Optimizing Gel Permeation Chromatography (GPC) using Phenogel™ Columns

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Gel Permeation Chromatography (GPC) is principally used to separate polymers and organic molecules based on their size. An important aspect in optimizing GPC separations is column selection, especially when multiple columns are used. Mobile phase selection and column temperature are also important factors that must be considered when developing a method, as they can influence the solubility and viscosity of diluent and analyte respectively. Column, mobile phase, and temperature selection will be discussed to optimize GPC methods using Phenogel GPC/SEC columns.

Introduction

GPC is one of the older "modern" chromatography methods with many of the methodologies and separation columns developed in the 1970's and 1980's. GPC is a type of size exclusion chromatography (SEC) where analytes are separated based on differences in their size in solution. Since the molecular weight of an analyte is typically directly proportional to its size in solution, SEC is often used as a method for estimating the molecular weight of an analyte. One type of SEC is gel filtration chromatography (GFC) where the separation method is used on proteins and biomolecules using an aqueous mobile phase. In the case of GPC, the separation method is performed using organic solvents as a mobile phase, and the HPLC columns employed typically are packed with porous polystyrene-divinylbenzene (PSDVB) particles.

The analytes separated by GPC are typically organic polymers, though the smaller pore size columns are also used to separate small molecules. Unlike reversed phase or other separation modes where separation is based on interaction with the particle surface, GPC separation of analytes is based mostly on their ability to permeate the porous particle; large analytes are fully excluded from the pores and elute first, followed by smaller analytes. The smallest analytes are able to permeate all the pores and thus elute last in the separation. Establishing the effective molecular weight range of a separation is based on the pore size of the column (or columns) used for a separation. Because backpressures are not typically an issue with GPC columns (because organic solvent is used for mobile phase), often several columns are used in series to increase the resolution or increase the molecular weight (MW) range of a separation. Optimizing such separation ranges and the choice of single pore size media versus the use of wide range (linear) columns will be discussed. In addition, the choice of mobile phase solvent can be critical when polymer solubility is in question; temperature is also used to address solubility issues as well as to match viscosity between diluent and mobile phase.

Results and Discussion

Pore Size

To develop any new polymer GPC separation method one must first have an idea of the separation range that they are addressing. All analytes larger than the pore size of a column will be equally excluded and elute as a single peak; similarly, all analytes that fully permeate the pores of a column will elute at the same time. It is only within a certain molecular weight range that analytes are separated, and that is column specific (**Figures 1 and 2**).

Figure 1.
Theoretical separation plot of a GPC column. Components D and E (between the total excluded and included size of a particular GPC column) will be separated by differences in their molecular weight. Components C and F (fully excluded and included, respectively) will likely be a mixture of multiple components. Reprinted with permission.[1]

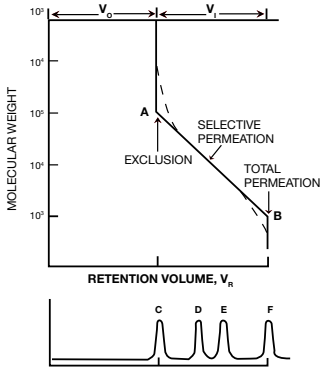
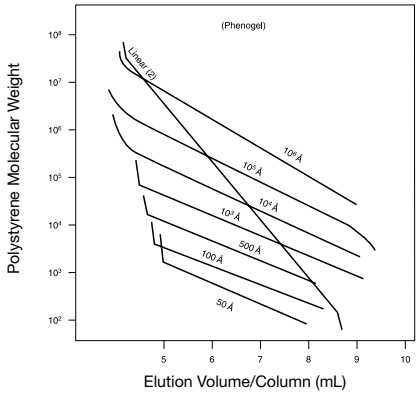


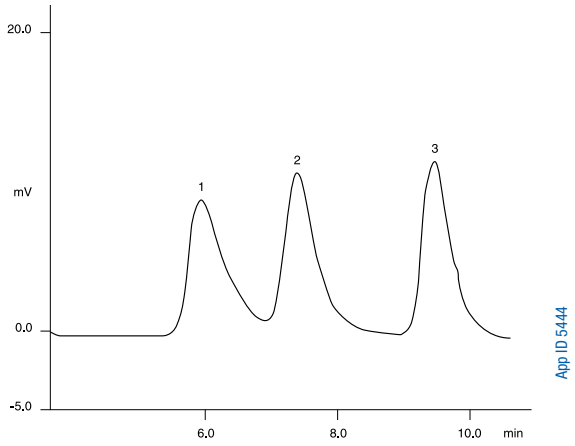
Figure 2.
Calibration MW curves of different Phenogel columns. Note that the different pore size columns give the highest resolution for a particular MW range. The mixed bed linear has the widest MW range.



For separation where a wide range of MW analytes are being separated, often a linear column can be used (**Figure 3**). Linear columns are a controlled mixture of different pore sized media designed to give the widest separation window. While linear columns are ideal for cases where the molecular weight is not known, a wide separation window comes at the cost of lower resolution of specific MW ranges. Adding additional linear columns in series will increase the

resolution of the entire MW separation and not specific MW ranges. For improved resolution of a particular MW range, a specific pore size column or a combination of adjacent pore columns should be used.

