Size Exclusion Chromatography (SEC)

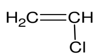
Fundamentals in Polymer Characterization

Definitions

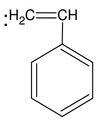
- Polymers: A chemical compound or mixture of compounds formed by polymerization and consisting essentially of repeating structural units (monomers)
- A Polymer sample is typically a mixture of polymer molecules having different chain lengths (molecular weights)

Common Monomers of Synthetic Polimers

Vinyl Chloride :



Styrene :



Ethylene : $H_2C{=}CH_2$

Propylene : $H_2C{=}CH{-}CH_3$

Ethylene and **propylene** are the most widely used monomers
PP + HDPE + LDPE + LLDPE : 25 % of main plastics world consumption

Different monomers combine to make a copolymer

Types of Polymers

- Natural occurring polymers
  - Natural rubber, starch, cellulose, proteins, chitosan, etc.
- Synthetic polymers
  - Polyethylene (PE), polypropylene (PP), polyhexamethylene adipamide (Nylon), polystyrene (PS), etc.
  - Widely used in our daily life (plastics, rubbers, and fibers).

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Molecular Characteristics of Polymers

- Molecular weight
- Composition
- Microstructure of copolymers
- Architecture

Molecular Characteristics of Polymers
Molecular Weight, M

$$— CH_2 — CH_2 — CH_2 — CH_2 — CH_2 — CH_2 —$$

M = Number of repeat units × molecular weight of one unit

A Polymer sample is a mixture of polymer chains varying in molecular weight distributions.

The molecular weight distribution and molecular weight averages are very important parameters for the polymer applications.

Principles of Molecular Weight Distribution

- Definition of MW averages :
  - A polymer is made of species (chains) of varying lengths. Each chain is characterized by its molecular weight, Mi, and its abundance ni. Then :

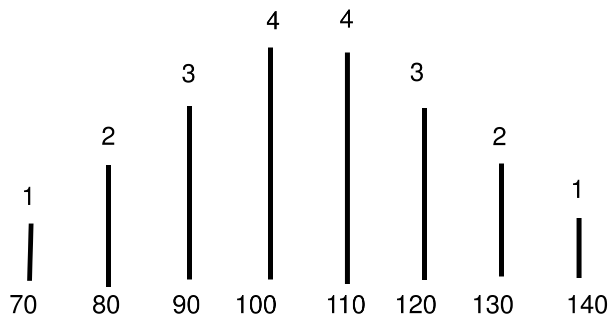
$$\mathbf{M_n} = \frac{\sum niMi}{\sum ni} \qquad \mathbf{M_z} = \frac{\sum niMi^3}{\sum niMi^2} \qquad \mathbf{I} = \frac{M_w}{M_n}$$

$$\mathbf{M_w} = \frac{\sum niMi^2}{\sum niMi} \qquad \mathbf{M_{z+1}} = \frac{\sum niMi^4}{\sum niMi^3}$$

I=Mw/Mn Mn<Mw<Mz<Mz+1

- Most of these values are necessary to characterize the polydispersity.
- These values can be calculated by different techniques but GPC is the most convenient and widely used method.

Mn Average



(70+160+270+400+440+360+260+140)/20 =105

1	70
1	80
1	80
1	90
1	90
1	90
1	100
1	100
1	100
1	100
1	110
1	110
1	110
1	110
1	120
1	120
1	120
1	130
1	130
1	140
20	2100

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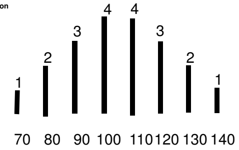
Mw Average



MW	ni	$MWxn_i$	$\frac{(MWxn_i)xMW}{\sum MWxn_i}$	$(MWxn_i)xMW$
70	1	70	2	4900
80	2	160	6	12800
90	3	270	12	24300
100	4	400	19	40000
110	4	440	23	48400
120	3	360	21	43200
130	2	260	16	33800
140	1	140	9	19600
	Σni 20	ΣMWxni 2100	$\frac{\sum (MWxn_i)xMW}{\sum MWxn_i}$ 108	$\sum (MWxn_i)xMW$ 227000 Average = **108** = 227000/2100

Molecular Weight Averages/ Physical Property Correlations

- Mn - can affect brittleness, flow and compression properties of the polymer.
- Mw - related to strength properties, and impact resistance
- Mz - related to elongation and flexibility (Elastomers)
- Mz+1 - related to die swell (Extrusion parameter)

