

Achieving the best GPC/SEC analysis

# Gel Permeation Chromatography and Size Exclusion Chromatography Reference Guide

GPC and SEC are liquid chromatography techniques that separate individual polymer chains on the basis of their size in solution.

Using GPC and SEC, you can measure 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/SEC is the *only* established method for obtaining a comprehensive understanding of a polymer's molecular weight distribution.

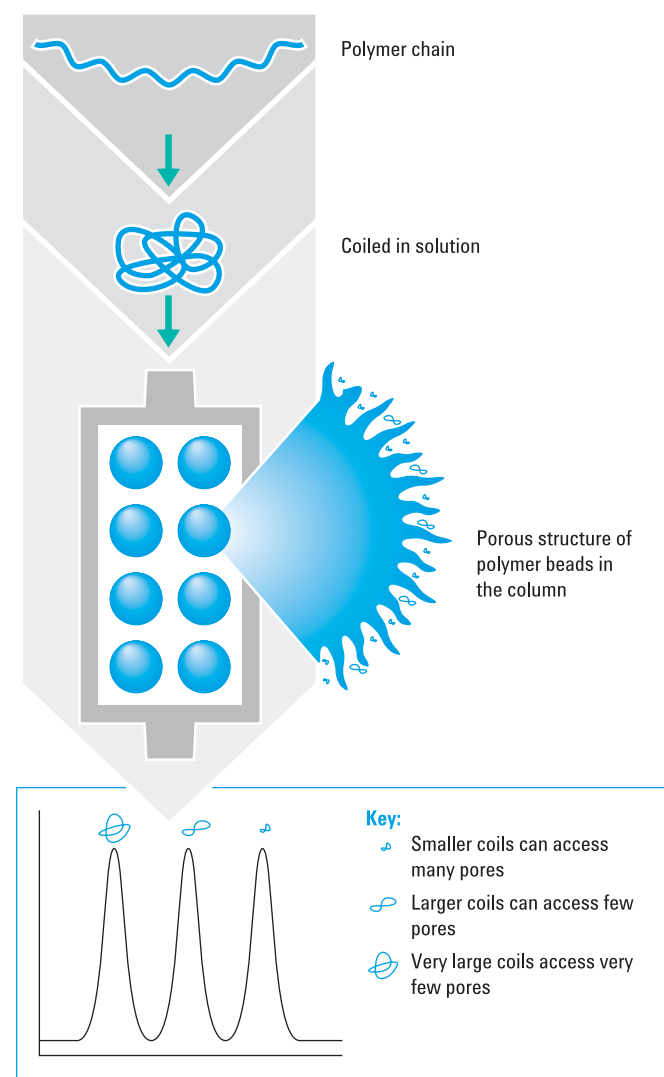
### We use...

- GPC to describe the analysis of polymers in organic solvents, such as tetrahydrofuran
- 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.