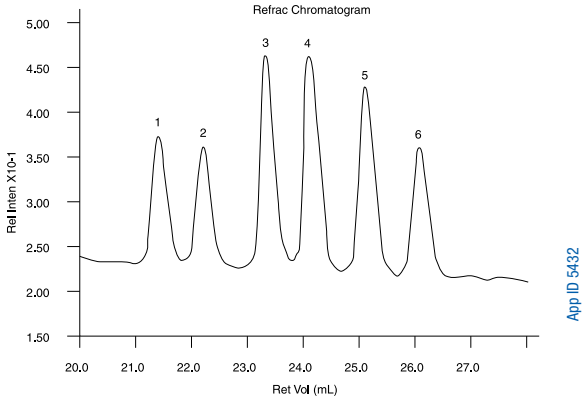
Figure 3.
Polystyrene standards separated on a Phenogel Linear (2) column (300 x 7.8 mm). Note that the Phenogel Linear column can separate very wide MW ranges of polymers. For higher resolution of a particular MW range, individual pore size columns (or columns placed in series) should be used.



Column: Phenogel 5 µm Linear(2)
Dimensions: 300 x 7.8 mm
Part No.: 00H-3259-K0
Mobile Phase: THF
Flow Rate: 1.0 mL/min
Detection: RI
Injection Volume: 50 µL
Temperature: 35 °C
Sample: Polystyrene standards injected
1. 2,860,000 MW
2. 277,000 MW
3. 9,350 MW

Using multiple columns of the same pore size or adjacent pore sizes is the better solution for situations where increased resolution of a particular MW range is needed. If a narrow MW range is needed, multiple columns (or a longer column) of the same pore size can be used to increase resolution between analytes. In most cases combining adjacent pore sized columns is the best solution where the MW separation range is slightly widened while increasing the separation window. An example of this is shown in **Figure 4** where long-chain hydrocarbons are well resolved by combining a 50 Å, 100 Å, and 500 Å in series. The typical convention for combining different pore-sized columns has one go from smaller pore to larger pore column in series, however in some cases the opposite orientation may give better results so it is often best to test both directions.

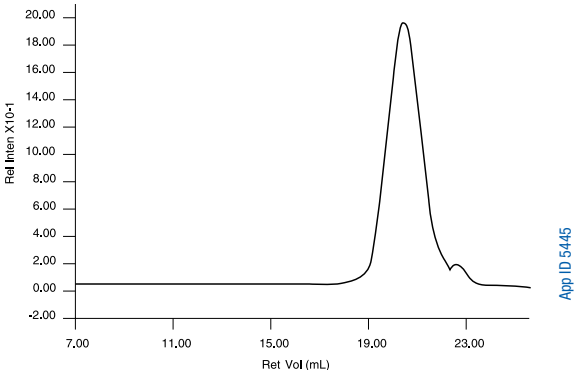
Figure 4.
Long-chain hydrocarbons separated on a 50 Å, 100 Å, and 500 Å Phenogel column attached in series. Note the excellent resolution across the narrow MW range that the series of fixed-pore columns provide for closely related hydrocarbons.



Column: Phenogel 5 µm 50 Å, 100 Å, 500 Å
Dimensions: 300 x 7.8 mm
Part No.: 00H-0441-K0 (50 Å)
00H-0442-K0 (100 Å)
00H-0443-K0 (500 Å)
Mobile Phase: THF
Flow Rate: 1.0 mL/min
Detection: Differential Refractometer
Injection Volume: 100 µL 0.25 % w/v
Temperature: Ambient
Sample:
1. C40 562 MW
2. C32 450 MW
3. C24 338 MW
4. C20 282 MW
5. C16 226 MW
6. C13 184 MW

Solvent and Temperature
While column selection is probably the more important aspect of method development for GPC methods, mobile phase and column temperature can have significant roles in obtaining the maximum resolution of a separation. Because many polymers demonstrate poor solubility in certain solvents, one must first verify analyte solubility in the chosen column mobile phase. Often THF is substituted with DMF or HFIP for certain polymers with limited solubility even though MW standards often deliver better performance using THF as a mobile phase. **Figure 5** is an example where a different solvent is used. Nylon is not soluble in a majority of organic solvents; HFIP is used as a mobile phase because it can solubilize nylon. A slightly elevated temperature (30 °C) is used to better match viscosity differences between the diluent and mobile phase. Because column interactions are minimized in GPC, any differences in viscosity between the diluent and mobile phase can lead to band broadening and peak distortion. Elevating the column temperature can help minimize such distortions; however, one must be careful to take into account the boiling point of the solvent to avoid outgassing; staying 50 °C below the boiling point of a solvent is a good idea. Finally, the use of a salt that is soluble in organic solvent can help minimize secondary interactions between the column and analytes; lithium bromide is sometimes used to minimize such interactions as well as break up aggregates.