Molecular Weight/ Physical Property Correlations

Property/Processs Parameter	Effect of High MW	Effect of Low MW
Impact Strength	⇧	⇩
Melt Viscosity	⇧	⇩
Processing Temp	⇧	⇩
Flex Life	⇩	⇧
Brittleness	⇧	⇩
Drawability	⇩	⇧
Softening Temp	⇧	⇩
Stress-crack Resistance	⇩	⇧
Melt Flow	⇩	⇧

Molecular Structure of Polymers Composition

— CH_2 — CH_2 —
ethylene unit A

CH_3
|
— CH_2 — CH_2 —
propylene unit B

– A – A – A – A – A – A – A – A – A – polyethylene

– B – B – B – B – B – B – B – B – B – polypropylene

– A – B – A – B – A – A – B – B – ethylene-propylene copolymer

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Molecular Structure of Polymers
Microstructures of Copolymers

A – ethylene   B – propylene

– A – A – B – A – B – A – A – B – B – random copolymer

– A – A – A – A – B – B – B – B – B – block-copolymer

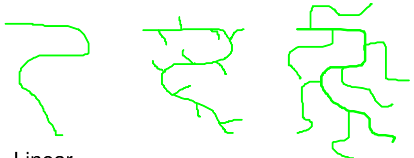
– A – B – A – B – A – B – A – B – A – alternating copolymer

< L > - average sequence length

Polymer Branching

A polymer chain can be linear or branched

in terms of its physical structure.



Predicting and Reproducing
Polymer Characteristics

- Molecular characteristics important for predicting performance characteristics
  - Molecular weight
    - molecular weight distribution
    - molecular weight averages
  - Composition
  - Microstructure
  - Architecture

Polymer Analysis Techniques

- High Performance Liquid Chromatography (HPLC)
  - mainly Size Exclusion Chromatography (SEC)
- Mass Spectroscopy (MS)
- Thermal Analysis (TA)
- Rheometry
- Nuclear Magnetic Resonance spectroscopy (NMR)
- Fourier Transform Infrared spectroscopy (FTIR)

Size Exclusion Chromatography (SEC)

Analysis of Polymers

- **GPC (or SEC)**
  - **RI, UV, or PDA**
  - **RI/Viscometry/Light Scattering**
- **GAP (or GPEC) gradient analysis of polymers or gradient polymer elution chromatography**
  - **UV, ELSD 2420 for additives and polymer blends or copolymers**
- **MS**
  - **LC/MS, GC/MS, MALDI-TOF-MS**
- **Thermal analysis**
  - **TGA, DSC**
- **NMR**
- **Fourier Transform Infrared FTIR**

Techniques for Analysis of Polymers

- Characterization of raw material
  - Room temp or high temp GPC
  - Thermal analysis
  - Rheology
- Polymer blends, alloys
  - GPEC or GAP
    - Gradient system with ELSD and/or UV detector
- Additives (antioxidants, slip agents, anti-UV)
  - Gradient analysis with UV/ELSD and/or MS detection
- More sophisticated detection
  - MS for additives and some polymers

Chromatography

- Size Exclusion Chromatography, or Gel Permeation Chromatography (GPC).
  - Separates molecules based on their hydrodynamic volume.
  - Isocratic mobile phase.
  - Most convenient in molecular weight determination.
- HPLC
  - Various separation mechanisms available.
  - Gradient or isocratic
  - Could be used to separate molecules based on their compositions.

GPC Basics

- GPC is used for the characterization of polymers :
  - Determining the average molecular weights
  - Determining the polydispersity, the distribution of MW
  - Obtaining branching information in large macromolecules
- All this information is important for :
  - Characterization of the raw material
  - Quality control
  - Control of degradation
  - Prediction of behavior. Physical properties are related to MW averages

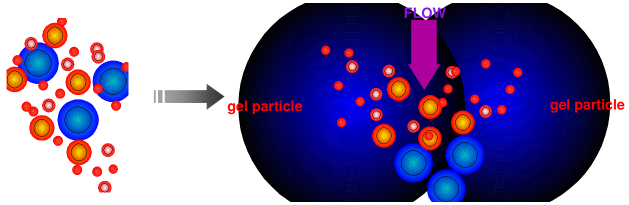
Size Exclusion Chromatography (SEC)

GPC Basics

- GPC known by many names
  - Gel Permeation Chromatography (GPC)
  - Size Exclusion Chromatography (SEC)
  - Gel filtration Chromatography (GFC)
- Separation is based on size exclusion
  - Actual behavior in solution, not molecular weight.
- All other interactions with the stationary phase (ion exchange, hydrophobic interaction) should be eliminated

