
PLgel 5 µm MIXED-D, 300 x 7.5 mm	200 to 400,000	PL1110-6504
PLgel 3 µm MIXED-E, 300 x 7.5 mm	up to 25,000	PL1110-6300
PLgel 3 µm 100Å, 300 x 7.5 mm	up to 5,000	PL1110-6320
PolyPore, 300 x 7.5 mm	200 to 2,000,000	PL1113-6500
ResiPore, 300 x 7.5 mm	up to 500,000	PL1113-6300
MesoPore, 300 x 7.5 mm	up to 25,000	PL1113-6325
OligoPore, 300 x 7.5 mm	up to 3,300	PL1113-6520

** Low shedding for light scattering applications*

Table 2. Ordering information for columns for mixed solvents

Mixed Solvent GPC columns		
Description	**MW Range (g/mol)**	**Part Number**
PolarGel-M, 300 x 7.5 mm	up to 500,000	PL1117-6800
PolarGel-L, 300 x 7.5 mm	up to 60,000	PL1117-6830
PLgel - see Table 1		

Table 3. Ordering information for columns for aqueous solvents

Aqueous GPC/SEC columns		
Description	**MW Range (g/mol)**	**Part Number**
PL aquagel-OH 15 µm 60, 300 x 7.5 mm	200,000 to 10,000,000	PL1149-6260
PL aquagel-OH 15 µm 40, 300 x 7.5 mm	10,000 to 200,000	PL1149-6240
PL aquagel-OH 8 µm MIXED-H, 300 x 7.5 mm	6,000 to 10,000,000	PL1149-6800
PL aquagel-OH 8 µm MIXED-M, 300 x 7.5 mm	1,000 to 500,000	PL1149-6801
PL aquagel-OH 8 µm 60, 300 x 7.5 mm	200,000 to 10,000,000	PL1149-6860
PL aquagel-OH 8 µm 50, 300 x 7.5 mm	50,000 to 600,000	PL1149-6850
PL aquagel-OH 8 µm 40, 300 x 7.5 mm	10,000 to 200,000	PL1149-6840
PL aquagel-OH 8 µm 30, 300 x 7.5 mm	100 to 60,000	PL1120-6830
PL aquagel-OH 5 µm 20, 300 x 7.5 mm	100 to 20,000	PL1120-6520

For a full list of GPC/SEC columns, go to **www.agilent.com/chem/gpcsec**

Table 4. Ordering information calibration standards

Calibration standards		
Description	**MW Range (g/mol)**	**Part Number**
Agilent EasiVial PEG/PEO 2 mL pre-weighed calibration kit	100 to 1,200,000	PL2080-0201
Agilent EasiVial PEG 2 mL pre-weighed calibration kit	106 to 35,000	PL2070-0201
Agilent PEG-10 polyethylene glycol calibration kit	106 to 20,000	PL2070-0100
Agilent PEO-10 polyethylene oxide calibration kit	20,000 to 1,000,000	PL2080-0101
Agilent SAC-10 pullulan polysaccharide calibration kit	180 to 850,000	PL2090-0100
Agilent PAA-10 polyacrylic acid Na salt calibration kit	1,000 to 1,000,000	PL2140-0100
Agilent PS-H EasiVial 2 mL pre-weighed polystyrene calibration kit	162 to 6,000,000	PL2010-0201
Agilent PS-M EasiVial 2 mL pre-weighed polystyrene calibration kit	162 to 400,000	PL2010-0301
Agilent PS-L EasiVial 2 mL pre-weighed polystyrene calibration kit	162 to 40,000	PL2010-0401
Agilent EasiCal PS-1 pre-prepared polystyrene kit	580 to 7,500,000	PL2010-0501
Agilent EasiCal PS-2 pre-prepared polystyrene kit	580 to 400,000	PL2010-0601
Agilent S H-10 polystyrene calibration kit	300,000 to 15,000,000	PL2010-0103
Agilent S-H2-10 polystyrene calibration kit	1,000 to 15,000,000	PL2010-0104
Agilent S-M-10 polystyrene calibration kit	580 to 3,000,000	PL2010-0100
Agilent S-M2-10 polystyrene calibration kit	580 to 300,000	PL2010-0102
Agilent S-L-10 polystyrene calibration kit	162 to 20,000	PL2010-0101
Agilent S-L2-10 polystyrene calibration kit	162 to 10,000	PL2010-0105
Agilent M-M-10 polymethylmethacrylate calibration kit	1,000 to 1,500,000	PL2020-0101
Agilent M-L-10 polymethylmethacrylate calibration kit	500 to 50,000	PL2020-0100

All the above polymer types are also available as nominal molecular weights

For a full list of calibration standards, go to **www.agilent.com/chem/gpcsec**

Agilent 1260 Infinity GPC/SEC Analysis System

Agilent GPC/SEC Analysis Systems

For easy and reliable polymer characterization, turn to the Agilent 1260 Infinity GPC/SEC Analysis System. The isocratic solvent delivery system provides the constant, stable flow rate that is essential to maintain the high resolution of the GPC/SEC column. And with its high flow precision and excellent temperature stability, you can be confident of the highest accuracy and precision for your molecular weight determinations.

The Agilent PL-GPC 50 Integrated GPC/SEC System is a standalone instrument containing all the components necessary for the analysis of a wide range of polymers. With pump, injection valve, column oven and optional degasser, as well as any combination of refractive index, light scattering and viscometry detectors, the PL-GPC 50 is an ideal choice when you are starting out in GPC or want the convenience of a single solution.

Agilent BioHPLC columns

Agilent also carries a full range of Bio SEC columns for analytical separation of proteins, peptides, and oligonucleotides. The Bio SEC materials utilize silica particles with a proprietary hydrophilic coating to provide reproducible, reliable separations based on size. The range of pore sizes enables high efficiency separations from small peptides and oligonucleotides through to large proteins and aggregates. For more information go to **www.agilent.com/chem/BioHPLC**

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