Polymer Characterization – Gel Permeation Chromatography

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Gormley Lab Group Meeting

October 9th, 2024

Learning Objectives

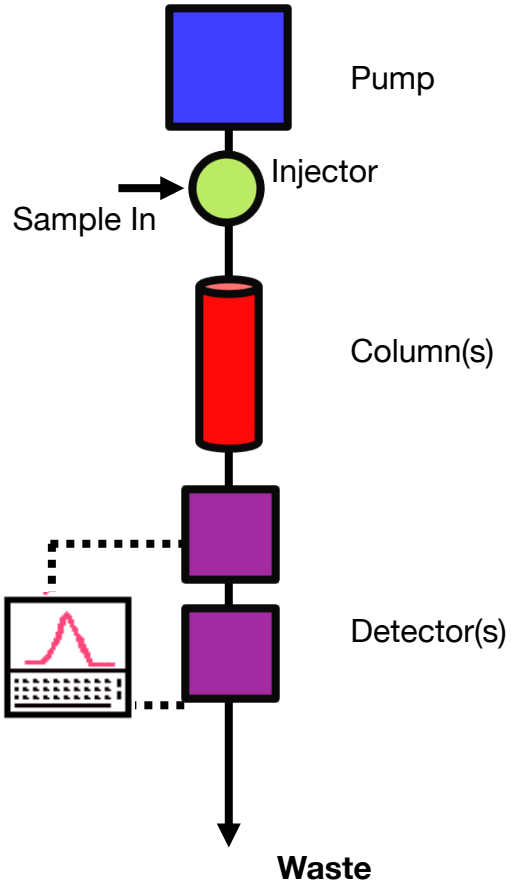
- Develop foundational understanding of gel permeation chromatography as a analytical technique to assess macromolecule physiochemical properties
- Review practical considerations for determining molecular weight of polymers via GPC to make well-informed decisions for experimental setup (i.e., column selection) and analysis (i.e., standard selection)
- Understand roles and tradeoffs of various detectors in multimodal GPC

Overview of Today's Presentation

Part 1:
Basics of Gel Permeation Chromatography as a Characterization Technique for Macromolecules

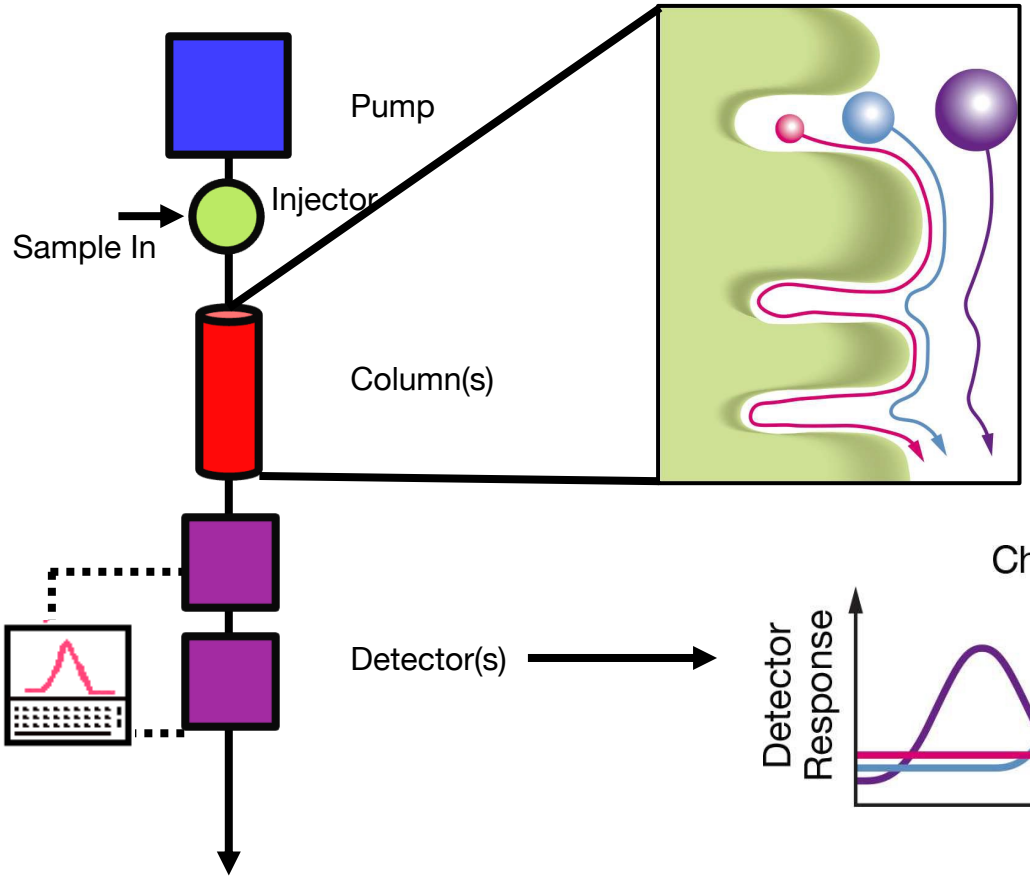
Part 2:
On-Line Multi-Angle Light Scattering (MALS) for Absolute Molecular Weight Characterization