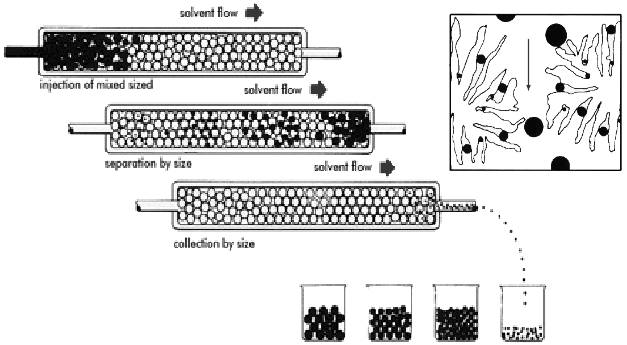
Separation Mechanism

- A dissolved polymer (comprised of a mixture of molecules) passes through a porous gel-based stationary phase
- The gel pores may be of uniform size or a blend of mixed sizes depending upon the column(s) chosen…



- Macromolecules eluted by decreasing order of molecular weight

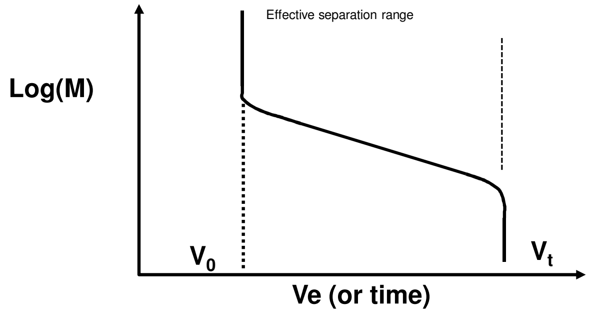
Separation Mechanism



Molecular size (molecular hydrodynamic volume) governs separation process.

Principles of Separation

- Simple relation between MW and elution volume.
- Above a limit all compounds are eluted at V_0.
- Under a limit all compounds are eluted at V_t.



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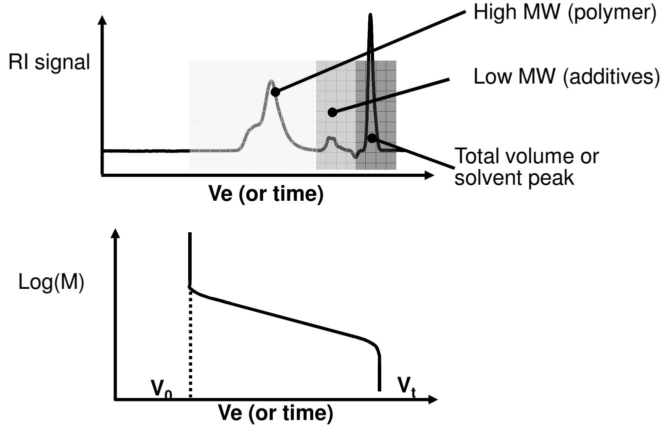
Size Exclusion Chromatography (SEC)

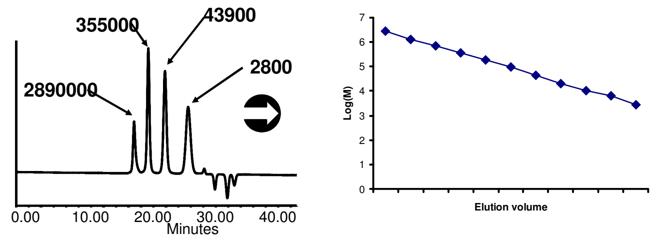
Principles of Separation



Principles of Calculations Step 1

Processing standards:

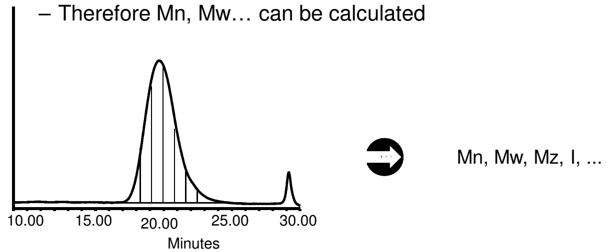
- Obtain Elution volume from data.
- Plot of log (M) versus f (Elution volume).



Principles of Calculations Step 2

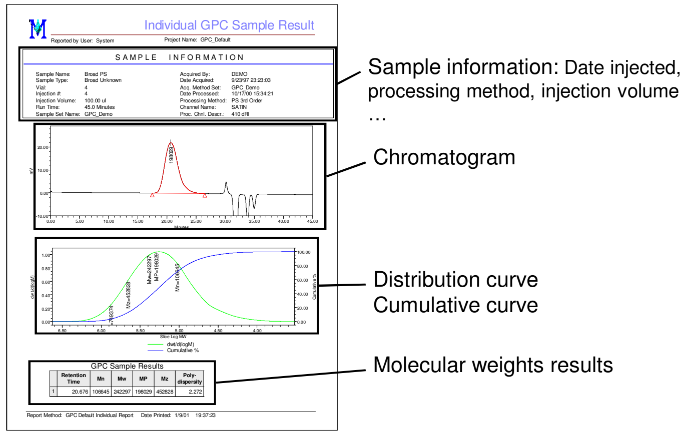
Processing unknowns:

- The signal is cut in slices (width dt)
- Each slice is characterized by
  - Elution volume, Vi
  - Area Hi.dt = niMi
- With the calibration curve Vi gives the MW in each slice, Mi
- ni for each slice is determined by the area and Mi
- Therefore Mn, Mw… can be calculated



Mn, Mw, Mz, I, ...

