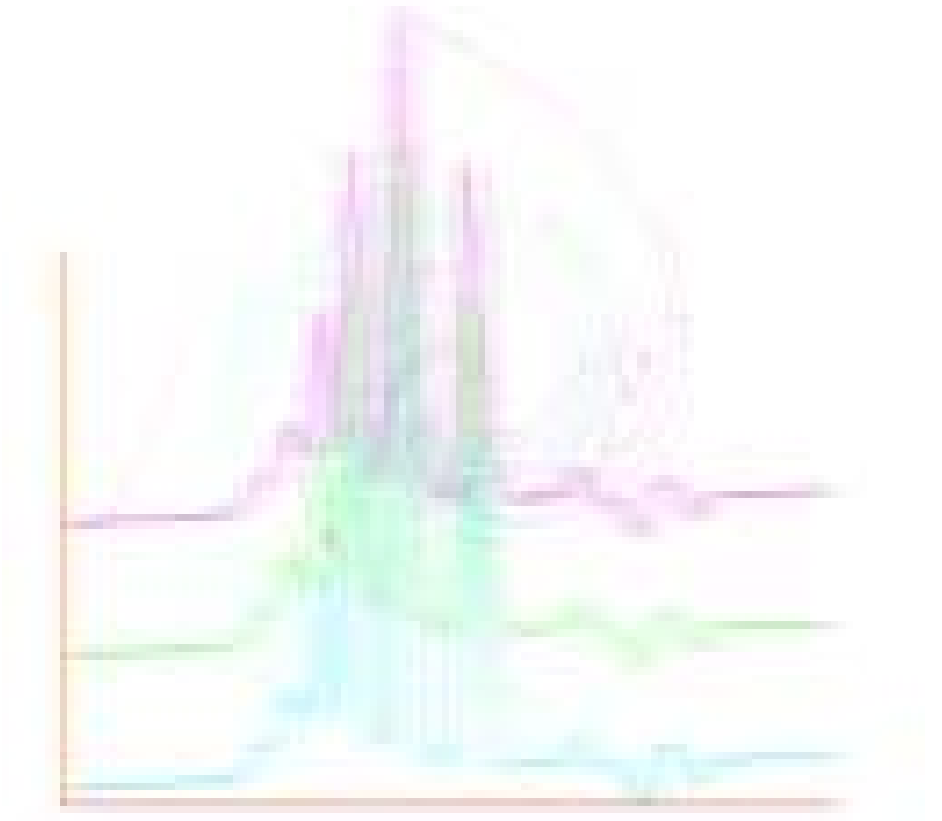
A User's Guide to

Gel Permeation Chromatography



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I. Introduction

Most HPLC separations today are performed using reversed-phase columns, with size-exclusion accounting for only about 7% of the total *(Ref. 1)*. For separation of macromolecules, however, size-exclusion accounts for about 22% of the total. With these facts in mind, it is logical to conclude that most chromatographers are familiar with the principles and operation of reversed-phase HPLC, but are less familiar with size-exclusion separations.

This guide focuses on some aspects of size-exclusion that are not necessarily logical extensions of reversed-phase HPLC. Principles will be explained in the simplest way possible, as it is intended to take some of the mystery out of size-exclusion separations.

II. SEC, GPC or GFC?

There are three names commonly applied to this technique:

Size-exclusion chromatography (SEC) is a general term and is used because in this chromatographic process molecules are separated on the basis of their exclusion from the controlled pores in the packing material. SEC applies to both polymeric and silica-based packings.

Gel-permeation chromatography (GPC) is used because most column packings are polymeric gels, and different-sized molecules permeate the pores to different extents. Generally, this term is used when one is using a polymer-based column with a non-aqueous mobile phase.

Gel-filtration chromatography (GFC) indicates that a filtration process takes place, in which the stationary phase acts as a kind of filter to separate molecules based on size. The term GFC often is limited to separations which use aqueous mobile phases in order to preserve biological activity of the analytes. GFC is occasionally referred to as "Aqueous GPC".

For the sake of simplicity, we will use the term "SEC" in this discussion of exclusion methods.

III. Principles Behind SEC: How does it differ from reversed-phase HPLC?

A. RETENTION

As its name suggests, the goal in SEC is to separate molecules based on size.

In Reversed-Phase Chromatography (RPC), retention is determined by molecular and chemical interactions between the bonded phase, sample molecules, the mobile phase, and other chromatographic parameters.

In SEC, retention is primarily a result of the molecules "physically" interacting with the packing pores (Fig 1). Very simply, a molecule has to be small enough to penetrate the pores. The longer it stays there, the longer the retention. Any molecule too big to penetrate the pores is excluded, exhibiting shorter or no retention.

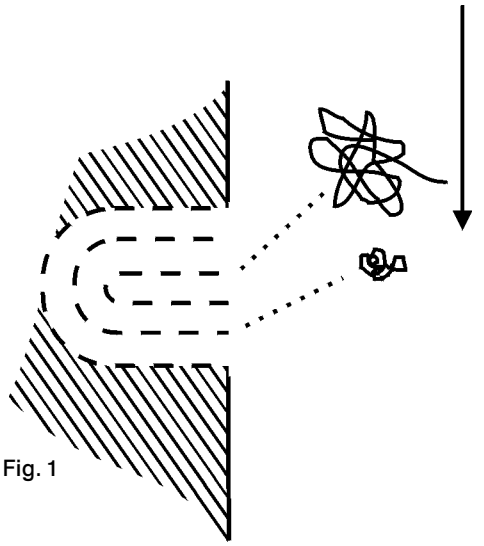


Fig. 1

In the absence of secondary interactions, retention is determined by the volume available to the center of the molecule.

This "effective" volume (the molecular diameter or volume available to the pore) can differ between compounds of the same molecular weight. For example, the size of the random coil of a polystyrene standard is relatively larger than that of dextran standards. Consequently, dextrans would have larger elution volumes (exhibit longer retention) than polystyrenes of the same molecular weight.

B. ELUTION ORDER

Unlike Reversed Phase Chromatography (RPC), all compounds elute before the column dead volume (Fig. 2). Therefore, the largest elution volume in any given SEC column is equal to the total mobile phase volume in the column. (Ref 8).

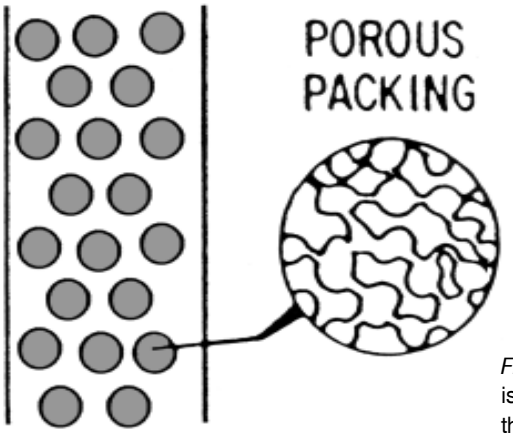


Figure 2. The column dead volume is the sum of the pore volume and the interstitial volume.

