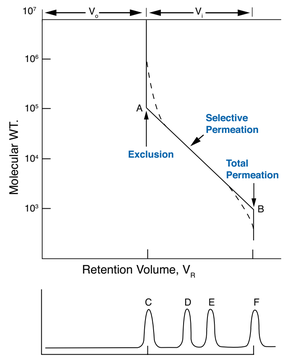
Introduction to GPC Method Development

Phenogel™
Non-Aqueous GPC/SEC Columns

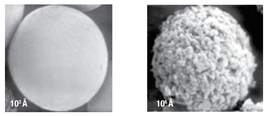
Understanding GPC

In **Gel Permeation Chromatography (GPC)**, molecules are separated based on their exclusion from the controlled pores in the packing material, which is usually determined by their molecular weight. GPC is operated with polymeric gel packing material with a non-aqueous mobile phase.

For an effective separation, the analytes should partially enter the pores of the packing material. If they are not able to enter the pores at all, they are completely excluded and elute first with V_0 as shown in peak C. The analytes that can fully enter the pores are totally permeated and will elute at the end of the chromatogram, as shown in peak F. All analytes that elute in between exclusion and total permeation are within the selective permeation where you can accurately qualify and quantify peaks.



SEM Photos of Phenogel Polymer Beads



Column Selection

The key to selecting a GPC column is the molecular weight range of the compounds being separated. Then the second decision weighs speed versus resolution. The following tables give you some guidance.

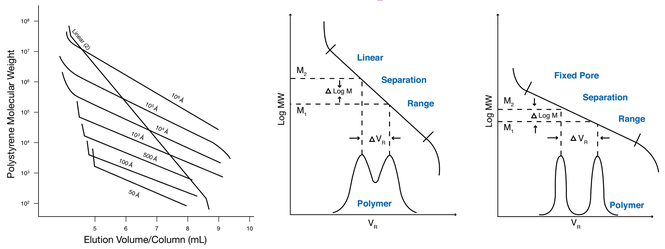
Aim	Mixed-Bed (Linear)	Fixed Pore
Maximum resolution		✓
Fast fingerprint over broad MW range	✓	
Known compound MW	✓	✓
Unknown compound MW	✓	

Sample Type	Molecular Weight	Phenogel Column
Small Organics	100 – 3 K 500 – 6 K 1 K – 15 K	50 Å 100 Å 500 Å
Resins	1 K – 75 K 5 K – 500 K 10 K – 1,000 K	10^3 Å 10^4 Å 10^5 Å
High MW Polymers	60 K – 10,000 K 100 – 10,000 K	10^6 Å Linear (2)

Effects of Coupling GPC Columns

Number of Analytical Columns	MW Range	Resolution	Run Time
1 Mixed-Bed Column	Broad	Moderate	Short
2 Mixed-Bed Columns	Broad	Good	Moderate
3 Mixed-Bed Columns	Broad	Better	Long
1 Fixed Pore Column	Narrow	Good	Short
2 Fixed Pore Columns (with different pore sizes)	Broad	Good	Moderate
3 Fixed Pore Columns (with different pore sizes)	Very Broad	Good	Long
2-3 Fixed Pore Columns (with same pore size)	Narrow	Better	Long

Calibration MW Curves of Different Phenogel Columns



ATTENTION
Never combine different mixed-bed (linear) columns. Never combine a mixed-bed column with a fixed pore column. When combining different fixed pore columns, ensure that their calibration curves have the same slope. Couple columns with either increasing pore size or decreasing pore size as long as the order is kept consistent.

Solvent Selection

The solvent selection needs to be determined by the following factors:

- *The analyte needs to be soluble in the solvent*
- *The stationary phase needs to be compatible with this solvent*
- *The solvent needs to ensure a secondary interaction-free, pure size exclusion separation*
- *To ensure that the viscosity of the mobile phase is not too high it is sometimes necessary to work at higher temperatures. In these cases ensure that the boiling point of the solvent is minimum 50 °C higher than the planned operating temperature*
- *Additives such as 0.1M Lithium Bromide are commonly used with polar solvents such as DMF, NMP, and DMAC to eliminate unwanted interactions with the analytes and mobile phase*

Recommended GPC Solvent	Sample	Suggested Temp.
THF	Polystyrenes Polybutadienes Epoxy Resins Phenolic Resins Polymethyl Methacrylates Polyethylene Glycol	Ambient Ambient
HFIP**	Polyamides (Nylon) PET/PETP (polyethyleneterephthalate)	30 °C
Dichloromethane	Naphthalene Diethylhexyl Phthalate	Ambient
Toluene	Polyisobutylene Polyisoprene Silicone Oils	Ambient
DMF	Polyethylene Oxide Polyvinylpyrrolidone (PVP) Cellulose Acetate Hydroxyethylcellulose (HEC) Chlorinated Rubber	50 °C 40 °C

**HFIP (hexafluoroisopropanol) allows polymers such as polyamides and PET that are analyzed at a temperature of 135 °C and higher to be analyzed at temperatures below 100 °C. The narrow particle distribution of Phenogel columns eliminates the problem of overlapping peaks and brand broadening that is typically associated with using this solvent with traditional GPC columns.