GPC, Principles of Calculations



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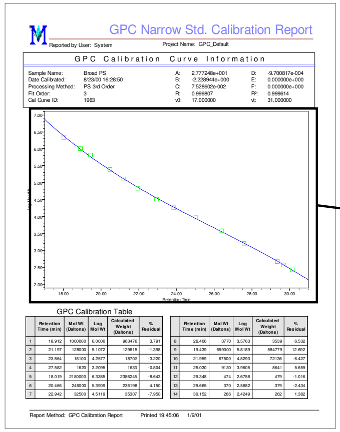
Size Exclusion Chromatography (SEC)

GPC, Principles of Calculations



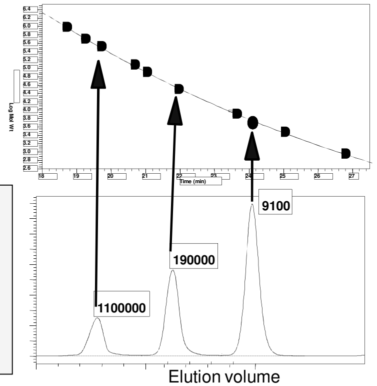
Which result is important ?

Calibration curve and points information

GPC Calibration Narrow Standards

To build a calibration curve :

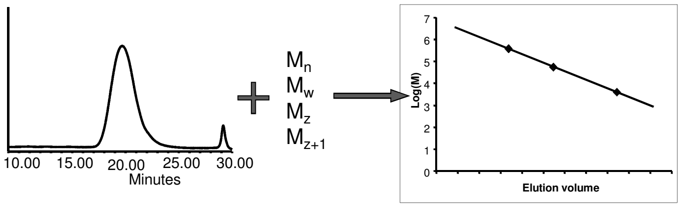
- Narrow disperse standards are injected
  - Polydispersity < 1.1
  - Elution volume at peak height
  - Curve Log(MW) = f(Ve)



Calibration:
1. >10 MW standards
2. Standards injected alone or as a mixture
3. Mixtures should have baseline resolved
4. Long process ! (But accurate)

GPC Calibration Broad Standards

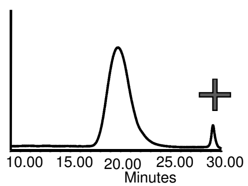
- Hamielec Method
  - A broad sample with known MW data (2 values at least) is injected
  - Processed as a Broad Standard
  - Calibration Curve is calculated



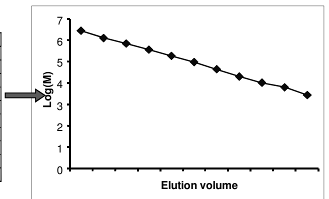
Saves time
Same broad reference for different labs

GPC Calibration Broad Standards

- **Cumulative Matching Method – Using *Distribution Table***
  - A broad sample with known distribution table (list a cumulative% - molecular weight) is injected
  - Information obtained from GPC or other technique
  - Processed as a Broad Standard
  - Calibration Curve is calculated



	RT	Mol Wt	Log Mol Wt	Cumulative %
1	18.207	1958675	6.291962	0.236
2	19.123	786046	5.895448	3.131
3	20.039	340400	5.531990	21.609
4	20.955	157437	5.197108	54.396
5	21.871	76970	4.886320	81.511
6	22.787	39368	4.595145	93.567
7	23.702	20850	4.319102	97.947
8	24.618	11316	4.053710	99.419
9	25.534	6230	3.794488	99.892
10	26.450	3443	3.536953	100.000



Saves time
Same broad reference for different labs
More accurate calibration curve with one injection

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Requirements for Successful GPC