Figure 3 shows the familiar separation characteristics of SEC columns.

- The first peaks to elute represent molecular weights that are totally excluded from the packing pores. These molecules co-elute as a single band (C) with a retention volume V_o.

V_o, also known as the interparticle volume, is the volume of the solvent within the column, not including the packing pores. (Ref 4)

- The last peaks (lowest molecular weights) elute at the void volume (e.g., peak F at volume B in Fig. 3). These extreme peaks (C & F) are often mixtures of compounds because selectivity is exhibited only in the region between these two extremes (also known as the fractionation Range).

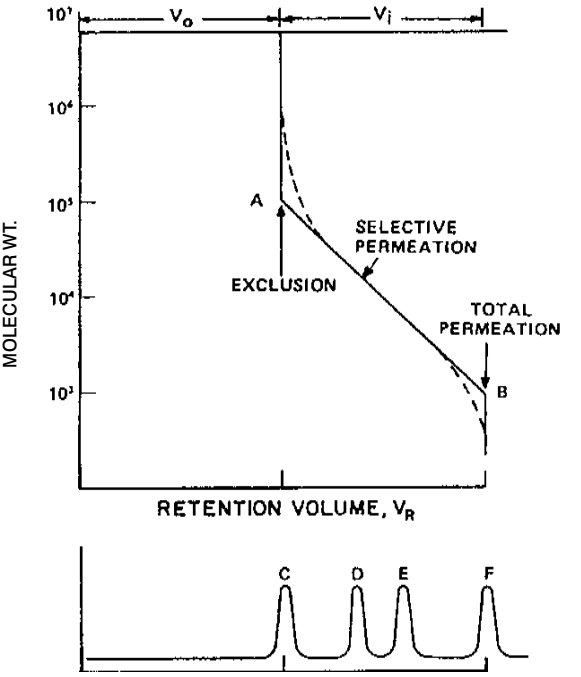


Figure 3. Hypothetical calibration plot for a SEC column. Reprinted from Ref. 3 with permission.

C. RESOLUTION

Resolution is determined by retention as a function of molecular size and column efficiency or bandwidth (Ref. 9).

1. Retention as a function of molecular size

Molecules, depending on size, will have either complete, partial, or no access to the available pores. The degree of access to the pores (partial vs. complete) results in

Figure 4 illustrates the typical relationship between the molecular weight and retention time of a given compound (in this case, polystyrene standards).

varying retention, which in turn affects resolution (Fig 4).

The range of molecular weights that can be separated (also known as fractionation range) is determined by the pore size of the column packing, not the column length.

SEC is limited in its ability to separate samples of similar molecular weights; often compounds with molecular weight differences of less than 10% cannot be resolved.

Peak separation is dependent on the solute molecular weight given the separation range of the column. Selectivity is exhibited primarily with the linear separation range of a given column (Fig 5).

Tip: Examine the slope of the calibration plot of a column with a given pore size. Between columns of the same pore size, a smaller calibration slope will give better resolution.

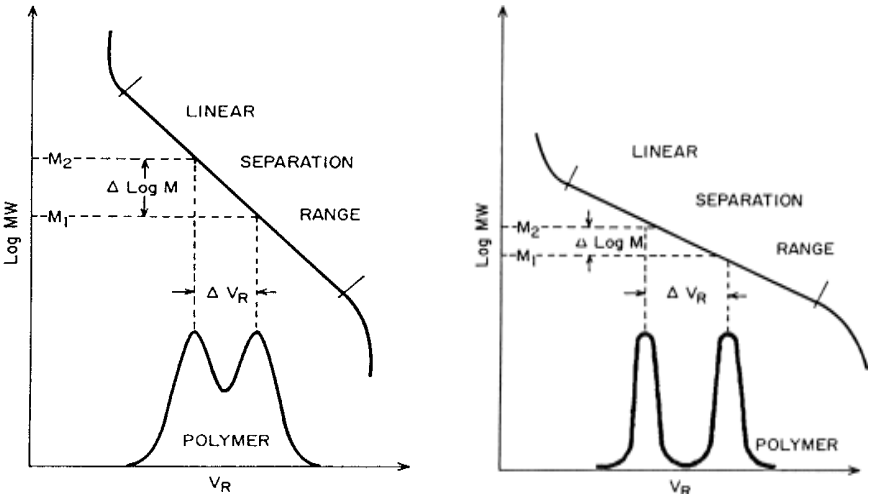


Figure 5. Selectivity is exhibited between the linear separation range. Peak separation is dependent on the solute molecular weight.

2. Column Efficiency or Bandwidth

In addition to having varying retention, good resolution between two peaks is strongly dependent upon the bandwidth or plate count. The higher the plate count, the sharper the peak, helping to increase resolution between peaks.

Tip: To help optimize resolution, columns can be used in series. Doing so will increase efficiency and help decrease the slope of the calibration curve (Ref.10)

D. CAPACITY

Because all compounds elute before the dead volume, SEC columns typically have a limited separation capacity. The usable separation capacity of an SEC column (the fractionation range) is the volume between the exclusion volume and V_o (Void volume).

The peak capacity of most SEC columns is therefore limited to about a dozen peaks (vs. sometimes hundreds of peaks in reversed phase). Because of this limited capacity, the column must have efficiencies high enough to produce sharp and well- resolved peaks.

IV. Considerations for Column Selection

A. SAMPLE EFFECTS

As with other types of HPLC, the usable sample size in SEC must be limited to prevent band broadening due to sample overload.

1. Sample Concentration

For most SEC columns, this means that injections of up to about 15 mg can be used before overload occurs and loss of resolution is experienced (Ref. 2). Overloading the column will not damage it, but resolution will decrease.

General Guideline for determining sample concentration:

Molecular Weight	*Sample Concentration*
50K	*0.25%*
50-500K	*0.10%*
500K-2.5M	*0.05%*
2.5M	*0.01%*

2. Sample Viscosity

Sample Viscosity can also play an important role in SEC. Sample viscosity is related to diffusion and thus to the column plate number N (i.e., column efficiency).

With low-molecular-weight separations by reversed-phase HPLC, sample viscosity is of less concern, but SEC often involves samples with molecular weights in the hundreds of thousands, and viscosity becomes quite important.

A rough rule of thumb is that sample viscosity should be no more than twice the viscosity of the mobile phase (Ref. 3).

Tip: Sample viscosity decreases with higher temperatures, so SEC systems that are operated at elevated temperatures will accommodate larger samples.

B. CHOOSING THE MOBILE PHASE

In SEC, the nature of the mobile phase should have no effect on the resolution. The solvent used should not create any unwanted secondary-retention interactions with the column.

Three primary criteria must be considered when selecting the SEC mobile phase:

1. Compatibility with the Column

a. Is the mobile phase compatible with the column?