Reduced Solvent Consumption

Phenogel-Narrow Bore Columns: An Improved Dimension in GPC Analysis

- *Decrease solvent consumption*
- *Retain same elution profile*
- *Reduce solvent disposal costs*

Cut Waste!

Solvent Consumption

The Phenogel-NB columns have a 4.6 mm column ID and run at 0.35 mL/min, reducing solvent consumption and disposal costs up to 65 %!

ATTENTION
With narrow bore GPC/SEC columns, the volume in which the sample elutes is significantly decreased, thus increasing the effective concentration of the sample. In GPC this leads to overloading effects and proportionally lower sample loadings must be used.

Solvent Compatibility Chart

Mobile Phase Solvent	Phenogel Fixed Pore: 50 Å	100 Å	500 Å	10^3 Å	10^4 Å	10^5 Å	10^6 Å	Linear & Mixed-Bed	Suggested Operating Temp.
Acetone	Y	Y	Y	Y	Y	Y	Y	Y	
Benzene	Y	Y	Y	Y	Y	Y	Y	Y	
Carbon Tetrachloride	Y	Y	Y	Y	Y	Y	Y	Y	
Chloroform	Y	Y	Y	Y	Y	Y	Y	Y	
30 % HFIP/Chloroform	Y	Y	Y	Y	Y	Y	Y	Y	
Diethyl Ether	Y	Y	Y	Y	Y	Y	Y	Y	
Dimethylacetamide (DMAC)	Y*	Y	Y	Y	Y	Y	Y	Y	60 °C
Dimethylformamide (DMF)	Y*	Y	Y	Y	Y	Y	Y	Y	60 °C
Dioxane	Y	Y	Y	Y	Y	Y	Y	Y	
DMSO	Y*	Y	Y	Y	Y	Y	Y	Y	60 °C
Ethyl Acetate	Y	Y	Y	Y	Y	Y	Y	Y	
Hexafluoroisopropanol (HFIP)	Y	Y	Y	Y	Y	Y	Y	Y	
Hexane	Y	Y	Y	Y	Y	Y	Y	Y	
M-Cresol	Y*	Y	Y	Y	Y	Y	Y	Y	100 °C
Methyl Ethyl Ketone	Y	Y	Y	Y	Y	Y	Y	Y	
Methylene Chloride	Y	Y	Y	Y	Y	Y	Y	Y	
0-Chlorophenol	Y*	Y	Y	Y	Y	Y	Y	Y	100 °C
0-Dichlorobenzene	Y*	Y	Y	Y	Y	Y	Y	Y	135 °C
Quinolin	Y*	Y	Y	Y	Y	Y	Y	Y	60 °C
Tetrahydrofuran	Y	Y	Y	Y	Y	Y	Y	Y	
Toluene	Y	Y	Y	Y	Y	Y	Y	Y	
Trichlorobenzene	Y*	Y	Y	Y	Y	Y	Y	Y	135 °C
Water	N	N	N	N	N	N	N	N	
Xylene	Y	Y	Y	Y	Y	Y	Y	Y	

*Not recommended on 5 µm 50 Å columns. N = Not Compatible Y = Compatible

Solvent Switching Considerations

You can extend the column life of your GPC columns if you dedicate them to certain solvents. This will also minimize the number of necessary solvent switches. If care is not taken, a void may occur.

