PL aquagel-OH MIXED Columns

FOR THE SEC ANALYSIS OF BROAD WATER-SOLUBLE POLYMERS

Aqueous size exclusion chromatography is used to determine molecular weight distributions of a variety of synthetic and naturally occurring water-soluble polymers. PL aquagel-OH columns are packed with a macroporous copolymer bead with an extremely hydrophilic polyhydroxyl functionality. The 'neutral' surface and the capability to operate across a wide range of eluent conditions provides high performance analyses.

PL aquagel-OH MIXED bed columns are packed with a combination of individual pore size materials blended to give a linear calibration over a set range.

This datasheet describes the columns available in the recently expanded PL aquagel-OH range of MIXED-bed columns, the PL aquagel-OH MIXED-H 8 µm column which resolves from 100-10,000,000 g/mol and the PL aquagel-OH MIXED-M 8 µm column which resolves up to 600,000 g/mol.

Key benefits include:

- **Simple column selection.** The resolving ranges of the two MIXED bed columns have been selected to suit typical applications. The PL aquagel-OH MIXED-H 8 µm column is suited to naturally occurring biopolymers such as starch and dextran, whereas the PL aquagel-OH MIXED-M 8 µm column is designed for polymers such as polyethylene glycols and oxides used in drug delivery. Matching resolving ranges to application areas simplifies column selection and ensures the highest resolution is obtained for an analysis.

- **Linear resolving range.** Blending individual components together produces PL aquagel-OH MIXED bed columns. The blending process is performed to ensure the most linear column calibration is obtained. The resulting linear calibration gives equal resolution across the resolving range, giving the best quality and most reliable results for the sample under analysis.

- **Dislocation free.** The PL aquagel-OH MIXED bed columns are produced such that artefacts from the crossover of calibration curves of the components used in the MIXED blend do not occur. As a result, no dislocation artefacts are seen on chromatograms, ensuring an accurate reflection of the sample is seen in the results.

- **Easy extrapolations.** In aqueous GPC it is common to extrapolate calibration curves to the exclusion limit of the column due to the limited molecular weight range of narrow standard calibrants available for use in water. With a linear calibration, extrapolation is straightforward, allowing the higher molecular weight resolving regions of the columns to be used without introducing errors into the analysis.

- **A quality product from one of the market leaders.** PL aquagel-OH MIXED bed columns come from one of the market leaders in size exclusion chromatography. With a reputation for quality and performance, users of the columns can expect only the best from their column set.

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PL aquagel-OH MIXED Columns

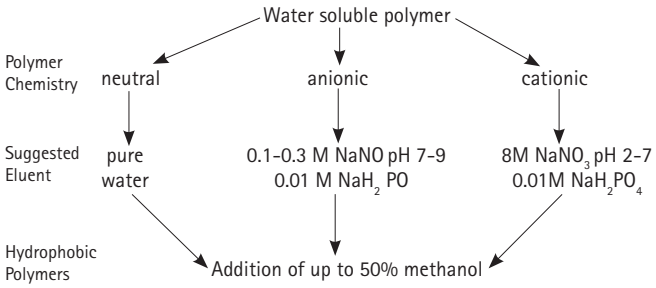
Features of the PL aquagel-OH range

- Compatible with eluent pH range of 2-10
- Compatible with organic solvent, up to 50% methanol by volume
- Mechanically stable up to 140 bar (2000 psi)
- Low column operating pressures
- 8 µm columns give plate counts of > 35,000 plates/m

Eluent selection guide

Like all PL aquagel-OH columns, the MIXED bed columns can be used in water, buffers and in salt solutions with up to 50% methanol by volume. The following solvent selection guide can be used to help select the correct eluent for an application.

Guide to eluent selection for PL aquagel-OH applications:



PL aquagel-OH MIXED calibration curves

PL aquagel-OH MIXED bed columns can be calibrated with polyethylene glycol/oxide standards. The following calibration curves in water illustrate the linearity and the resolving ranges of the two MIXED bed columns.