Check with the column manufacturer to be sure the desired mobile phase is one which is recommended for the column. Some mobile phases (e.g., DMF) may be limited to certain column configurations. For most columns designed for use with organic solvents, water should never be used in the mobile phase.

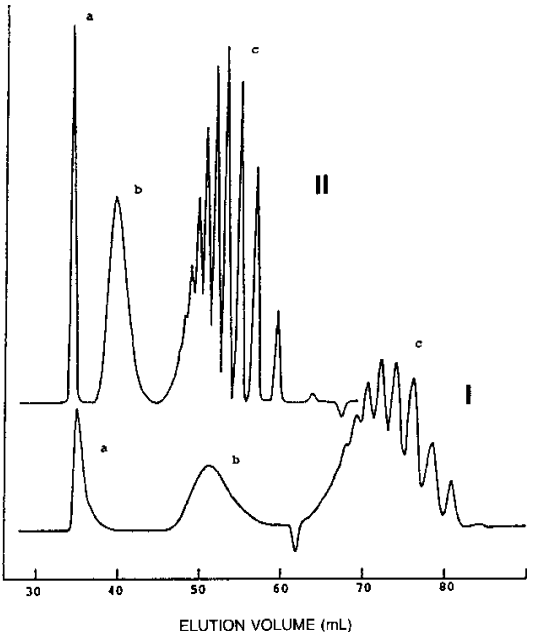


Figure 6. Separation of polystyrenes in DMF (I) and THF (II). Samples: (a) 37000MW; (b) 4000MW; (c) 600MW. Columns: 8 x 250mm, 100Å pore size, flow rate 1mL/min. Reprinted from Ref. 5 with permission.

Some of the results of the mobile phase choice are illustrated in Figures 6 and 7. In this case, the sample was a melamine-formaldehyde resin, which was soluble in DMF, but insoluble in THF. The brand of column selected was available packed either in THF or DMF, but the solvents were not interchangeable within a single column.

Figure 6 shows a chromatogram of polystyrene standards on nominally equivalent sets of each column. It is clear that, even though the specified plate number was the same for the two columns, the column with THF as a mobile phase gives a superior separation. Note also that with DMF the column was operated at 60°C (vs. 23°C for THF) in order to reduce the solvent viscosity. A separation of the melamine-formaldehyde resin in DMF is seen in Figure 7.

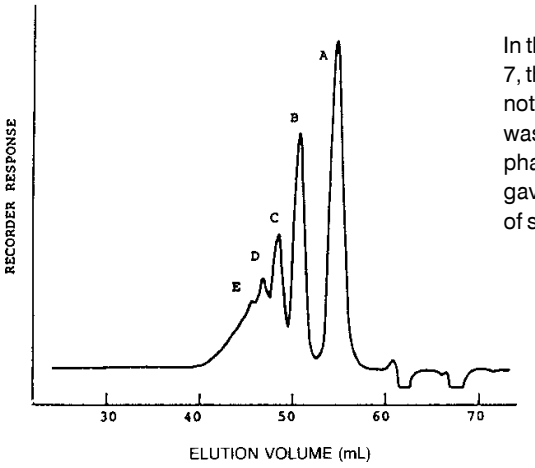
b. The solvent itself should not create any unwanted secondary-retention interactions with the column packing.

Tip: Should there be secondary interactions, you can try the following: If the interaction is hydrophobic, add an organic solvent into the mobile phase. If the interaction is hydrophilic, add a salt.

Please be mindful of the column limitations when trying to reduce secondary interactions and consult the column manufacturer prior to modifying the mobile phase.

2. Solubility of the Sample

Ideally, the sample solvent should be used as mobile phase in order to prevent problems with detection.



In the example of Figure 7, the resin sample was not soluble in THF, so DMF was used as a mobile phase even though THF gave a better separation of standards (see Fig. 6).

Figure 7. Chromatograph of a melamine-formaldehyde resin in DMF. Conditions as in *Figure 6*. Reprinted from Ref. 5 with permission.

Tip: The sample solvent should not promote any sample aggregation, as well as any interaction between the sample and the column packing. Should there be any sample aggregation, salts such as lithium bromide can be added to the mobile phase.

3. Operating Conditions

Is the mobile phase suitable for the operating conditions? Many times the SEC column will be operated at elevated temperature to decrease the solvent and sample viscosity and to increase the sample solubility.

Tip: When working at elevated temperatures, the boiling point of the mobile phase being used should be 50°-60°C above the column operating temperature and the maximum temperature used to dissolve the sample (Ref. 4). Failure to do so may cause outgassing, which can then upset the column packing bed.

If one must work at elevated temperatures, a higher-boiling-point solvent is desirable, such as trichlorobenzene or o-dichlorobenzene.

C. COLUMN TYPE

The following are some general characteristics of the most common commercially available SEC columns:

1. Polymer-Based Columns

A. Aqueous Mobile Phase

- **Material:** Polyvinyl alcohol, polyhydroxymethacrylate
- Best for particularly small or particularly large molecular weights (i.e. < 1000 or > 100,000 Da)
- Recommended for polydisperse compounds such as polysaccharides and synthetic polymers
- **Mechanism:** In addition to size exclusion, some hydrophobic and ionic interaction can occur between the sample and the packing
- **Limitations:** Has very limited capacity for organic solvents; lower efficiencies compared to silica-based column
- **Advantages:** Higher temperature limits, higher pH range

B. Organic Mobile Phase

- **Material:** styrene/divinyl benzene copolymer.
- Typically has a wide pore size selection (single, linear mixed and mixed pore sizes).
- **Mechanism:** Size exclusion with some hydrophobic interaction and ionic interaction.
- **Limitations:** Can only tolerate minimal amounts (< 5%) of water; susceptible to swelling and shrinkage of the column packing depending on the solvent used.
- **Advantages:** Can handle most organic solvents; Higher temperature range (typically 140°C-210°C).

2. Silica-Based Columns

- **Mechanism:** Pure size exclusion, but free silanols can interact with samples.
- **Limitations:** pH range is only 2.5 to 7; lower temperature limitations.
- **Advantages:** Can handle higher concentrations of organics such as MeOH, ACN, etc.
- Higher efficiency than polymer-based columns.
- Suitable for monodisperse biopolymers such as proteins and nucleic acids due to its higher resolving power.

D. SELECTION OF PORE SIZE OR PORE SIZES

- Typically, several pore sizes are used in series, both for increasing plate count and the fractionation range (Fig. 9).
- For samples that have a range of molecular weights, a range of pore sizes must be used in order to achieve separation. This is frequently achieved by coupling columns in series.