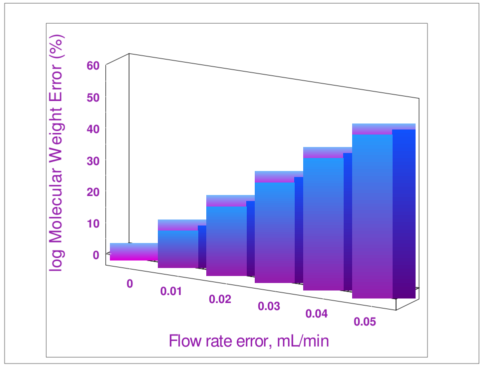
- Considerations
  - Mobile Phase
  - Sample Preparation
  - Column
  - Solvent Delivery System (Pump)

Solvent Delivery System

- Flow rate reproducibility/precision is critical
- Elution volume (or retention time) is converted to a mass with the calibration curve log(M)=f (Ve)
- A small difference in elution volume gives a large difference in the calculated mass.
- Minimize pulse or pressure changes
  - Increased sensitivity
  - Increased column lifetime

Solvent Delivery System

Precise flow is essential for precise GPC.



Non Aqueous GPC

Size Exclusion Chromatography (SEC)

Mobile Phase Selection

- In which solvent(s) is the sample completely soluble?
  - Organic or aqueous GPC
  - Common organic solvents:
    - THF (Tetrahydrofuran)
    - Toluene
    - DMF (Dimethylformamide)
    - TCB (Trichlorobenzene)
    - Special solvents such as HFIP (Hexafluoroisopropanol)
- At what temperature is the sample completely soluble?
  - Room temperature
  - Elevated temperature (40^{o}C to 85^{o}C)
  - High temperature (135^{o}C to 180^{o}C)

Common Solvents for Materials

- Mobile phase, solvents :
  - Synthetic elastomers (polybutadiene, polyisoprene)
    - Toluene, 75℃
  - PS, PVC, Styrene-Butadiene Rubber, Epoxy resins
    - Tetrahydrofuran, 40℃
  - Polyolefins (PE, PP, HDPE,LDPE)
    - Tri-chloro-benzene, 145℃ (or ODCB)
  - Polyurethane, ABS
    - Di-methylformamide 85℃
  - Other solvents : Hexafluoroisopropanol, m-cresol, N,N-Dimethylacetamide (DMAC)
  - Proteins, polysaccharides
    - Water / Buffers

Mobile Phase Preparation

- Filter and degas mobile phase
  - Filter mobile phase solvents using 0.5 micron filter to remove any particular impurities such as dusts, insoluble salts. This is to prevent any clog on columns. Gas in solvent contributes to baseline drift in RI response.
- Additives in mobile phase
  - Antioxidant is added to trichlorobenzene to keep solvent stable in high temperature.
  - Other additives eliminate adsorption or interaction of solutes with column packing materials

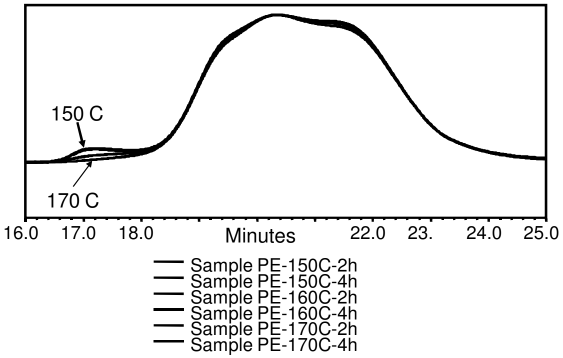
Sample Preparation

- Concentration
  - About 1mg/ml, usually less than 3mg/ml
  - Samples with broad molecular weight distribution may require higher concentrations.
- Injection volume
  - 50 – 100 μL/column
- Dissolution time
  - Usually overnight
- Agitation and filtration
  - Generally filtration is required to remove insoluble impurities and agitation is allowed.
  - Do not agitate and filter samples that contain very high MW (>1 million). Consider centrifugation.

Size Exclusion Chromatography (SEC)

Sample Preparation

Effect of temperature and time dissolution on the chromatogram shape (molecular weight sensitive detector)



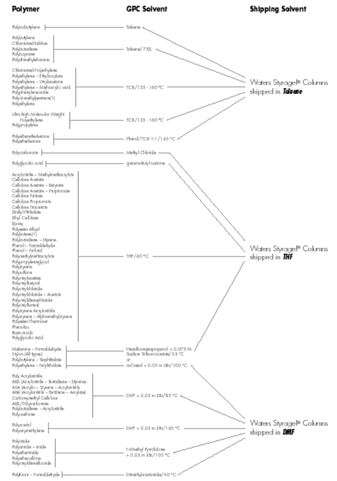
GPC Column Selection

- Considerations
  - Column shipping solvent
    - Compatible with Mobile phase / Sample solubility
  - Column effective molecular weight range
    - Molecular weight range of sample
      - Known / unknown
  - Particle size of column packing material
    - Resolution requirements
    - Shearing of high molecular weight samples
  - Temperature requirements
    - Total sample solubility
  - Column dimensions
    - Conventional 7.8 x 300mm
    - Solvent efficient 4.6 x 300mm
    - High Speed 6.0 x 150mm