Conditions

Column: PL aquagel-OH MIXED-H 8 µm or PL aquagel-OH MIXED-M 8 µm (300 x 7.5 mm)
Eluent: Water
Flow rate: 1.0 mL/min
Detector: PL-GPC 50 Plus differential refractive index
Injection loop: 200 µL

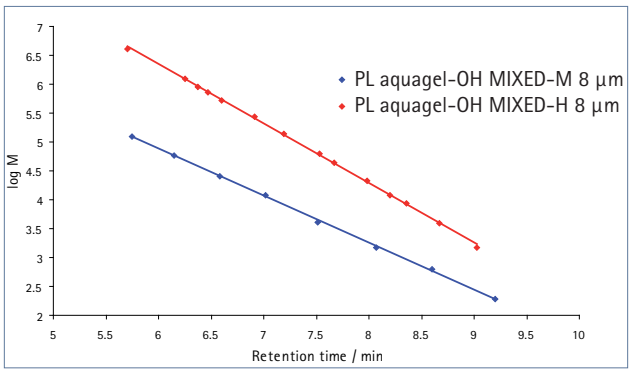


Figure 1. Overlaid calibration curve of the PL aquagel-OH MIXED bed columns showing their excellent linearity.

Applications on PL aquagel-OH MIXED columns

The following applications on the PL aquagel-OH MIXED columns illustrate data that may be obtained using these columns.

Analysis of Hyaluronic Acid on PL aquagel-OH MIXED-M 8 µm Columns

Hyaluronic acid is a high molecular weight linear polymer with repeating disaccharide units, each containing one residue of D-glucuronic acid and N-acetyl-D-glucosamine linked by glycosidic bonds. Hyaluronic acid has unique rheological properties because of its polyanionic character and stiffness in the chains. Commercial preparations of hyaluronic acid are often isolated from the intercellular matrix of animal connective tissues or fermented from bacteria, with the rooster comb the most common source. Hyaluronic acid is used in pharmaceutical products such as viscoelastic fluids in ophthalmological surgery and in viscosupplementary products for orthopaedic disorders. The application and effectiveness of hyaluronic acid is significantly affected by its molecular weight and molecular weight distribution, which can be readily determined by aqueous SEC. A set of two PL aquagel-OH MIXED-M 8 µm columns is ideal for this application as the material has a broad distribution. Obtaining an accurate reflection of the peak shape is important as even small changes at high molecular weight strongly influence the material's physical properties. A sample of hyaluronic acid was dissolved in the eluent and analyzed as follows:

Conditions

Columns: 2 x PL aquagel-OH MIXED-M 8 µm, 300 x 7.5 mm
Eluent: 0.2M $NaNO_3$, 0.01M NaH_2PO_4, pH 7
Flow Rate: 1.0 mL/min
Detector: PL-GPC 50 differential refractive index

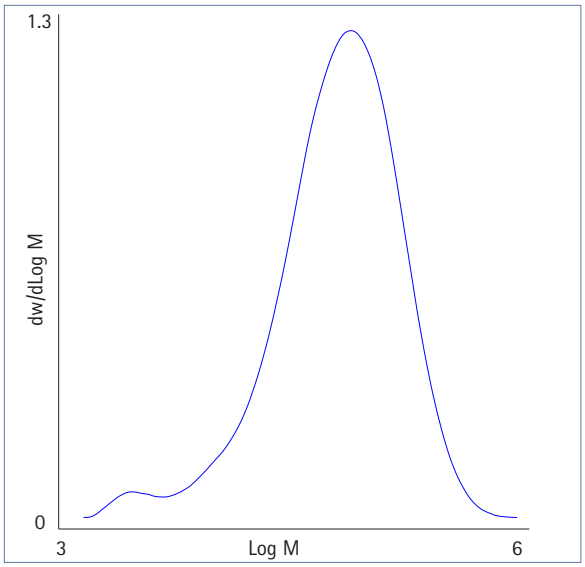


Figure 2. Molecular weight distribution of hyaluronic acid, showing the smooth peak shapes obtained on PL aquagel-OH MIXED-M 8 µm columns.