Tip: When coupling columns in series, make sure the pore sizes overlap to increase the effective fractionation range (the molecular weight range) of the columns. (Fig. 8)

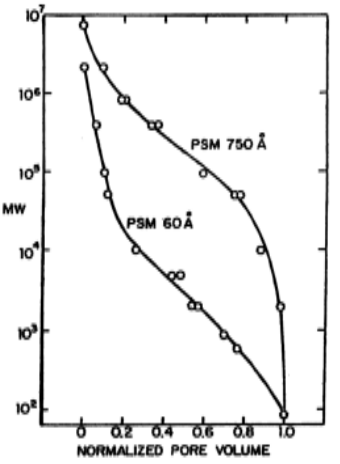


Figure 8. Comparison of the fractionation range for SEC columns of differing pore size. Porous silica SEC column, THF as mobile phase at 2.5mL/min with polystyrene standards. Reprinted from Ref. 4 with permission.

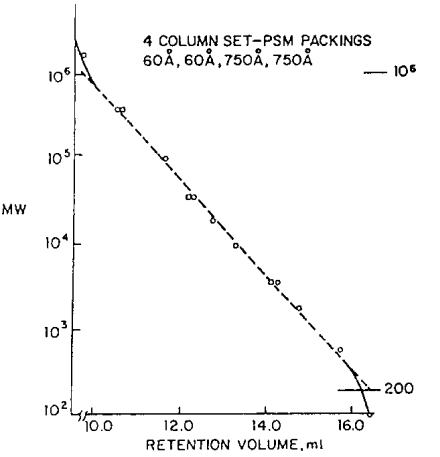
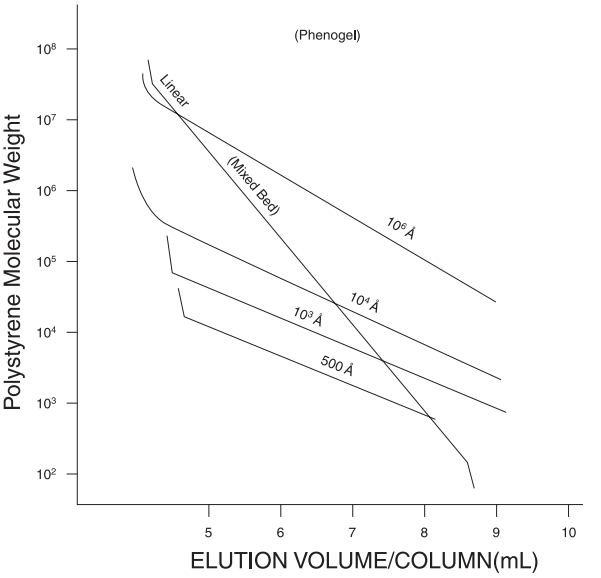


Figure 9. Extending the fractionation range of SEC columns by using a bimodal column set from Figure 8. Reprinted from Ref. 4 with permission.

Mixed Bed vs. Single Pore Size

- Mixed bed columns (includes linear mixed) have a wider molecular weight range than a single pore size.
- Mixed bed columns tend to have more uniform selectivity from low to high molecular weight. Over the middle range, single pore size columns show inferior selectivity (Fig. 10) (Ref. 1).
- If the molecular weight range of a sample is known, choose a single pore size. The molecular weight range is narrower; selectivity and resolution are significantly better.

Fig. 10



E. INCREASING EFFICIENCY

After choosing the appropriate column and mobile phase conditions that would minimize secondary interactions, the only other way to improve separation is to increase efficiency (Ref. 8).

1. Particle Size

Generally, columns with smaller particle sizes (typically 4-5μ in GPC) have higher efficiencies.

Columns with smaller particle sizes will typically run at higher backpressures. Since most SEC columns have lower backpressure limitations compared to other HPLC columns, this is usually more of a concern.

Tip: With an unacceptable increase in backpressure, one can compensate by decreasing the flow rate and/or increasing the column temperature to decrease mobile phase and sample viscosity.

2. Coupling Columns in Series

As mentioned in previous sections, one can put columns in series to effectively increase efficiency. The molecular weight calibration curves should overlap in order to effectively increase the efficiency, resolution and separation capacity.

On the other hand, if the molecular weight calibration curves overlap significantly and resolution is more than enough, it may be possible to use just one column from that range.

The best source of this information is the manufacturer. Most manufacturers publish the range of molecular weights the column can separate. These ranges are obtained using known standards of known molecular weights. For polymer-based materials, for example, the range is calculated using polystyrenes.

When coupling columns in series, conventional wisdom indicates that the columns must be placed in order of increasing pore size (Ref. 7). However, in the absence of more definitive experiments on this subject, it is best to experimentally determine the best pore size order for your method.

Tip: Since one cannot obtain pure standards for every sample, chromatographers typically use commercially available standards for calibration.

Make sure to use standards whose hydrodynamic volume or diameter is similar to your compound of interest.

3. Decreasing Extra Column Effects

Optimizing the system dead volume to reduce extra column effects, decreases band broadening and effectively increases efficiency. Make sure that you are using the correct pore size columns in series. Any length of column packing which does not contribute to the separation just adds to dead volume, which in turn contributes to band broadening.

V. CALIBRATION

The purpose of most size-exclusion separations is the determination of molecular weight distribution of polymers. Since the elution volume of a specific molecule is determined by its size, which in turn is a function of molecular weight, one can use the elution volume to determine the molecular weight of the unknown (Ref. 8).

Different calibration methods are used to define the relationship between the molecular weight (or its logarithm) and retention volume (or retention time), given the permeation range of the columns used (Ref. 10).

The following is a brief description of commonly used calibration methods:

Direct Standard Calibration

The simplest calibration method; it utilizes narrowly distributed standards of the same sample being analyzed. The retention volume at the peak maximum of each standard is then equated with its stated molecular weight.

Polystyrene, polyethylene oxide and polyethylene glycol are examples of these commercially available standards.

Polydisperse or Broad Standard Calibration

In the absence of narrowly distributed standards, the polydisperse or Broad Standard Calibration method is used. As the name implies, one uses broadly distributed polymer standards of similar molecular diameter and chemical type as the sample.

Universal Calibration

This method utilizes the relationship between the intrinsic viscosity and hydrodynamic volume of the sample and the standard being used. This "correlation" is then expressed as a constant which, when equated to the known molecular weight of the standard, can be used to develop a calibration plot applicable to the sample. This would then be possible to use a set of narrow molecular weight standards to obtain molecular weight calibration regardless of the sample used (Ref. 10).

Note: It can be difficult to find commercially available molecular weight standards similar to ones compound of interest.

In these cases, molecular-weight-sensitive detectors (such as light scattering and viscometry detectors) are commonly used to provide direct means of calibration without the need for external standards.

VI. COLUMN CARE CONSIDERATIONS

SEC columns packed with polymeric gels are much more susceptible to damage than are silica-based columns. The following guidelines highlight some important aspects of SEC column care.

Note: The following are general recommendations. Please consult the column manufacturer for their column care considerations.