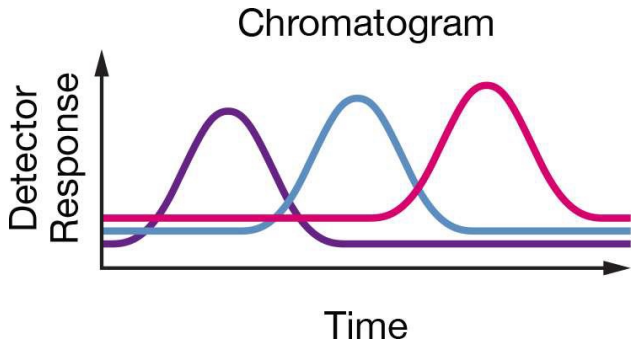
Principles of GPC



- Gel permeation chromatography (GPC) or size exclusion chromatography (SEC) is one of numerous types of chromatography used to separate and fractionate heterogenous mixtures of analytes
- In GPC, molecules are separated based on their size, or in more technical terms, their hydrodynamic volume.
- Assumes that no additional interaction occurs between column and analyte (i.e. charge-charge interaction, hydrophobic interaction)

Principles of GPC



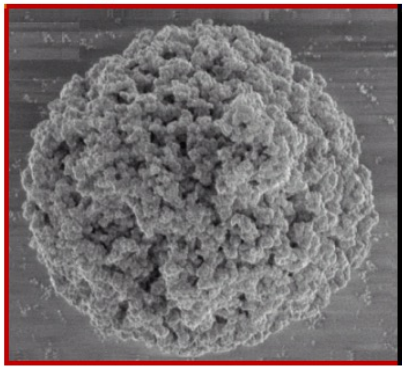


Large particles cannot enter the gel and are excluded. They have less volume to traverse and elute sooner.

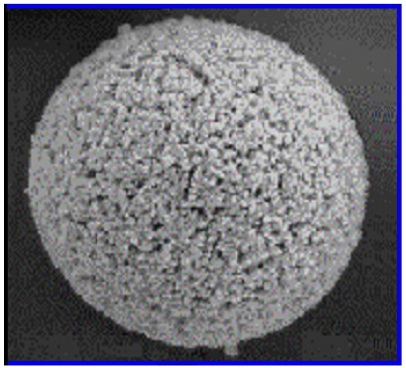
Small particles can enter the gel and have more volume to traverse. They elute later.