### Mechanism of Gel Permeation Chromatography/Size Exclusion Chromatography

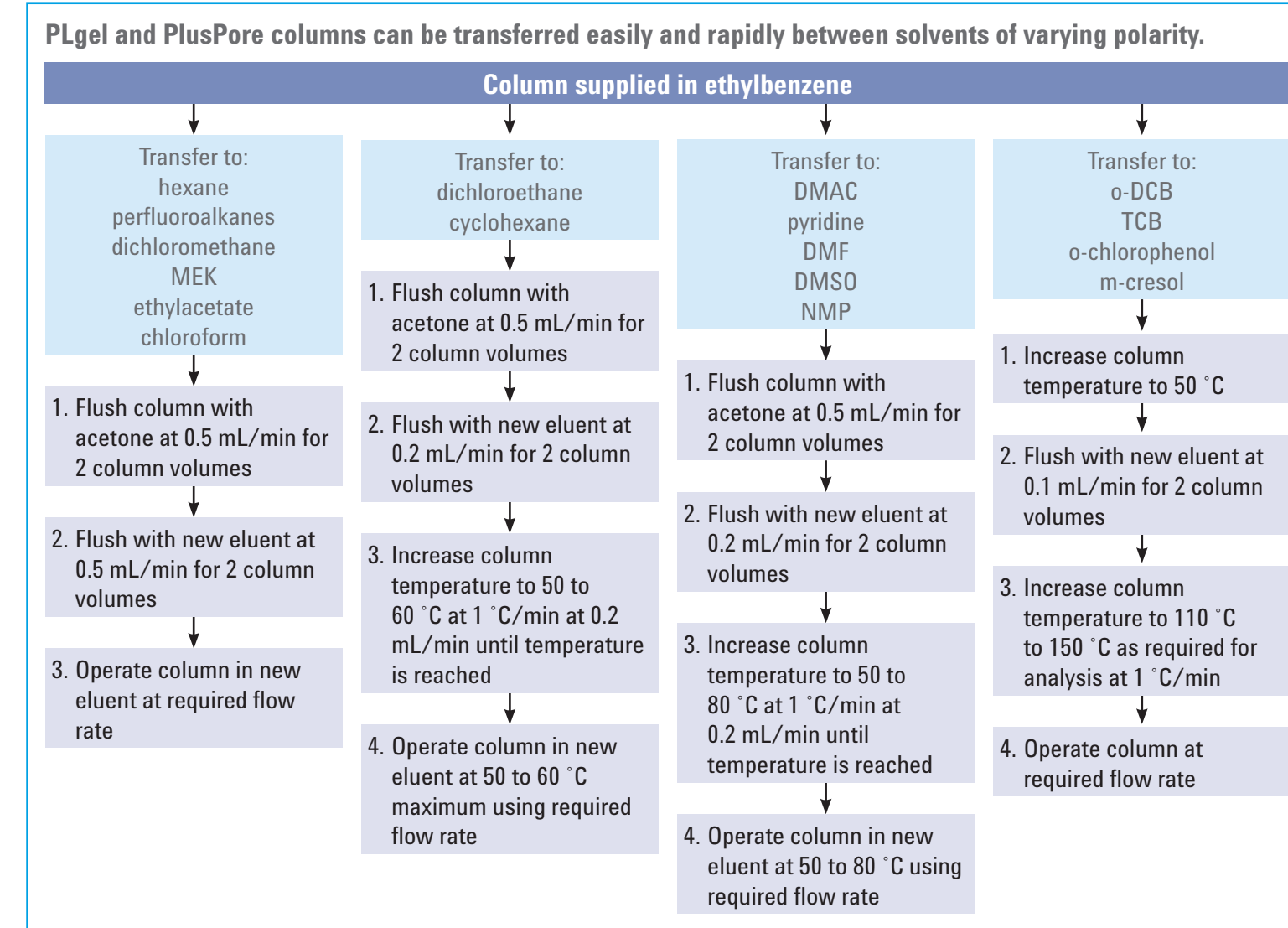
- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- Polymer coils introduced to eluent flowing through a column
- Column packed with insoluble porous beads with well-defined pore structure
- Size of pores similar to that of polymer coils
- Polymer 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



### Troubleshooting guidelines

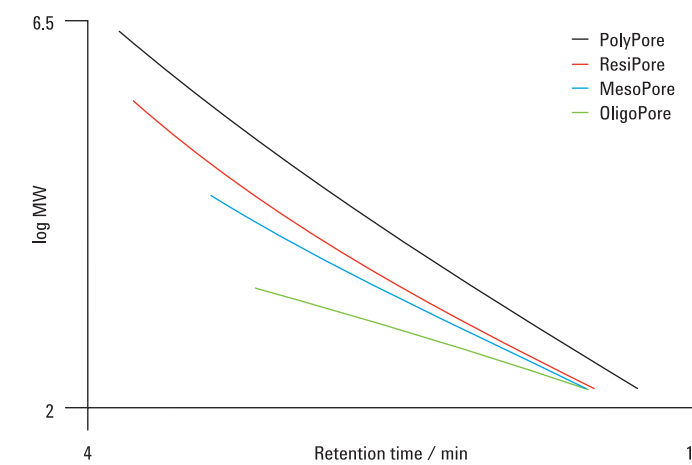
- Establish familiarity and good working-order parameters for all equipment
- Keep records of normal operating conditions and chromatograms
- Refer to manufacturer's handbook, call supplier for advice and discuss problems with other users
- Be methodical in solving problems by systematically removing items one at a time and only replacing with known working equipment
- Use a control sample to classify symptoms
- Anticipate problems with good system notes and maintenance records