Analysis of Pectin on PL aquagel-OH MIXED-H 8 µm Columns

Pectin is a natural product used extensively as a jellifying, thickening and stabilizing agent in the food industry. Pectin is produced from plants such as apple, citrus and beet. Extracts from these foodstuffs are processed to produce pectins with specific properties. Although the chemical composition of pectin is key to its final application, the rheological behavior is also critical to performance. The determination of the molecular weight distribution can help to predict rheological behavior. PL aquagel-OH MIXED-H 8 µm columns with their wide molecular weight resolving range and high efficiency are the columns of choice for this application.

Four samples of pectin for comparison were prepared at 2 mg/mL, left to fully dissolve overnight and filtered over a 0.45 µm membrane prior to injection.

Conditions

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm
Eluent: 0.2M $NaNO_3$, 0.01M NaH_2PO_4, pH 7
Flow Rate: 1.0 mL/min
Detector: PL-GPC 50 differential refractive index

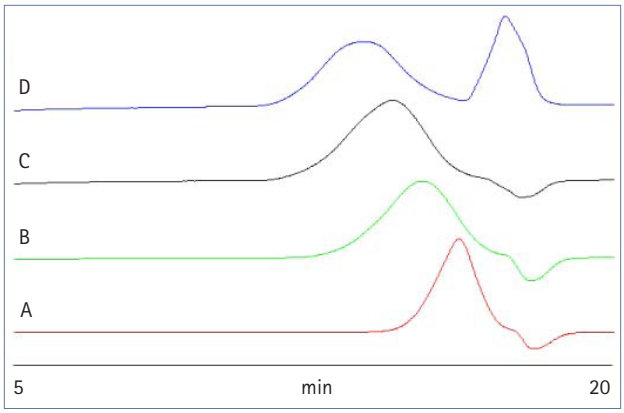


Figure 3. Chromatograms of four samples of pectin; the wide resolving range of the PL aquagel-OH MIXED-H 8 µm columns allows the samples to be easily distinguished.

Analysis of Chitosan on PL aquagel-OH MIXED-H 8 µm Columns

Chitosan is a naturally occurring polysaccharide. The term chitosan does not refer to a uniquely defined compound, but merely refers to a family of copolymers with various fractions of acytylated chitin units. Application areas of chitosan include biomedical (e.g. wound healing, burn treatment and use as a hemostatic agent), paper production, textile finishes, photographic products, cements, heavy metal chelating agents and waste removal. GPC/SEC can be used as a quality control tool for the determination of the molecular weight distribution, with different molecular weights appropriate to particular applications. Three grades of chitosan were analyzed using a column set comprising 2 x PL aquagel-OH MIXED-H 8 µm columns. These columns offer resolution over a wide molecular weight range and are therefore well-suited to this application.

Due to the cationic nature of the samples, they were prepared in strong acid and were allowed to stand overnight to aid dissolution. They were analyzed in 0.5 M sodium nitrate buffer and at low pH.

Conditions

Columns: 2 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm
Eluent: 0.5M $NaNO_3$, 0.01M NaH_2PO_4, pH 2
Flow Rate: 1.0 mL/min
Detector: PL-GPC 50 differential refractive index

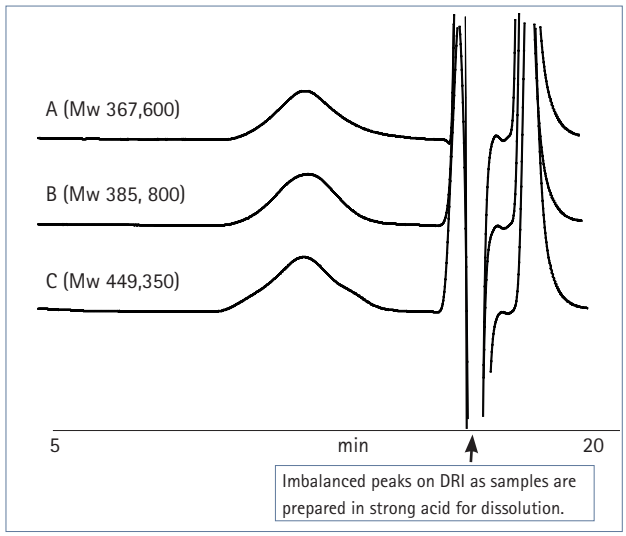


Figure 3. Chromatograms of the three samples of chitosan showing imbalanced peaks due to the sample preparation - the resolution of the PL aquagel-OH MIXED-H 8 µm column set is sufficient to see small differences in the molecular weight distributions.