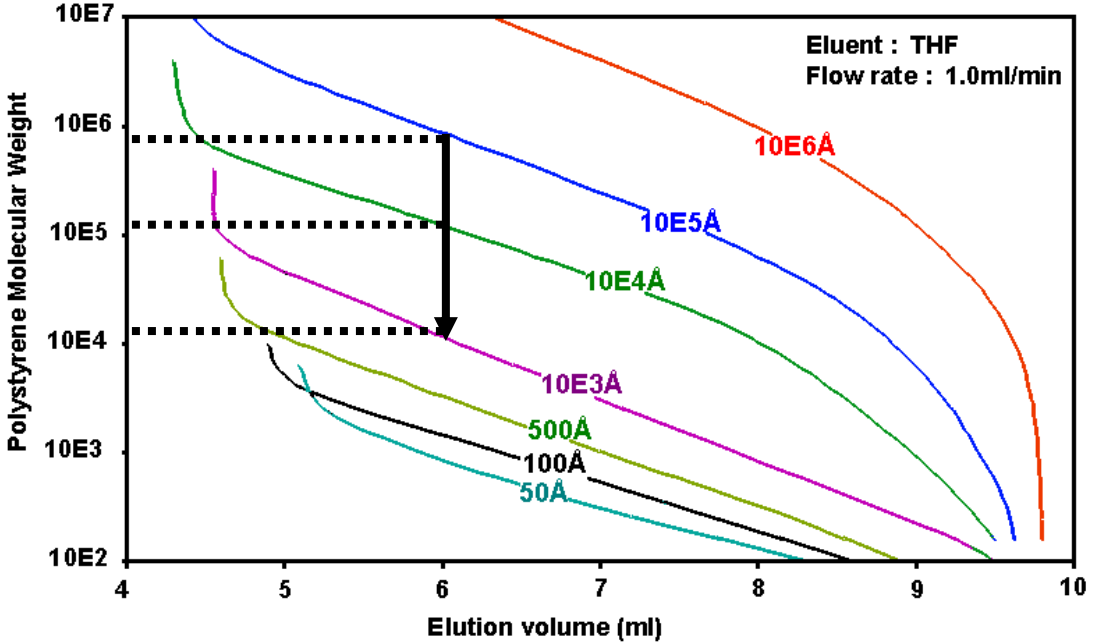
Pore Size Effects Separation Range

PLgel 10 um 10^6A



PLgel 10 um 10^3A





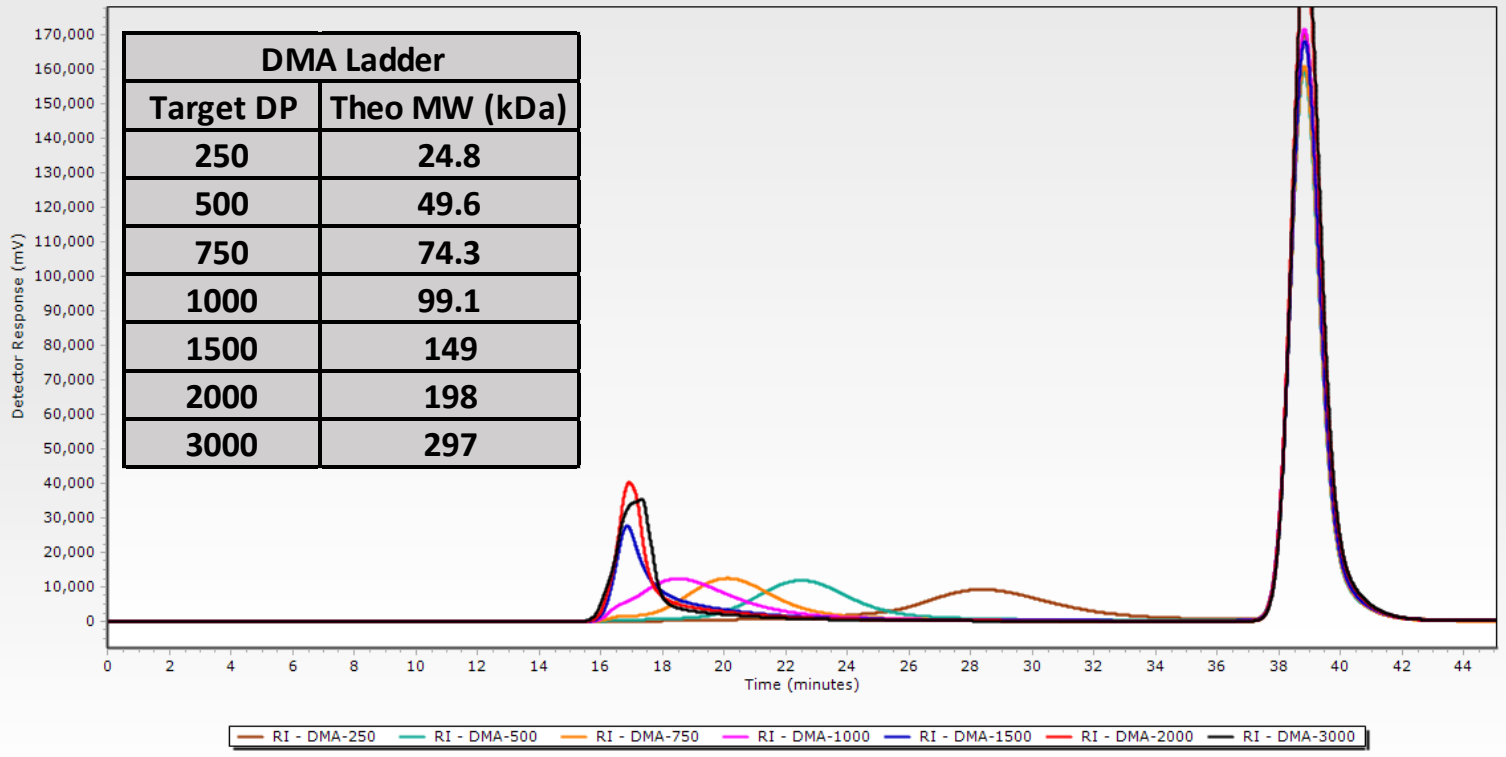
Smaller pore size shifts separation range to lower MWs

https://www.agilent.com/cs/library/slidepresentation/public/Maximizing_Performance_Through_Column_Selection_May_2015.pdf

Column Selection Matters!