### Eluent transfer guide for Agilent PLgel/Agilent PlusPore 7.5 mm ID column



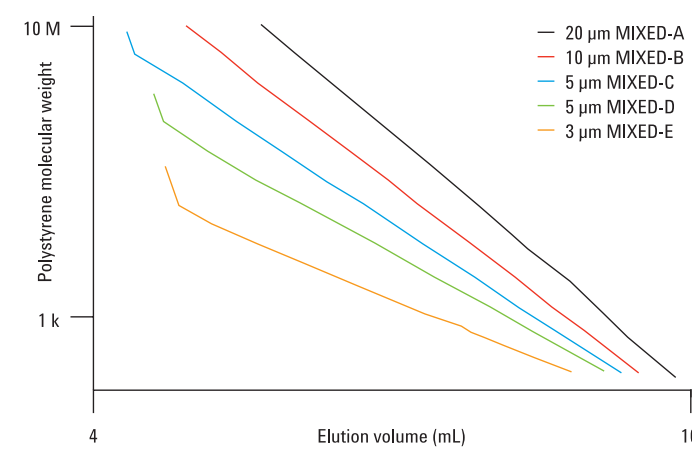
### Selected GPC/SEC column calibrations

PlusPore high resolution organic GPC columns



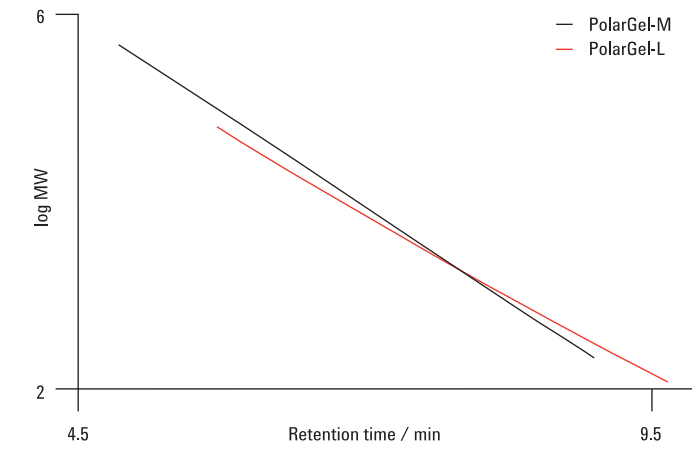
- High pore volume, high resolution
- Wide pore size distribution
- Optimized separation range
- Full solvent compatibility
- No MWD distortions

PLgel organic GPC columns



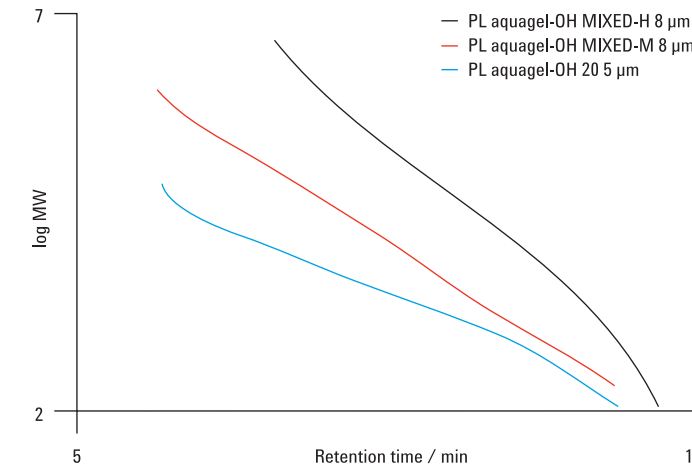
- Unequalled solvent compatibility for transfers between polar and non-polar organic eluents
- Outstanding physical rigidity for extended lifetimes especially at high temperatures and in aggressive solvents
- PLgel columns can be used at temperatures up to 220 °C and operating pressures up to 150 bar (2200 psi)