Figure 5.
Separation of nylon on a Phenogel Linear column. HFIP is used as a mobile phase. Different mobile phase solvents must sometimes be used to properly solubilize an analyte. Temperature should also be used to minimize differences between diluent and mobile phase viscosity.



Column: Phenogel 10 µm Linear(2) x 2
Dimensions: 300 x 7.8 mm
Part No.: 00H-3260-K0
Mobile Phase.: HFIP (0.01 M NATFAT)
Flow Rate: 1.8 mL/min
Detection: Differential Refractometer
Injection Volume: 100 µL 0.25 % w/v
Temperature: 30 °C
Sample: Nylon 6
14,500 MW

Conclusion

GPC is a separation method that has unique rules for optimizing a separation. Column type (mixed bed or fixed-pore) as well as pore size is the primary method for optimizing a separation method. For wide MW range separations “mixed bed” Phenogel Linear columns should be used. For more defined MW ranges one can use a combination of fixed-pore columns to optimize the resolution for a particular analyte mixture. While the pore size of the column plays the biggest role in the selectivity of a GPC method, the solvent used can also be important. Using a mobile phase that analytes are soluble in is key. Finally, column temperature is used to minimize viscosity differences between the mobile phase and diluent, which can perturb peaks shapes and result in lower resolution.

References

1. L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2nd Ed., Wiley-Interscience, New York, 1979

APPLICATIONS

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Ordering Information

5 µm Columns (mm)

					Guards
		300 x 7.8	600 x 7.8	300 x 21.2	50 x 7.8
Pore Size	**MW Range**				
50 Å	100-3 K	00H-0441-K0	—	—	03B-2088-K0
100 Å	500-6 K	00H-0442-K0	00K-0442-K0	—	03B-2088-K0
500 Å	1 K-15 K	00H-0443-K0	—	—	03B-2088-K0
10^3 Å	1 K-75 K	00H-0444-K0	—	—	03B-2088-K0
10^4 Å	5 K-500 K	00H-0445-K0	00K-0445-K0	00H-0445-P0	03B-2088-K0
10^5 Å	10 K-1,000 K	00H-0446-K0	00K-0446-K0	00H-0446-P0	03B-2088-K0
10^6 Å	60 K-10,000 K	00H-0447-K0	—	00H-0447-P0	03B-2088-K0
		300 x 7.8	600 x 7.8	—	50 x 7.8
Mixed Beds					
Linear(2)	100-10,000 K	00H-3259-K0	00K-3259-K0	—	03B-2088-K0

5 µm Narrow Bore (NB) Columns (mm)

			Guards
		300 x 4.6	30 x 4.6
Pore Size	**MW Range**		
50 Å	100-3 K	00H-0441-E0	03A-2088-E0
100 Å	500-6 K	00H-0442-E0	03A-2088-E0
500 Å	1K-15 K	00H-0443-E0	03A-2088-E0
10^3 Å	1K-75 K	00H-0444-E0	03A-2088-E0
10^4 Å	5K-500 K	00H-0445-E0	03A-2088-E0

10 µm Columns (mm)

						Guards
		300 x 7.8	600 x 7.8	300 x 21.2	600 x 21.2	50 x 7.8
Pore Size	**MW Range**					
50 Å	100-3 K	00H-0641-K0	00K-0641-K0	00H-0641-P0	00K-0641-P0	03B-2090-K0
100 Å	500-6 K	00H-0642-K0	00K-0642-K0	00H-0642-P0	00K-0642-P0	03B-2090-K0
500 Å	1 K-15 K	00H-0643-K0	00K-0643-K0	—	00K-0643-P0	03B-2090-K0
10^3 Å	1 K-75 K	00H-0644-K0	00K-0644-K0	00H-0644-P0	00K-0644-P0	03B-2090-K0
10^4 Å	5 K-500 K	00H-0645-K0	00K-0645-K0	00H-0645-P0	00K-0645-P0	03B-2090-K0
10^5 Å	10 K-1,000 K	00H-0646-K0	00K-0646-K0	00H-0646-P0	00K-0646-P0	03B-2090-K0
10^6 Å	60 K-10,000 K	00H-0647-K0	00K-0647-K0	00H-0647-P0	00K-0647-P0	03B-2090-K0
		300 x 7.8	600 x 7.8	300 x 21.2	—	50 x 7.8
Mixed Beds						
Linear(2)	100-10,000 K	00H-3260-K0	00K-3260-K0	00H-3260-P0	—	03B-2090-K0

Other Shipping Solvents:
Methanol, Methylene Chloride, Cyclohexane, Ethyl Acetate, NMP, DMAC, DMF
Size (mm)
30 x 4.6
50 x 4.6
300 x 4.6
300 x 7.8
600 x 7.8
300 x 21.2
600 x 21.2

NOTE: Phenogel columns are routinely shipped in THF. Columns can be shipped in Toluene and Chloroform upon request at no additional charge.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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