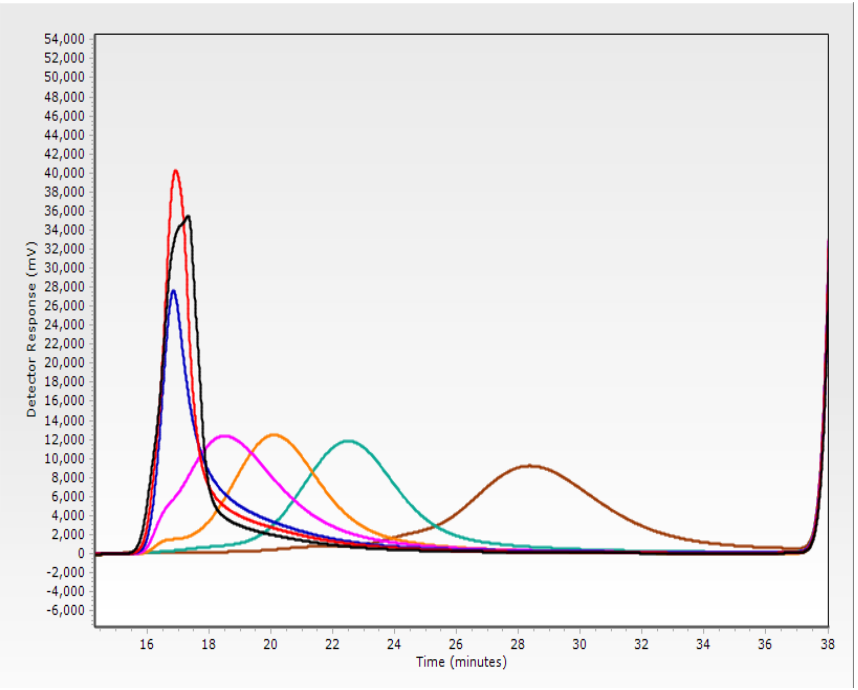
Superose 12 10/300 GL

DMA Ladder	
Target DP	Theo MW (kDa)
250	24.8
500	49.6
750	74.3
1000	99.1
1500	149
2000	198
3000	297

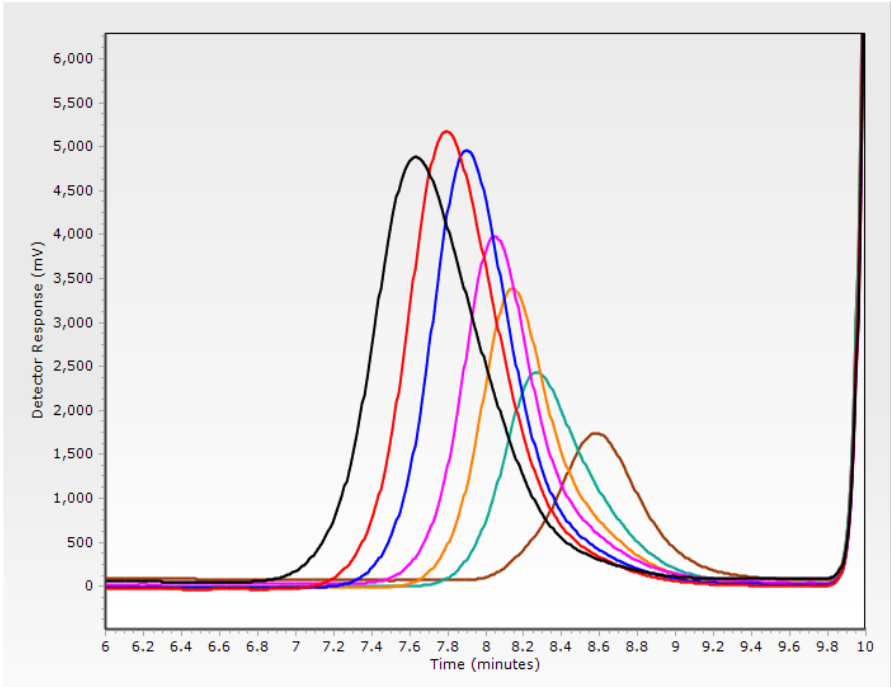


Column Selection Matters! (Cont.)