Storage

For short-term storage (e.g., overnight), columns can be stored in the mobile phase. When longer-term storage is anticipated, it is wise to return the column to its original solvent for maximum stability; this solvent must always be degassed. Be sure to tightly cap the column ends so the column does not dry out, because gel shrinkage can result in a disruption of the packing bed.

Backpressure

Because the polystyrene-divinylbenzene SEC packings are relatively soft, care must be taken to limit the column pressure, or the packing bed can collapse, making the column useless. Check the column data sheet for the recommenced pressure limits (usually 1000-2000 psi); it is wise to set the pump high-pressure shutoff to about 75-80% of the recommended limit. For similar reasons, don't shock the column with sudden changes in flow rate. For example, increase the flow rate in 0.5 mL/min increments until the desired flow rate is reached.

Solvent Switching

Most solvent-compatible polystyrene divinyl benzene columns exhibit wide solvent compatibility. Different solvents, however, produce different swell characteristics (Table 1). Improper solvent switches can result in a void. For this reason, we recommend that you dedicate columns to specific solvents.

If you need to switch solvents, PLEASE consult the column manufacturer. The following are generalized guidelines for switching solvents:

1. Reduce flow rate to 0.2mL/min.

2. Backpressure must NEVER exceed 650 psi.
3. Always check solvent miscibility in a beaker or follow the solvent miscibility table on page 15 before proceeding with ANY solvent switch.
4. Compare the swell characteristics of solvent 1 (old solvent) to solvent 2 (new solvent) and use the following guidelines:

- If solvent 1 and solvent 2 belong to the same swell category (Table 1), check the solvent miscibility and proceed with the switch.
- If solvent 1 and solvent 2 belong to successive swell categories as indicated by the arrows on Table 1, check the miscibility and proceed with the switch.
- If solvent 1 and solvent 2 DO NOT belong to the same OR successive swell categories, switch to an intermediate solvent FIRST, as indicated by the arrows on Table 1.

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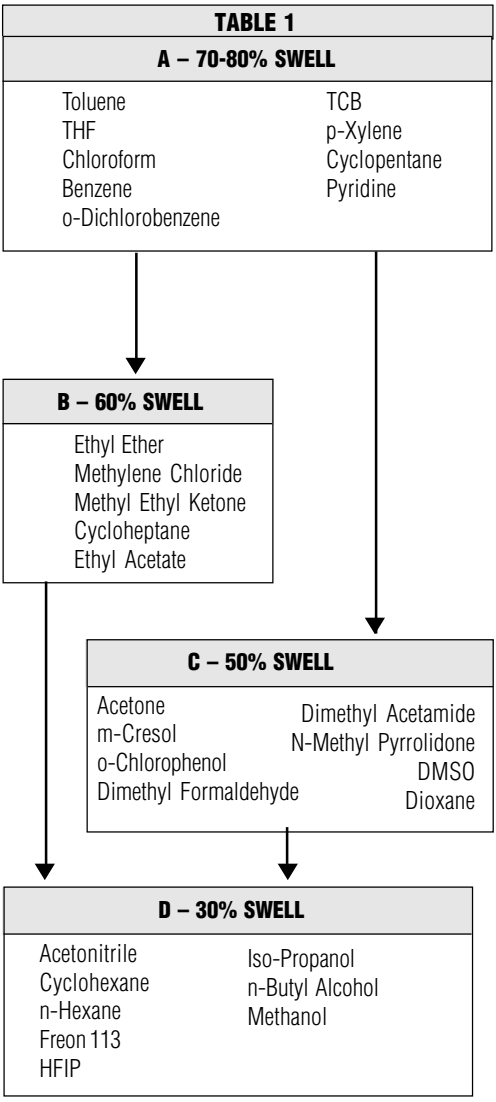


TABLE 1
A – 70-80% SWELL
Toluene
THF
Chloroform
Benzene
o-Dichlorobenzene
TCB
p-Xylene
Cyclopentane
Pyridine
B – 60% SWELL
Ethyl Ether
Methylene Chloride
Methyl Ethyl Ketone
Cycloheptane
Ethyl Acetate
C – 50% SWELL
Acetone
m-Cresol
o-Chlorophenol
Dimethyl Formaldehyde
Dimethyl Acetamide
N-Methyl Pyrrolidone
DMSO
Dioxane
D – 30% SWELL
Acetonitrile
Cyclohexane
n-Hexane
Freon 113
HFIP
Iso-Propanol
n-Butyl Alcohol
Methanol

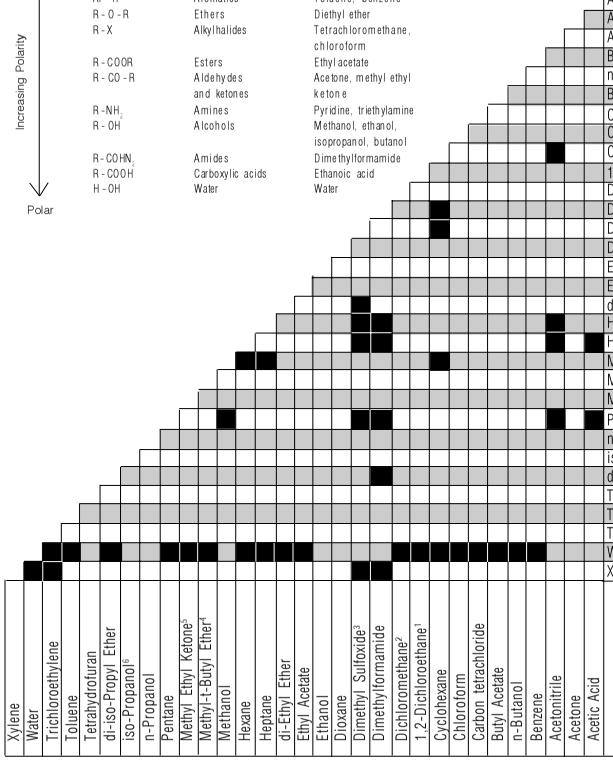
SOLVENT POLARITY CHART

Relative Polarity	Compound Formula	Group	Representative Solvent Compounds
Nonpolar	R-H	Alkanes	Petroleum ethers, ligroin, hexanes
	Ar-H	Aromatics	Toluene, benzene
	R-O-R	Ethers	Diethyl ether
	R-X	Alkylhalides	Tetrachloromethane, chloroform
	R-COOR	Esters	Ethyl acetate
	R-CO-R	Aldehydes and ketones	Acetone, methyl ethyl ketone
	$R-NH_2$	Amines	Pyridine, triethylamine
	R-OH	Alcohols	Methanol, ethanol, isopropanol, butanol
	R-CONR$_2$	Amides	Dimethylformamide
	R-COOH	Carboxylic acids	Ethanoic acid
Polar	H-OH	Water	Water

Increasing Polarity

TABLE 2 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



■ Immiscible*

□ Miscible

SYNONYM TABLE
[1] Ethylene Chloride
[2] Methylene Chloride
[3] Methyl Sulfoxide
[4] tert-Butyl Methyl Ether
[5] 2-Butanone
[6] 2-Propanol

*Immiscible means that in some proportions two phases will be produced.