4 quick steps to ensure proper solvent switching:

1. *Reduce flow rate to 0.2 mL/min (7.5 – 8 mm ID)*
2. *Keep backpressure < 1500 psi*
3. *Check for solvent miscibility on chart*
4. *Compare the swell characteristics of old and new solvent and use the following guidelines*

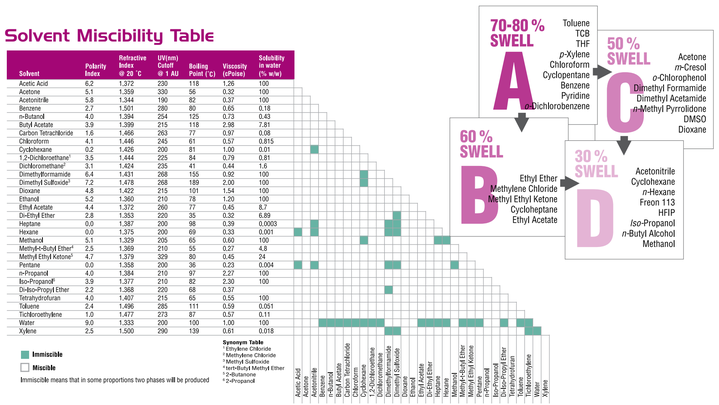
Swell Class	A	B	C	D
A	D	D	D	I
B	D	D	I	D
C	D	I	D	D
D	I	D	D	D

Direct Switches (D), Intermediate Step Required (I)

ATTENTION
Make sure the old and new solvents are miscible with each other before proceeding with the solvent switch.

Solvent Miscibility Table

Solvent	Polarity Index	Refractive Index @ 20 °C	UV(nm) Cutoff @ 1 AU	Boiling Point (°C)	Viscosity (cPoise)	Solubility in water (% w/w)
Acetic Acid	6.2	1.372	230	118	1.26	100
Acetone	5.1	1.359	330	56	0.32	100
Acetonitrile	5.8	1.344	190	82	0.37	100
Benzene	2.7	1.501	280	80	0.65	0.18
n-Butanol	4.0	1.394	254	125	0.73	0.43
Butyl Acetate	3.9	1.399	215	118	2.98	7.81
Carbon Tetrachloride	1.6	1.466	263	77	0.97	0.08
Chloroform	4.1	1.446	245	61	0.57	0.815
Cyclohexane	0.2	1.426	200	81	1.00	0.01
1,2-Dichloroethane[1]	3.5	1.444	225	84	0.79	0.81
Dichloromethane[2]	3.1	1.424	235	41	0.44	1.6
Dimethylformamide	6.4	1.431	268	155	0.92	100
Dimethyl Sulfoxide[3]	7.2	1.478	268	189	2.00	100
Dioxane	4.8	1.422	215	101	1.54	100
Ethanol	5.2	1.360	210	78	1.20	100
Ethyl Acetate	4.4	1.372	260	77	0.45	8.7
Di-Ethyl Ether	2.8	1.353	220	35	0.32	6.89
Heptane	0.0	1.387	200	98	0.39	0.0003
Hexane	0.0	1.375	200	69	0.33	0.001
Methanol	5.1	1.329	205	65	0.60	100
Methyl-t-Butyl Ether[4]	2.5	1.369	210	55	0.27	4.8
Methyl Ethyl Ketone[5]	4.7	1.379	329	80	0.45	24
Pentane	0.0	1.358	200	36	0.23	0.004
n-Propanol	4.0	1.384	210	97	2.27	100
Iso-Propanol[6]	3.9	1.377	210	82	2.30	100
Di-Iso-Propyl Ether	2.2	1.368	220	68	0.37	
Tetrahydrofuran	4.0	1.407	215	65	0.55	100
Toluene	2.4	1.496	285	111	0.59	0.051
Trichloroethylene	1.0	1.477	273	87	0.57	0.11
Water	9.0	1.333	200	100	1.00	100
Xylene	2.5	1.500	290	139	0.61	0.018



Sample Concentration and Injection Volume

These recommendations are based on molecular weight and the polydispersity index (PDI) of the sample.

Sample	Recommended Concentration [mg/mL], (%)
100 – 10,000 Da (narrow PDI)	2 (0.2 %)
10,000 – 1,000,000 Da (narrow PDI)	1-2 (0.1 – 0.2 %) (lower for higher molar masses)
>1,000,000 Da (narrow PDI)	0.5 (0.05 %) (lower for higher molar masses)
Broad PDI (≥ 2)	4-5 (0.4 – 0.5 %)

Number of Analytical Columns	Injection Volume [µL]
≥ 4	200 – 250
3	100
2	50
1	20

ATTENTION
Please take into consideration that samples tend to have a greater viscosity as the molecular weight gets higher. Use lower concentrations of these samples to avoid degradation by sheering.

www.phenomenex.com/understandingGPC

guarantee

If Phenogel analytical columns do not provide at least equivalent separation as compared to a competing column of the similar particle size, phase, and dimensions, send in your comparative data to a similar product with the Phenogel column within 45 days for a FULL REFUND.

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