Superose 12 10/300 GL



PL Aquagel-OH MIXED-H, 7.5 x 300 mm, 8 µm



Other Factors to Consider in Column Selection

- Efficiency/ Resolution – Ability of column to cleanly separate peaks of small MW differences
  - Function of length, particle size, and MW range
- Solvent Compatibility
  - Need to select solvent that both solubilizes the analyte of interest and minimzies interaction with the column
  - Organic: Polar, Non-polar
  - Aqueous: pH range, Tolerance to organic co-solvent

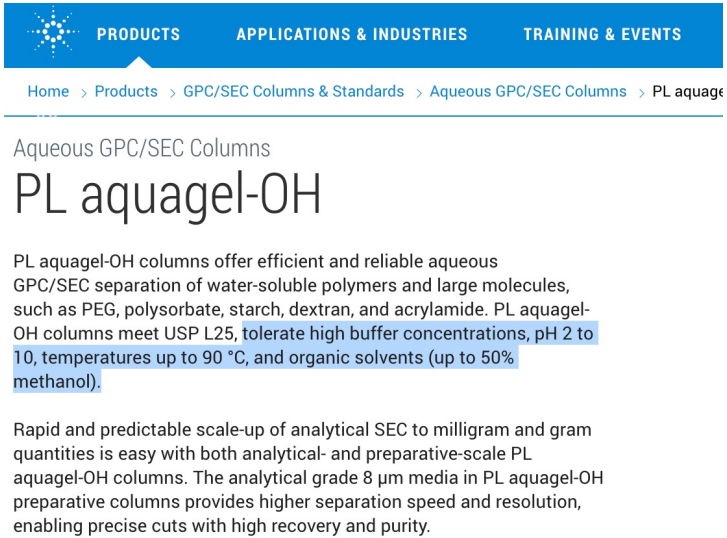
Other Factors to Consider in Column Selection (Cont.)

Just google it!