Agilent PolarGel columns for the analysis of polymers soluble in polar solvents

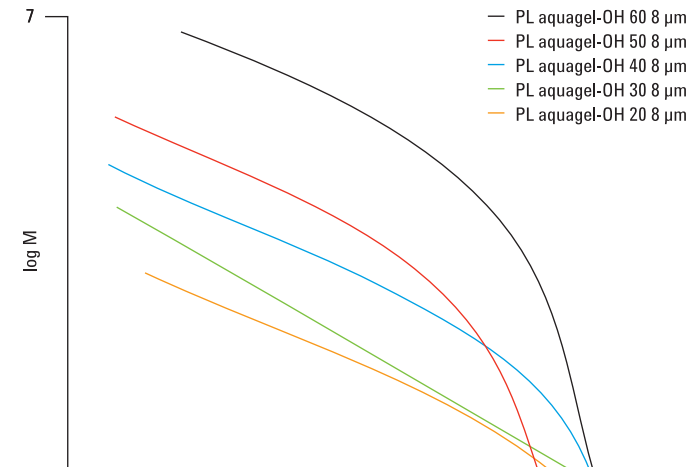


- Packed with 8 µm particles, PolarGel-M resolves up to 1,000,000 g/mol (PEG/PEO in water) and PolarGel-L up to 30,000 g/mol (PEG/PEO in water)
- Balanced hydrophobic and hydrophilic surfaces for polar compound selectivity
- No interaction between sample and stationary phase, ensuring accurate measurement of molecular weights
- Low swell and high mechanical stability ensure longer column lifetimes

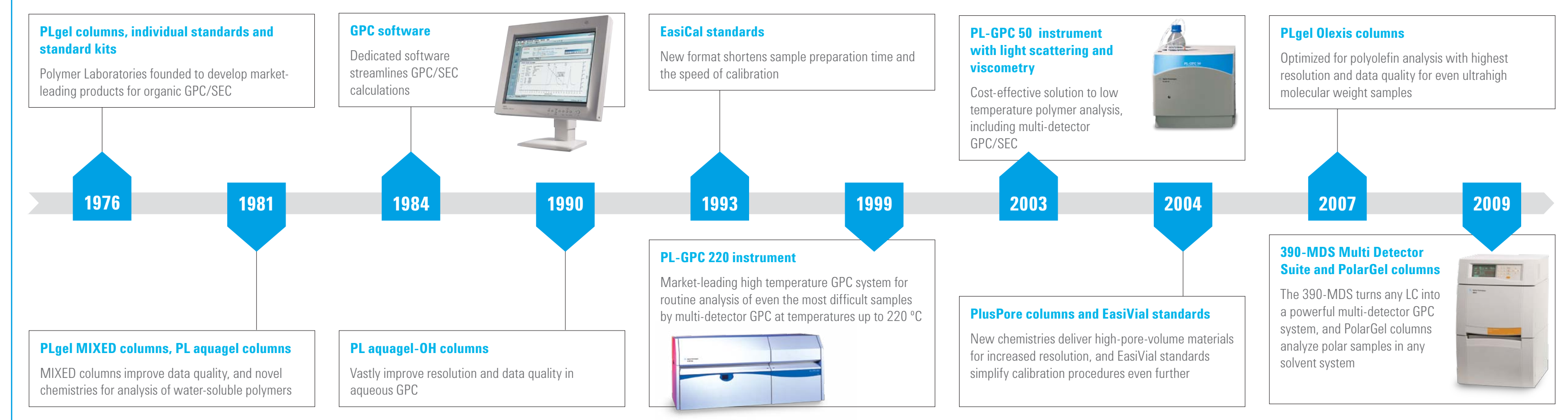
Agilent PL aquagel-OH aqueous SEC columns



- pH range 2 – 10
- Compatible with organic solvent, up to 50% methanol
- Mechanical stability up to 140 bar (2000 psi)
- Low column operating pressures
- 8 µm columns > 35,000 plates/m
- 15 µm columns > 15,000 plates/m