SOLVENT COMPATIBILITY CHART FOR PHENOGEL GPC COLUMNS

TABLE 3

MOBILE PHASE SOLVENT	PHENOGEL PORE SIZE 50Å	100Å	500Å	10^3Å	10^4Å	10^5Å	10^6Å	LINEAR & MIXED	SUGGESTED OPERATING TEMPERATURE
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	
METHYL 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 5μ 50Å columns. N=NOT COMPATIBLE Y=COMPATIBLE

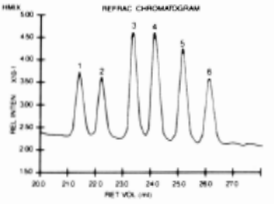
VII. PHENOGEL™ APPLICATIONS

CLOSELY RELATED HYDROCARBONS

Columns:	Phenogel 5μ 50Å, 100 Å, 500Å
Dimensions:	300 x 7.8mm
Solvent:	THF
Flow Rate:	1.0 ml/min
Detector:	Differential Refractometer
Injection Volume:	100μl 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

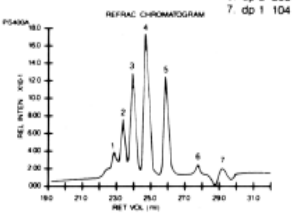


POLYSTYRENE 400

Columns:	Phenogel 5μ 50Å, 100Å, 500Å
Dimensions:	300 x 7.8mm
Solvent:	THF
Flow Rate:	1.0 ml/min
Detector:	Differential Refractometer
Injection Volume:	100μl 0.25% w/v
Temperature:	Ambient

Sample:

1. dp 7 728 MW
2. dp 6 624 MW
3. dp 5 520 MW
4. dp 4 416 MW
5. dp 3 312 MW
6. dp 2 208 MW
7. dp 1 104 MW

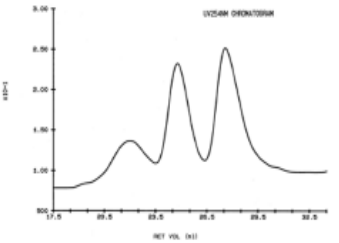


HIGH MOLECULAR WEIGHT POLYSTYRENE

Columns:	Phenogel 10μ 10⁶ x 3
Dimensions:	300 x 7.8mm
Solvent:	THF
Flow Rate:	1.0 ml/min
Detector:	UV @ 254 min
Injection Volume:	100 μl 0.05% w/v
Temperature:	30°C

Sample:

1. 12,250,000 MW
2. 1,560,000 MW
3. 300,000 MW

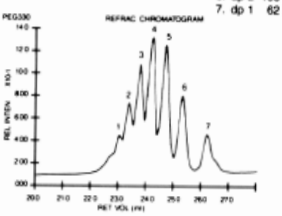


POLYETHYLENE GLYCOL 330

Columns:	Phenogel 5μ 50Å, 100Å, 500Å
Dimensions:	300 x 7.8mm
Solvent:	THF
Flow Rate:	1.0 ml/min
Detector:	Differential Refractometer
Injection Volume:	100μl 0.25% w/v
Temperature:	Ambient

Sample:

1. dp 7 546 MW
2. dp 6 458 MW
3. dp 5 370 MW
4. dp 4 282 MW
5. dp 3 194 MW
6. dp 2 106 MW
7. dp 1 62 MW

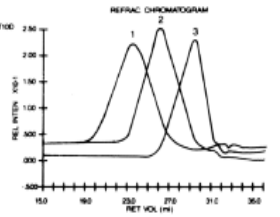


DEXTRANS

Columns:	Phenogel 10μ 10⁴Å, 10³Å, 10²Å
Dimensions:	300 x 7.8mm
Solvent:	DMSO
Flow Rate:	1.0 ml/min
Detector:	Differential Refractometer
Injection Volume:	100 μl 0.25% w/v
Temperature:	50°C

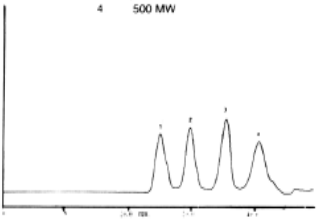
Sample:

1. 500,000 MW
2. 70,000 MW
3. 10,000 MW



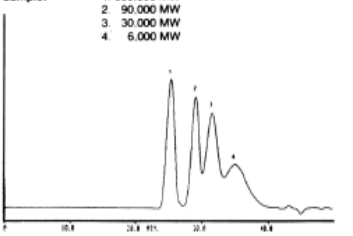
POLYBUTADIENES (WIDE MW RANGE)

Columns: Phenogel 5μ 10^3Å, 10^4Å, 10^3Å, 500Å
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Differential Refractometer
Injection Volume: 100 μl 0.25% w/v
Sample:
1. 420,000 MW
2. 24,000 MW
3. 2,500 MW
4. 500 MW



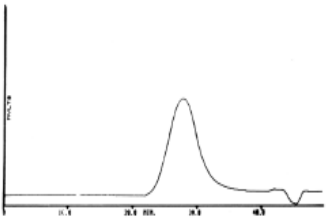
POLY (α-METHYL STYRENE) (WIDE MW RANGE)

Columns: Phenogel 5μ 10^3Å, 10^4Å, 10^3, 500Å
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Differential Refractometer
Injection Volume: 100 μl 0.25% w/v
Sample:
1. 680,000 MW
2. 90,000 MW
3. 30,000 MW
4. 6,000 MW



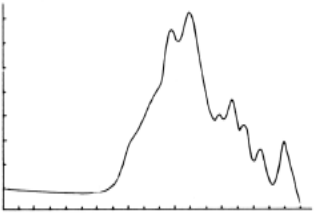
POLYVINYL BUTYRAL

Columns: Phenogel 5μ 500Å, 10^3Å, 10^4Å, 10^5Å
Dimensions: 300 x 7.8mm
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Differential Refractometer
Injection Volume: 100 μl 0.25% w/v
Temperature: 25 C
Sample: 1. 300,000 MW



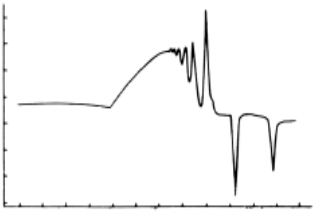
PHENOL FORMALDEHYDE RESIN

Columns: Phenogel 5μ 100Å, 10^3Å
Dimensions: 300 x 7.8mm
System: Spectra Physics GPC
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Spectra Physics 8430 RI
Injection Volume: 10 μl
Temperature: 25°C
Sample: PVC and Additives MW 50-70K