Ordering information

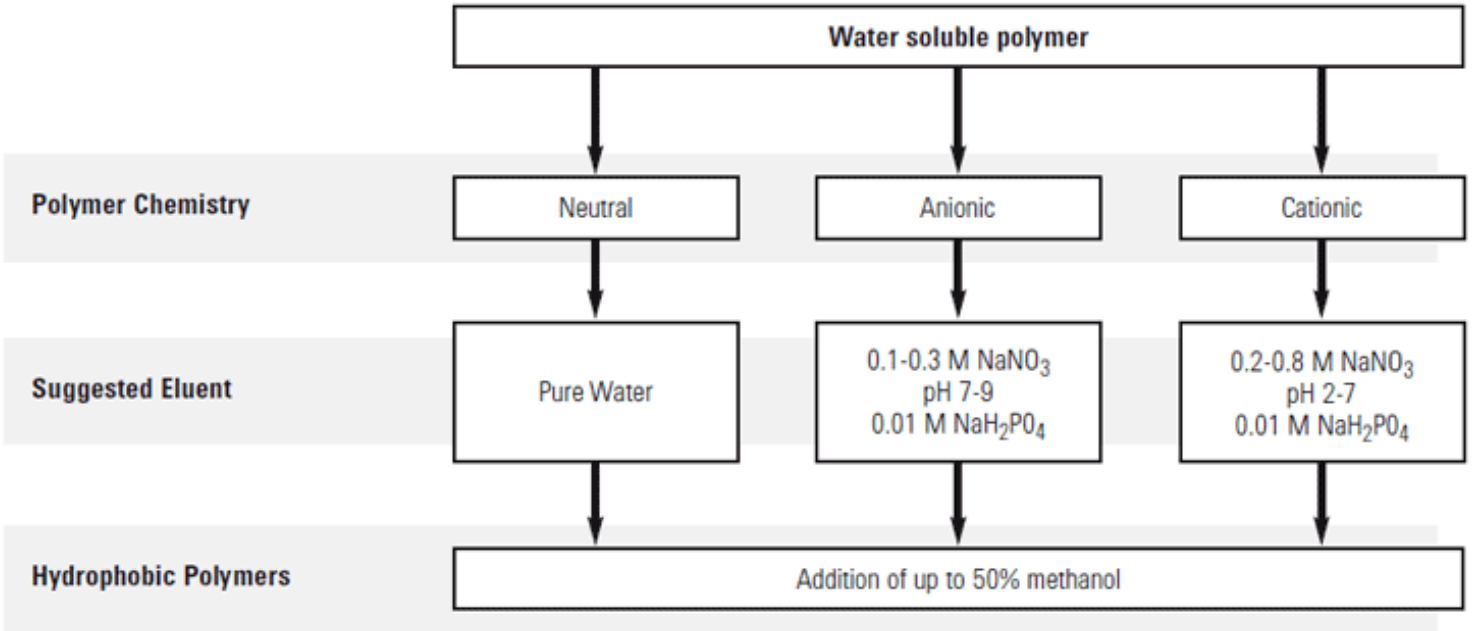
PL aquagel-OH Columns, 300 x 7.5 mm

Description	Particle size (µm)	MW range (g/mol) (PEG/PEO)	Guaranteed Efficiency (p/m)
PL aquagel-OH 20	5	100 to 10,000	>55,000
PL aquagel-OH 30	8	100 to 30,000	>35,000
PL aquagel-OH 40	8	10,000 to 200,000	>35,000
PL aquagel-OH 40	15	10,000 to 200,000	>15,000
PL aquagel-OH 50	8	50,000 to 1,000,000	>35,000
PL aquagel-OH 50	15	50,000 to 1,000,000	>15,000
PL aquagel-OH 60	8	200,000 to >10,000,000	>35,000
PL aquagel-OH 60	15	200,000 to >10,000,000	>15,000
PL aquagel-OH MIXED-H	8	100 to 10,000,000	>35,000
PL aquagel-OH MIXED-M	8	>600,000	>35,000



Solvent Selection – An Example

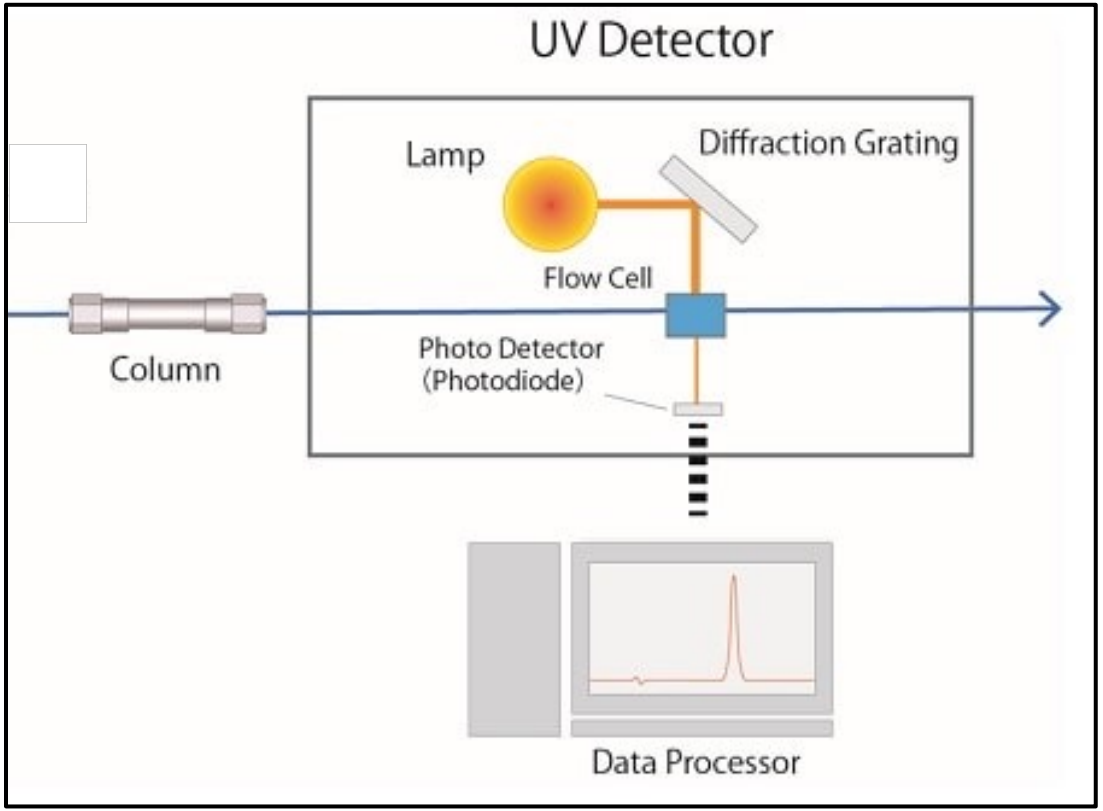
PL Aquagel-OH SEC Analytical Guide to Eluent Selection



* Only applies to PL Aquagel columns!

https://www.agilent.com/en/products/gpc-sec/gpc-sec-columns/aqueous-polar-organic-gpc-sec-columns/pl-aquagel-oh-sec-analytical/guides