GPC Column Selection

- Column shipping solvent
  - The choice of column shipping solvent depends on the mobile phase required to obtain complete sample solubility
  - Organic GPC columns are shipped in a variety of solvent choices
    - Toluene, THF, DMF, ODCB
  - In most cases sample solubility (mobile phase) can be achieved with one of these choices
    - Temperature elevation is sometimes required
  - For samples not soluble in the available shipping solvents, choose a column with a shipping solvent that has similar polarity to the mobile phase required for sample solubility
    - Minimize the shrinking or swelling of the packing material
      - Will result in mechanical failure of the packed bed

GPC Solvent Selection Guide



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Size Exclusion Chromatography (SEC)

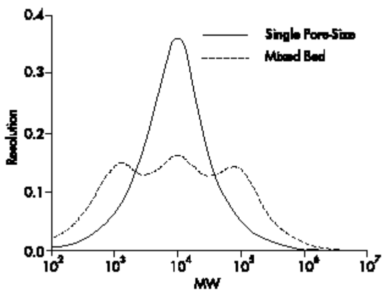
GPC Column Selection

- Column effective molecular weight range
  - Choosing columns with the appropriate effective molecular weight range depends entirely on the molecular weight range of your sample
    - The effective molecular weight range of your column bank should not exceed the molecular weight range of your sample
  - The pore size of porous packing materials is related to the column working molecular weight range
  - For organic GPC the molecular weight range of the column is relative to PS. May not be the true molecular weight range for a specific polymer
  - Columns are available as individual pore-size or mixed bed (extended range) columns

GPC Column Selection

- Individual pore-size columns vs. Mixed bed columns
  - Individual pore-size columns deliver greater pore volume and resolution in a more concentrated molecular weight range
  - Mixed bed (extended range) columns are ideal for use as scouting columns when the molecular weight range of your sample is unknown or for working on samples with broad molecular weight distributions.
- Choosing the columns with right molecular weight range is a challenge.
  - Typically use different column sets for different MW ranges

Individual Pore vs. Mixed Bed Resolution Comparison



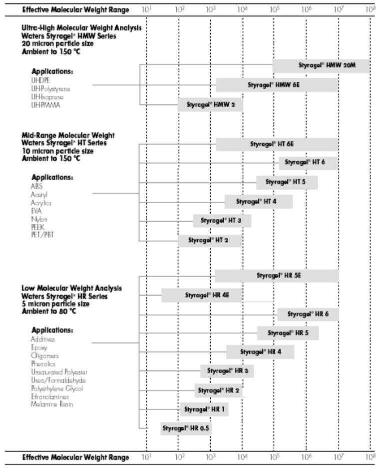
Individual pore-size columns deliver up to 2.5 times the resolution of mixed-bed columns.

GPC Column Selection

- Choosing the right particle size of column packing material
  - Conventional GPC columns are available in different particle sizes
    - 5µm, 10µm, 20µm
  - Choosing the right particle size is sample-dependent
    - 5µm: offer the highest resolution:
      - Ideal for low-to-mid molecular weight range samples where maximum resolution and efficiency is often required (Styragel® HR series)
    - 10µm: more robust than 5µm
      - Ideal for mid-to-high molecular weight samples. Can be used at ambient or high temperature (Styragel® HT series)
    - 20µm:
      - Designed for the analysis of shear-sensitive, ultrahigh molecular weight polymers (Styragel® HMW series)

Size Exclusion Chromatography (SEC)

GPC Column Selection Guide



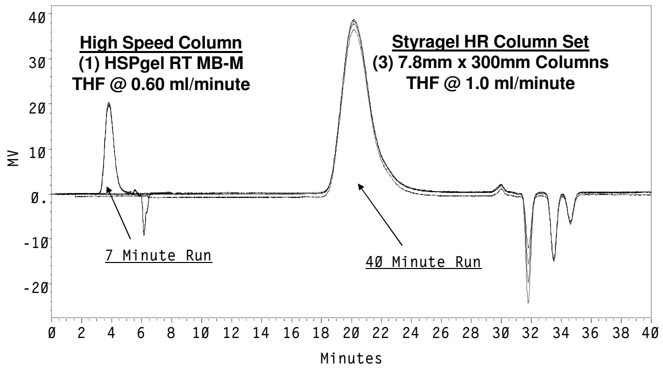
GPC Column Selection

- Choosing the right column dimensions
  - Waters offers analytical GPC columns in three dimensions
    - 7.8 x 300mm Conventional GPC (5, 10 or 20µm particles)
      - 1mL/minute flow rate
      - Typically run in banks of three or four columns
      - Run times: 45-60 minutes
      - Offer maximum pore volume / efficiency
    - 4.6 x 300mm Solvent Efficient GPC columns (5, 10 or 20µm particles)
      - 0.3mL/minute flow rate
      - Typically run in banks of three or four columns
      - Run times: 45-60 minutes
      - Offer 2/3 solvent reduction compared to conventional GPC columns