## Over 30 years' experience in GPC/SEC



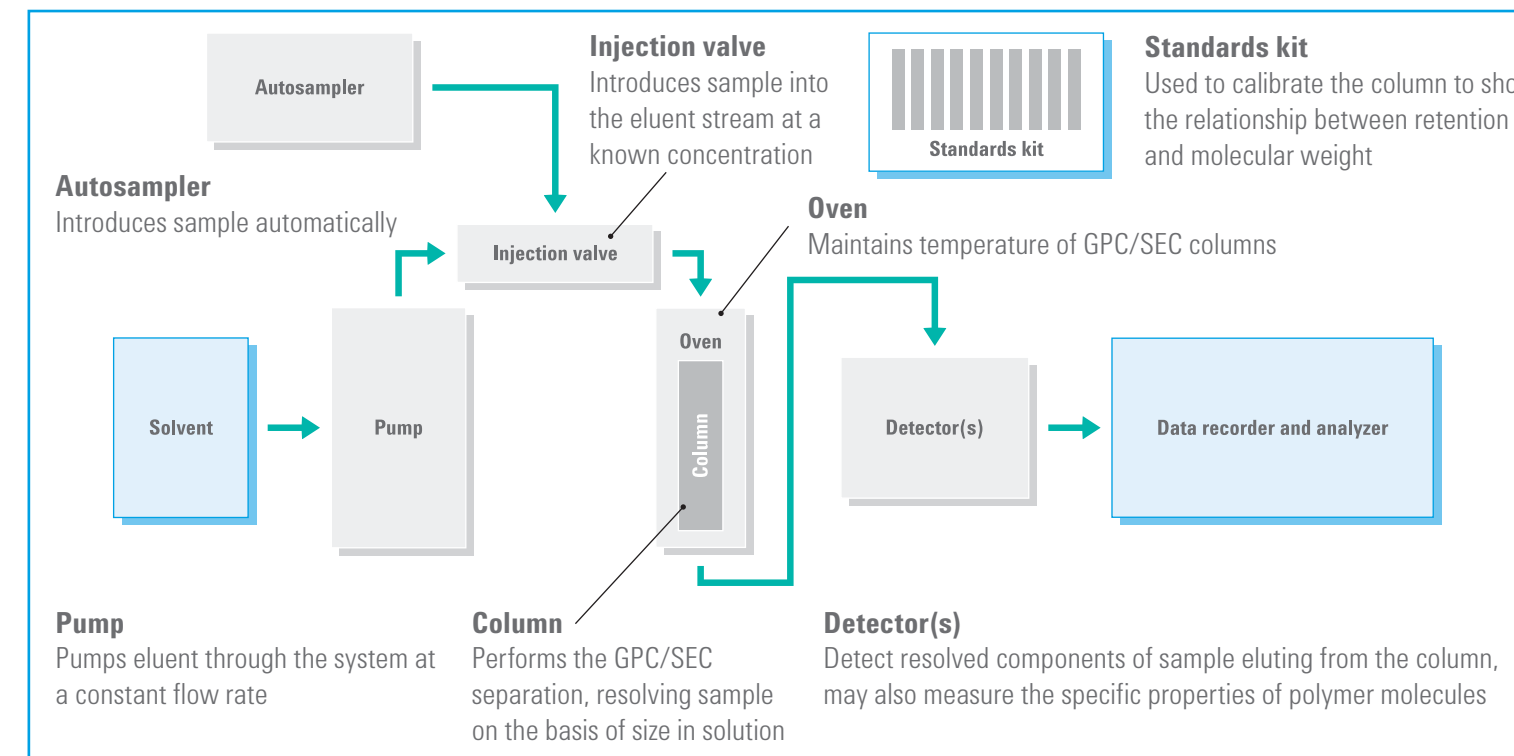
### Specialist detectors for GPC/SEC

Detectors	Use	Features
Light scattering detectors	Measure accurate molecular weights, molecular sizes and polymer branching	No column calibration required. Independent of polymer chemistry. Sensitive to high molecular weight
Viscometers	Measure accurate molecular weights, molecular viscosities (related to density), and polymer branching	Independent of polymer chemistry. Sensitive to high molecular weight. Wide molecular weight range. Simple to use
Refractive index detectors	Measure polymer concentrations	A 'universal' detector suitable for most polymers. Combines well with light scattering and viscometry
UV/Vis detectors	Measure polymer concentrations	Suitable for polymers with chromophores, very sensitive