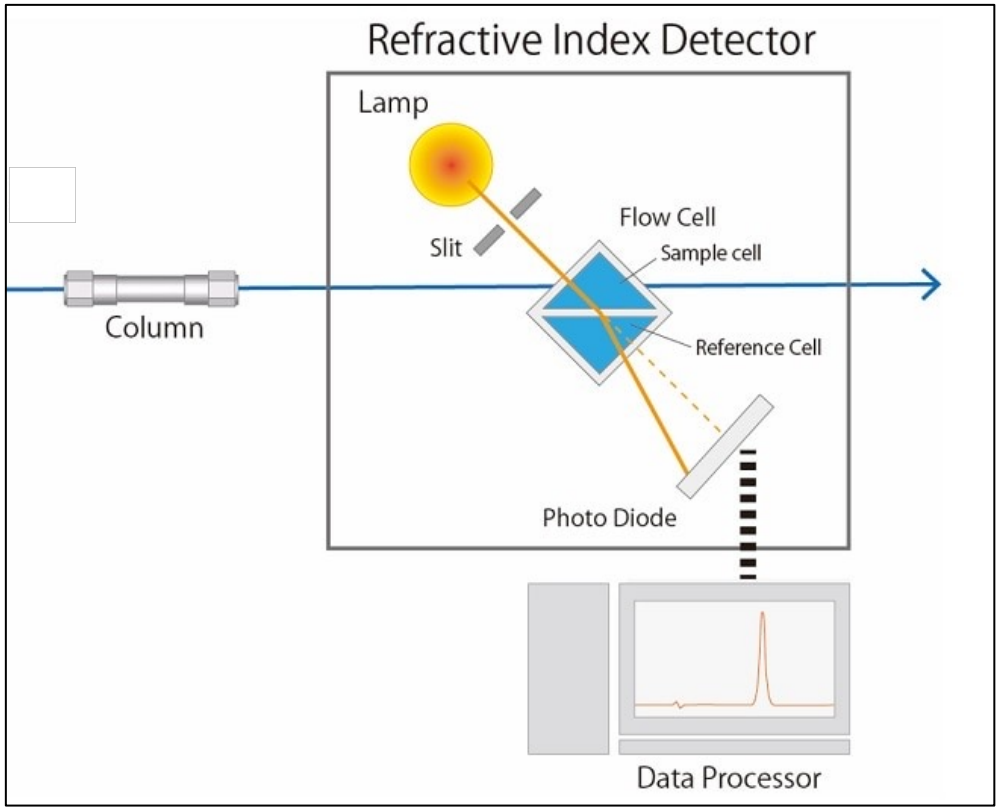
Principles and Considerations for UV Detector



- Photo detector measures absorbance of analyte as it passes through flow cell
- Note: Need to keep in mind absorbance spectra of macromolecule and select appropriate wavelength to maximize intensity and/or specificity of signal!
- Other considerations for RAFT polymers: MW-dependence of extinction coefficient, extent of livingness, etc.

https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/basic/absorbance_detector.html