GPC Column Selection

- Choosing the right column dimensions
  - 6.0 x 150mm High Speed GPC columns (HSPgel™ series)
    - Smaller particles 3, 3.5, 5 & 10µm options
    - Provides sufficient pore volume and molecular weight resolution for accurate and precise molecular weight measurements
    - Flow rate ~0.6mL/minute
    - Run times as low as 7 minutes
      - Up to 6x faster than conventional GPC columns
    - Solvent reduction of 80 – 90% compared to conventional GPC
    - Requires high-precision solvent delivery HPLC system (Waters Alliance® System)
    - Requires broad standard calibration techniques
      - More information available in Waters "High Speed GPC" seminar

High Speed GPC vs. Conventional GPC



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Size Exclusion Chromatography (SEC)

Basic Column Information

- Columns are put together in series to form a bank (>2)
- Highest MW column first
  - Reduces back pressure on the most fragile column (low molecular weight column)
- Ramp the flow up slowly
  - 0.1ml/minute – 1.0 ml/minute in small increments
- Change over solvent at a 0.1 ml/minute flow rate
  - Typically done over night

Sample Concentration

1) > 1,000,000MW, let sit for 12 to 24 hrs. Agitate very gently for dissolution.
2) Strong agitation may degrade the sample.
3) Solute concentration ~ 0.01 to 0.30%
4) Injection volume ~20 to 100ul per column

Molecular weight	Sample concentration on column	Injection volume per column	
		4.6mm i.d.	7.8mm i.d.
< 5,000	less than 0.30 w/v%	20 - 50µl	50 to 100µl
5,000 to 25,000	less than 0.20%		
25,000 to 200,000	less than 0.10%	10 - 30µl	
200,000 to 2,000,000	less than 0.05%		
> 2,000,000	less than 0.025%		

Water Soluble Polymers

Aqueous GPC Analysis

Ionic Considerations

- Neutral Polymers
  - Polyethylene Oxide, Polysaccharides, Polyvinyl Alcohol
- Anionic Polymers
  - Polyacrylic Acid, Polyalginates, Hyaluronic Acid
- Cationic Polymers
  - Polyvinyl Amine, Chitosan, Polylysine

Size Exclusion Chromatography (SEC)

Column Chemistry

- Hydroxylated Methacrylate Gels – Ultrahydrogel
  - pH 2 – 12
  - Maximum temperature 80°C
  - Maximum organic loading 50%
    - Acetonitrile, methanol
    - Introduce organic slowly (gradient)
  - Packing has an overall anionic charge

Calibration in Aqueous GPC

- Somewhat limited in choice of narrow standards
  - Poly(ethylene oxides)
    - ~18,000 – 900,000
  - Poly(ethylene glycols)
    - monomer - ~12,000
  - Pullulans, (trioses)
    - ~10,000 – 850,000
- Calibration fit order critical for high MW
  - Many water soluble polymers have MWs >1,000,000
  - Linear fit may be best

Non-Size Exclusion Effects in Aqueous GPC

- Intramolecular Electrostatic Interactions
- Ionic Interactions
  - Ion exclusion
  - Ion exchange
- Adsorption
  - Hydrogen bonding
  - Hydrophobic interactions

Non-Size Exclusion Effects (cont'd)

- Solvophobic Interactions
- Memory Effects

Size Exclusion Chromatography (SEC)

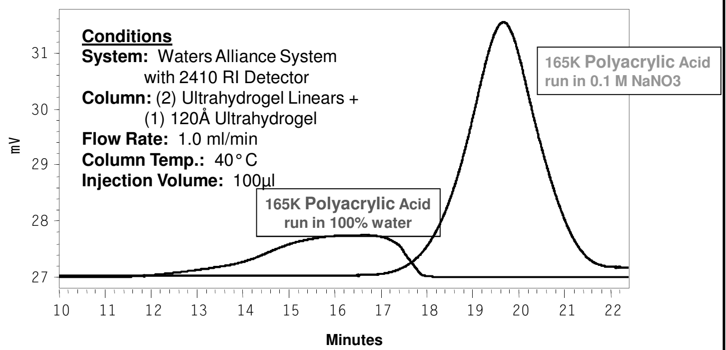
Intramolecular Electrostatic Interactions