### Typical analysis conditions for common polymers

Polymer sample	Suitable GPC/SEC solvent	Typical analysis temperature	Suitable GPC/SEC standards
Natural and synthetic rubbers, polydimethyl siloxane, polyethylene wax, polyethylene copolymers with under 10% ethylene	Toluene	Ambient to 80 °C	Polystyrene
High density, low density, linear low density and ultra high molecular weight polyethylene, polyethylene copolymers with over 10% ethylene, polypropylene, general polyolefins	Trichlorobenzene or o-dichlorobenzene	140 to 160 °C	Polystyrene or polyethylene
Polycarbonate, polylactide, polyglycolide and copolymers	Tetrahydrofuran or chloroform	Ambient to 40 °C	Polystyrene or polymethyl methacrylate
Styrenic polymers and copolymers, acrylic polymers and copolymers, methacrylic polymers and copolymers, vinyl polymers and copolymers, low polarity condensation resins, alkyl resins, epoxy resins, polyesters, polyesteramide, polyurethanes, polyols, polyvinyl chloride, asphalt, hydrocarbons, polyvinyl alcohol (partially hydrolyzed), polyvinyl acetate	Tetrahydrofuran	Ambient to 40 °C	Polystyrene or polymethyl methacrylate
Polyesters, polyamides, nylons	Hexafluoroisopropanol	40 °C	Polymethyl methacrylate
Polar condensation resins, phenol-formaldehyde resins, urea-aldehyde resins, melamine resins, formaldehyde-based resins, cellulose derivatives	Dimethylformamide or n-methyl pyrrolidone or dimethyl sulfoxide + 0.1% LiBr	50 to 80 °C	Polymethyl methacrylate or polyethylene glycol/oxide
Polyethylene glycol/oxide, low molecular weight starches, low molecular weight cellulose, polysaccharides, chitosan, dextran, pectin, polyvinyl alcohol, gums (guar, arabic), polyacrylamide	Water/buffer pH 2-9	Ambient to 40 °C	Polyethylene glycol/oxide or polysaccharide
Starches	Dimethyl sulfoxide + 0.1% LiBr	50 to 80 °C	Polyethylene glycol/oxide or polysaccharide
Polyphenylene sulfide	o-chloronaphthalene	220 °C	Polystyrene
Lignin, PVP, cellulose, polyurethanes	Dimethyl acetamide + 0.1% LiBr	50 to 80 °C	Polysaccharide

### Components of a GPC/SEC system



### Agilent Bio SEC columns for high resolution, size-based separations of biomolecules

#### Agilent Bio SEC-3 HPLC columns – for fast size separations with good recovery

With a 3 µm particle size and three pore sizes, 100Å, 150Å and 300Å, high efficiency separations are achieved for most peptides and proteins. The additional separation power of a 3 µm particle makes these columns ideal for QC applications where quantitation of aggregation levels is required.

#### Agilent Bio SEC-5 HPLC columns – for improved recovery for a wide range of molecular sizes

With a larger range of pore sizes, 100Å, 150Å, 300Å, 500Å, 1000Å and 2000Å, the Agilent Bio SEC-5 columns provide good recovery and column lifetime when characterizing bio-molecules over a broad size range.

### Calibration standards

To determine the sizes of the polymer components, a calibration must first be generated from polymer standards of a known size. Values from the unknown sample are then compared against this calibration to calculate the polymer size of the sample. Polymer standards are available in a wide range of molecular weights, in various kit formats and as individual molecular weights for maximum choice.

Agilent manufactures traditional 10-individual molecular weight kits and a time-saving range of EasiVial and EasiCal kits. EasiVial kits contain three vials, each containing a mixture of four accurately pre-weighed polymer standards to provide a 12-point calibration. Since the mass of every polymer is known, adding a fixed volume of eluent results in an accurate and precise solution ready for injection. EasiVial kits are available as polystyrene, polymethyl methacrylate and polyethylene glycol/oxide standards.

EasiCal is a pre-prepared calibration kit, consisting of two different combs, each with ten detachable spatulas supporting a mixture of five polymer standards. The thin film of polystyrene on the tip of the spatulas rapidly dissolves to provide two calibration solutions. To use EasiCal standards, remove a spatula from each comb and place it in a vial, then add the appropriate volume of solvent and allow to dissolve. Inject once from each vial to generate a 10-point calibration.

Learn more:  
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