Analysis of Polystyrene Sulphonates on PL aquagel-OH MIXED-H 8 µm Columns

Polystyrene sulfonate standards are amongst very few water soluble standards that also contain a chromophore. They can therefore be used with both RI and UV detection. To counteract ionic interactions between the sample and the column packing, the eluent must be modified by the addition of salt. Also, due to the hydrophobic nature of these samples, the eluent must be further modified by the addition of a weak organic solvent (methanol). The excellent stability of the PL aquagel-OH packing material allows such modifications while retaining the high column efficiency (>35,000 plates/meter).

A mixture of polystyrene sulfonate standards were analyzed that explored the wide resolving range of the PL aquagel-OH MIXED-H 8 µm columns under the following conditions:

Conditions

Columns: 3 x PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm
Eluent: 0.2M $NaNO_3$, 0.01M NaH_2PO_4, pH 7 + 30% Methanol
Flow Rate: 1.0 mL/min
Detector: PL-GPC 50 differential refractive index

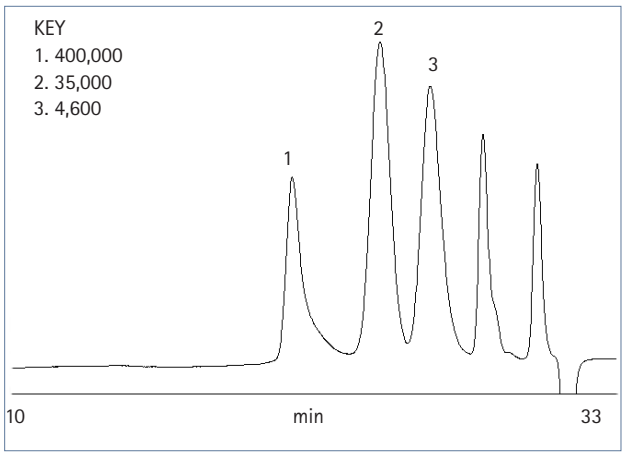


Figure 4. Chromatograms of polystyrene sulfonate standards showing the excellent resolution of the PL aquagel-OH MIXED-H 8 µm column over a broad molecular weight range.

Ordering Information

Description	Part number
PL aquagel-OH 10 8 µm, 300 x 7.5 mm	PL1149-6810
PL aquagel-OH 20 5 µm, 300 x 7.5 mm	PL1120-6520
PL aquagel-OH 20 8 µm, 300 x 7.5 mm	PL1149-6820
PL aquagel-OH 30 8 µm, 300 x 7.5 mm	PL1120-6830
PL aquagel-OH 40 8 µm, 300 x 7.5 mm	PL1149-6840
PL aquagel-OH 50 8 µm, 300 x 7.5 mm	PL1149-6850
PL aquagel-OH 60 8 µm, 300 x 7.5 mm	PL1149-6860
PL aquagel-OH MIXED-H 8 µm, 300 x 7.5 mm	PL1149-6800
PL aquagel-OH MIXED-M 8 µm, 300 x 7.5 mm	PL1149-6801
PL aquagel-OH 40 15 µm, 300 x 7.5 mm	PL1149-6240
PL aquagel-OH 50 15 µm, 300 x 7.5 mm	PL1149-6250
PL aquagel-OH 60 15 µm, 300 x 7.5 mm	PL1149-6260
PL aquagel-OH 5 µm Column Repair Gel	PL1449-0520
PL aquagel-OH 8 µm Column Repair Gel	PL1449-0801
PL aquagel-OH 15 µm Column Repair Gel	PL1449-0201
PL aquagel-OH Guard 8 µm, 50 x 7.5 mm	PL1149-1840
PL aquagel-OH Guard 15 µm, 50 x 7.5 mm	PL1149-1240

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