- Polyelectrolytes have ionic groups present
  - Charged groups repel one another
  - Polymer chain expands
  - Polymer elutes at much earlier retention time
  - Independent of column chemistry
- Add 0.05M – 0.10M salt
  - $NaNO_3$, for example
  - Salt effectively shields the charge

Ion Exclusion

- Polyelectrolyte has same charge as column packing
  - Permeation into pores is hindered
  - Retention time is earlier than expected
  - Addition of 0.10M salt will shield the effect
  - Vary the salt level until retention time stabilizes
- Example: Polyacrylic acid on methacrylate gel packings

Ion Exclusion Effects Polyacrylic Acid



Ion Exchange

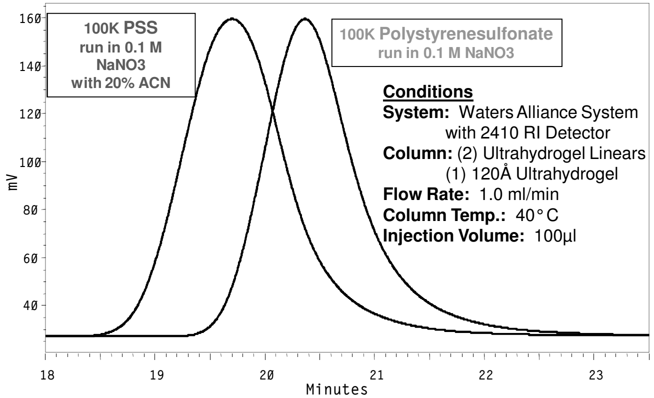
- Polyelectrolyte has different charge than column packing
- Packing charges can act as weak ion exchange sites
  - Carboxyl group in methacrylate gels
  - Polymer will adsorb onto column packing and may not elute
- Example: Chitosan (a glucosamine)
- Adjust pH of eluent (~0.50M Acetic Acid)
- Add electrolyte (0.30M Sulfate works well)

Size Exclusion Chromatography (SEC)

Hydrophobic Interactions

- Attraction between “nonionic” portion of polyelectrolyte and “non ionic” portion of packing
  - Polyelectrolyte adsorbs onto column packing
  - Polyelectrolyte is not truly hydrophilic
  - Eluent must be modified
  - Addition of 20% acetonitrile or methanol
  - Sulfonated polystyrene is an example

Hydrophobic Interactions
Sulfonated Polystyrene



Solvophobic Association

- Polyelectrolyte is not dissolved into a true solution
- Some association occurs, (agglomeration, “gelling”)
  - Sample elutes early
  - Particles may be filtered out, giving non-reproducible MW results
  - Addition of 0.10M salt shields effect

Memory Effects

- Cationic Polymers on Methacrylate Gels
- If there is any adsorption occurring:
  - Subsequent injections of cationic polymer will be attracted to adsorbed material
  - Condition worsens as more injections made
  - Elution occurs later and later, (lower and lower MWs)
  - Loss of pore volume, pressure may increase
- Correct eluent must be chosen up front

Size Exclusion Chromatography (SEC)

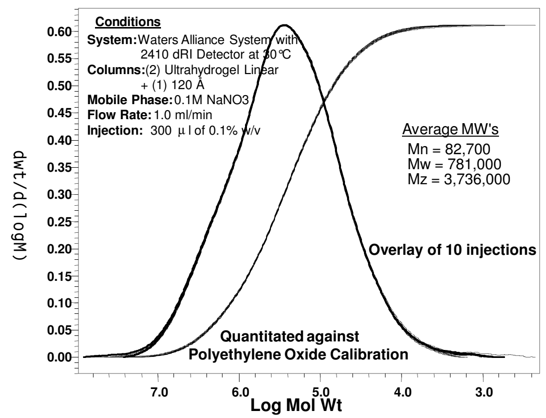
Aqueous GPC Eluent Guide

Eluent	Neutral Polymers	Anionic Polymers	Cationic Polymers
Pure Water	May be O.K.	Exclusion	Adsorption
0.10M NaNO3	O.K., if not, then ↓	O.K., if not, then ↓	Adsorption
0.10M NaNO3/ACN, (80:20)	O.K.	O.K.	Adsorption

Eluent Guide (Cont'd)

Eluent	Neutral Polymers	Anionic Polymers	Cationic Polymers
0.50M -0.80M NaNO3	NA	NA	O.K., if not, then ↓
0.30M sulfate/0.5M Acetic Acid	NA	NA	O.K.

Hydroxyethyl Cellulose



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