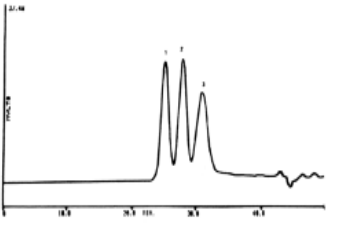
PHENOLIC RESINS

Columns: Phenogel 5μ 500Å X 2, 10^3Å, 10^4Å
Dimensions: 300 x 7.8mm
System: Spectra Physics GPC
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Spectra Physics 8430 RI
Injection Volume: 5 μl
Temperature: 25°C
Sample: Phenolic Aldehyde Resin MW 500 to 470K



POLYMETHYL METHACRYLATES (WIDE MW RANGE)

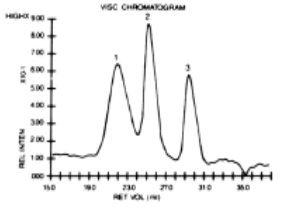
Columns: Phenogel 5μ 10^5Å, 10^4Å, 10^3Å, 500Å
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Differential Refractometer
Injection Volume: 100 μl 0.25% w/v
Sample:
1. 700,000 MW
2. 107,000 MW
3. 27,000 MW



HIGH MOLECULAR WEIGHT POLYSTYRENE

Columns: Phenogel 10μ 10⁴Å x 3
Dimensions: 300 x 7.8mm
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Differential Viscometer
Injection Volume: 100μl 0.05% w/v
Temperature: 30°C

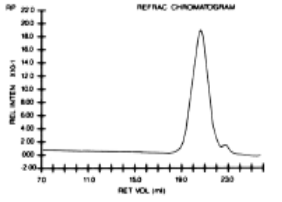
Sample:
1. 7,800,000 MW
2. 1,200,000 MW
3. 260,000 MW



NYLON 6 IN HFIP

Columns: Phenogel 10μ Linear x 2
Dimensions: 300 x 7.8mm
Solvent: HFIP (0.01M NATFAT)
Flow Rate: 1.0 ml/min
Detector: Differential Refractometer
Injection Volume: 100 μl 0.25% w/v
Temperature: 30°C

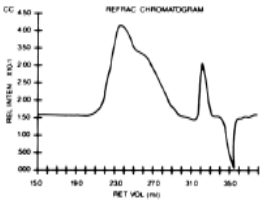
Sample:
1. 14,500 MW



POLYETHYLENE OXIDE (PEO)

Columns: Phenogel 10μ 10⁵Å, 10⁴Å, 10³Å
Dimensions: 300 x 7.8mm
Solvent: DMF (0.1M LiBr)
Flow Rate: 1.0 ml/min
Detector: Differential Refractometer
Injection Volume: 100 μl 0.125% w/v
Temperature: 50°C

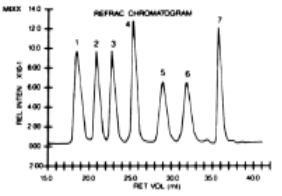
Sample: 1. 400,000 MW



POLYSTYRENES (WIDE MOLECULAR WEIGHT RANGE)

Columns: Phenogel 10μ 10⁵Å, 10⁴Å, 10³Å
Dimensions: 300 x 7.8mm
Solvent: THF
Flow Rate: 1.0 ml/min
Detector: Differential Viscometer
Injection Volume: 100 μl 0.25% w/v
Temperature: Ambient

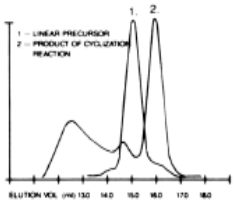
Sample:
1. 1,560,000 MW
2. 260,000 MW
3. 94,000 MW
4. 30,000 MW
5. 6,100 MW
6. 845 MW
7. 146 MW



CYCLIC POLYMER CHARACTERIZATION

Columns: Phenogel 10μ Linear (600 x 7.8mm)
Dimensions: 300 x 7.8mm
Solvent: THF with 1% TEA
Flow Rate: 1.3 ml/min
Detector: UV 268nm
Injection Volume: 40μl (0.2% w/v)
Temperature: Ambient

Sample: poly (2-vinylpyridine) [0.05% – 0.25% (w/v)]

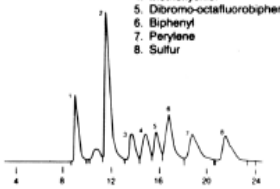


Chromatograms courtesy of Dr. William Toreki III

ENVIRONMENTAL SAMPLE CLEANUP

Columns: Phenogel Prep-100 10μ 100Å (two in series)
Dimensions: 250 x 22.5mm
Mobile Phase: Methylene Chloride
Flow Rate: 7 ml/min
Detection: UV @ 254
Sample Size: 500 μl

Sample (1-20μg/ml):
1. Polystyrene
2. Corn Oil (500 ug/ml)
3. Bis (ethyl-hexyl) Phthalate
4. Methoxychlor
5. Dibromo-octafluorobiphenyl
6. Biphenyl
7. Perylene
8. Sulfur



VIII. PHENOGEL™ ORDERING INFORMATION

60 DAY RISK-FREE TRIAL OFFER ON PHENOGEL™ GPC COLUMNS

We believe Phenogel GPC columns are the best GPC columns on the market, and we guarantee it! Evaluate PHENOGEL GPC columns in your lab for 60 days. If you are not convinced that PHENOGEL is an outstanding GPC column, simply return the column(s) with comparative data for a complete refund. That's confidence.

5µ GPC Columns

Pore Size	MW Range	Columns (mm)			Guards (mm)
		300 x 7.8	600 x 7.8	300 x 21.2	50 x 7.8
50Å	100 — 3K	00H-0441-K0	00K-0441-K0	00H-0441-P0	03B-2088-K0
100Å	500 — 6K	00H-0442-K0	00K-0442-K0	00H-0442-P0	03B-2088-K0
500Å	1K — 15K	00H-0443-K0	00K-0443-K0	00H-0443-P0	03B-2088-K0
10-3Å	1K — 75K	00H-0444-K0	00K-0444-K0	00H-0444-P0	03B-2088-K0
10-4Å	5K — 500K	00H-0445-K0	00K-0445-K0	00H-0445-P0	03B-2088-K0
10-5Å	10K — 1,000K	00H-0446-K0	00K-0446-K0	00H-0446-P0	03B-2088-K0
10-6Å	60K — 10,000K	00H-0447-K0	00K-0447-K0	00H-0447-P0	03B-2088-K0
Mixed Beds		**300 x 7.8**	**600 x 7.8**	**300 x 21.2**	**50 x 7.8**
Linear	100—10,000K	00H-0449-K0	00K-0449-K0	00H-0449-P0	03B-2088-K0
Linear (2)	100—10,000K	00H-3259-K0	00K-3259-K0	00H-3259-P0	03B-2088-K0
M1	400—1,200	00H-3262-K0	00K-3262-K0	00H-3262-P0	03B-2088-K0
M2	300—50,000	00H-3025-K0	00K-3025-K0	00H-3025-P0	03B-2088-K0
M3	500—75,000	00H-3263-K0	00K-3263-K0	00H-3263-P0	03B-2088-K0
MXL	100—100K	00H-3087-K0	00K-3087-K0	00H-3087-P0	03B-2088-K0
MXM	5K—500K	00H-3088-K0	00K-3088-K0	00H-3088-P0	03B-2088-K0
MXH	75K—10,000K	00H-3089-K0	00K-3089-K0	00H-3089-P0	03B-2088-K0

5µ Phenogel-NB (Narrow Bore)

Pore Size	MW Range	Columns (mm)	Guards (mm)
		300 x 4.6	30 x 4.6
50Å	100 — 3K	00H-0441-E0	03A-2088-E0
100Å	500 — 6K	00H-0442-E0	03A-2088-E0
500Å	1K — 15K	00H-0443-E0	03A-2088-E0
10-3Å	1K — 75K	00H-0444-E0	03A-2088-E0
10-4Å	5K — 500K	00H-0445-E0	03A-2088-E0
10-5Å	10K — 1,000K	00H-0446-E0	03A-2088-E0
10-6Å	60K — 10,000K	00H-0447-E0	03A-2088-E0
Mixed Beds		**300 x 4.6**	**30 x 4.6**
Linear	100 — 10,000K	00H-0449-E0	03A-2088-E0
Linear (2)	100 — 10,000K	00H-3259-E0	03A-2088-E0
MXL	100 — 100K	00H-3087-E0	03A-2088-E0
MXM	5K — 500K	00H-3088-E0	03A-2088-E0
MXH	75K — 10,000K	00H-3089-E0	03A-2088-E0

Phenogel-UT, UltraTemp Columns (100-205°C)

Pore Size	Exclusion Limits*	Columns (mm)	Guards (mm)
		300 x 7.8	50 x 7.8
10-3Å	20,000	00H-4100-K0	03B-4100-K0
10-6Å	20,000,000	00H-4101-K0	03B-4101-K0
10-7Å	200,000,000	00H-4102-K0	03B-4102-K0

*Exclusion limit based on the molecular weight of polystyrene.

20µ GPC Columns

Pore Size	MW Range	Columns (mm)		Guards (mm)
		300 x 7.8	600 x 7.8	50 x 7.8
100 Å	500 — 6K	00H-0842-K0	00K-0842-K0	03B-2091-K0
500 Å	1K — 15K	00H-0843-K0	00K-0843-K0	03B-2091-K0

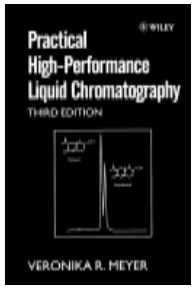
10µ GPC Columns

Pore Size	MW Range	Columns (mm)				Guards (mm)
		300 x 7.8	600 x 7.8	300 x 21.2	600 x 21.2	50 x 7.8
50Å	100 — 3K	00H-0641-K0	00K-0641-K0	00H-0641-P0	00K-0641-P0	03B-2090-K0
100Å	500 — 6K	00H-0642-K0	00K-0642-K0	00H-0642-P0	00K-0642-P0	03B-2090-K0
500Å	1K — 15K	00H-0643-K0	00K-0643-K0	00H-0643-P0	00K-0643-P0	03B-2090-K0
10-3Å	1K — 75K	00H-0644-K0	00K-0644-K0	00H-0644-P0	00K-0644-P0	03B-2090-K0
10-4Å	5K — 500K	00H-0645-K0	00K-0645-K0	00H-0645-P0	00K-0645-P0	03B-2090-K0
10-5Å	10K — 1,000K	00H-0646-K0	00K-0646-K0	00H-0646-P0	00K-0646-P0	03B-2090-K0
10-6Å	60K — 10,000K	00H-0647-K0	00K-0647-K0	00H-0647-P0	00K-0647-P0	03B-2090-K0
Mixed Beds		300 x 7.8	600 x 7.8	300 x 21.2	600 x 21.2	50 x 7.8
Linear	100 — 10,000K	00H-0649-K0	00K-0649-K0	00H-0649-P0	00K-0649-P0	03B-2090-K0
Linear (2)	100 — 10,000K	00H-3260-K0	00K-3260-K0	00H-3260-P0	00K-3260-P0	03B-2090-K0
MXL	100 — 100K	00H-0200-K0	00K-0200-K0	00H-0200-P0	00K-0200-P0	03B-2090-K0
MXM	5K — 500K	00H-0201-K0	00K-0201-K0	00H-0201-P0	00K-0201-P0	03B-2090-K0
MXH	75K — 10,000K	00H-0202-K0	00K-0202-K0	00H-0202-P0	00K-0202-P0	03B-2090-K0

PRACTICAL HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

3rd ed. Veronika R. Meyer 1999, 338pp.

This seminal work discusses theoretical principles and concepts of HPLC including the major separation modes of normal and reversed phase chromatography, ion-exchange and ion-pairing techniques, and size-exclusion chromatography. The separation of enantiomers is given special attention. Aspects of column selection and method development are presented. Analytical and preparative chromatographic techniques are treated in detail, along with discussions of the selection and use of various system components from injectors to detectors. The separations of individual compounds are reviewed, tabulated and indexed. An overview of commercially available stationary phases for HPLC is presented.

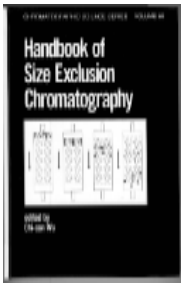


Order No.: AA0-1710

HANDBOOK OF SIZE EXCLUSION CHROMATOGRAPHY

Chromatographic Science Series Vol. 69. Chi-San Wu 1995, 453pp.

This outstanding single-source reference details the practical use of size exclusion chromatography (SEC) in characterizing the molecular weight distribution of important polymeric materials - addressing problems encountered in SEC of specific substances, including copolymers, polyamides, polyvinyl alcohol and acetate, lignin derivative, proteins and starch. SEC column technology is thoroughly reviewed, and the use of semirigid polymer gels and modified silica-based packing materials is clearly delineated. Written by more than 25 internationally renowned authorities in their respective fields, the *Handbook of Size Exclusion Chromatography* is an invaluable resource for polymer chromatographers.



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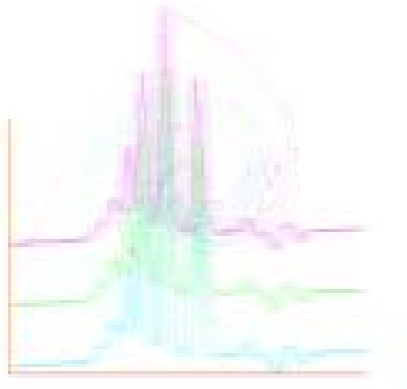

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