Principles and Considerations for RI Detector

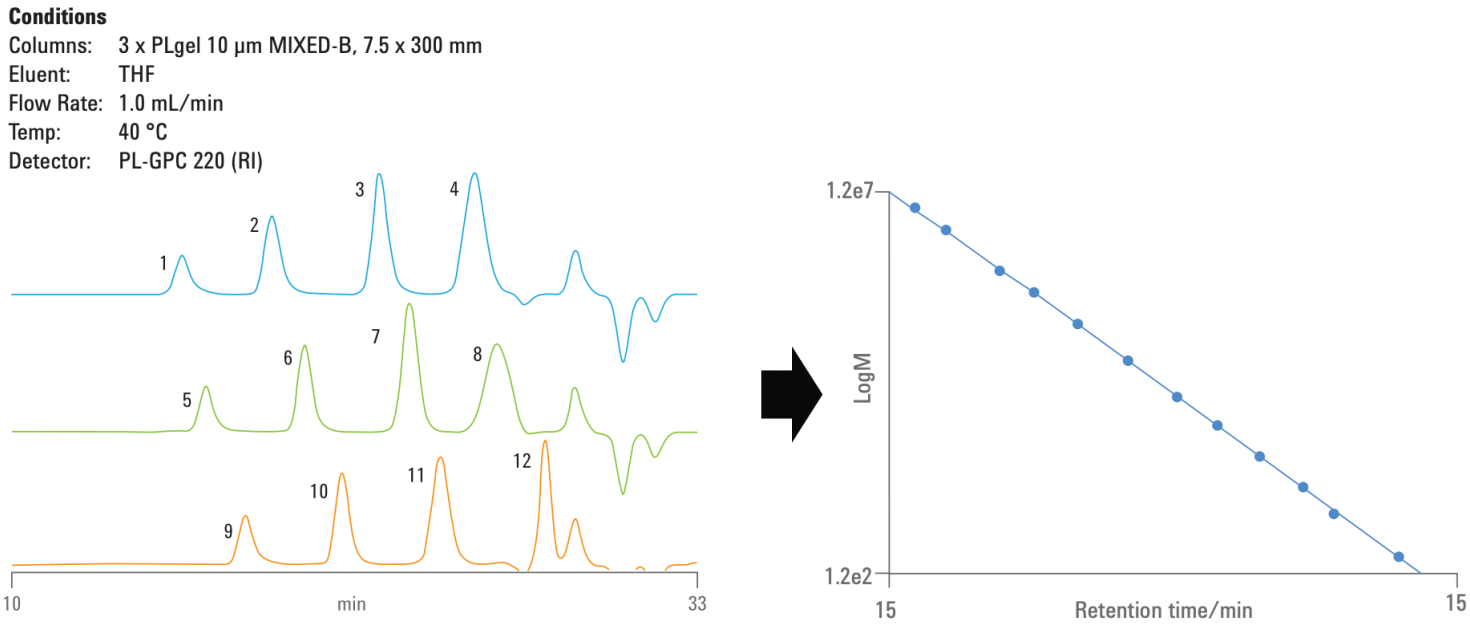


- If sample cell only contains mobile phase, no refraction because both cells have same refractive index (dRI ~0)
- However, as analyte passes through light refracted at interface of sample cell and reference cell
- RI increases as concentration of analyte increases
- Note: much more sensitive than UV for most RAFT polymers. However, also very sensitive to any other species in solution.

https://www.shimadzu.com/an/service-support/technical-support/analysis-basics/basic/refractive_index_detection.html

Determining MW from GPC Chromatograph

GPC standards aim to produce a mathematical model for log M versus retention time ("calibration curve") that can then be applied to unknown samples



https://www.agilent.com/cs/library/brochures/5990-7996E_GPC-standards.pdf

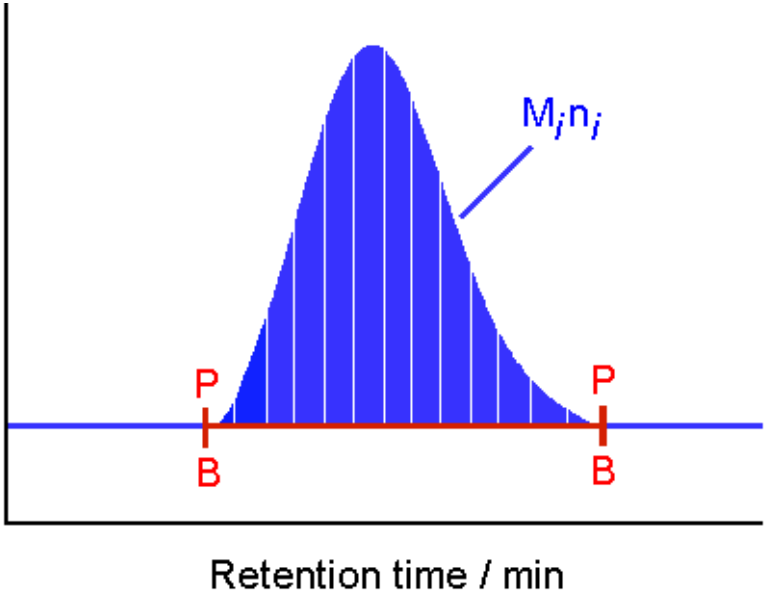
Determining MW from GPC Chromatograph (Cont.)

- Selection of Standard
  - PEG- and PMMA-based calibration curves produce different MWs because hydrodynamic size of polymer coils varies!
  - MW values reported assume your samples share the exact same conformation as the reference standards
  - For accurate MW results, should compare against standards with most similarity to your polymer!

Common Macromolecule Standards for Calibration		
Polymer Type	**GPC Compatibility**	**Notes**
Polystryene (PS)	Organic	Hydrophobic
Polymethylmethacrylate (PMMA)	Organic	Hydrophobic
Polyethylene (PE)	Organic	Hydrophobic
Polyethylene Glycol (PEG)	Organic/ Aqueous	Hydrophilic
Pullulan	Organic/ Aqueous	Polysaccharide
Polyacrylic Acid Sodium Salt	Aqueous	Charged
Protein	Aqueous	Self-Explainatory

How MW Calculations Actually Work

Chromatograph is segmented into discrete slices.
MW is then calculated for each slice based on calibration curve.
Averages (i.e. Mn and Mw) are calculated from this discretized MW profile.



Number-Average MW

$$M_n = \frac{\sum M_i N_i}{\sum N_i} = \sum M_i x_i$$

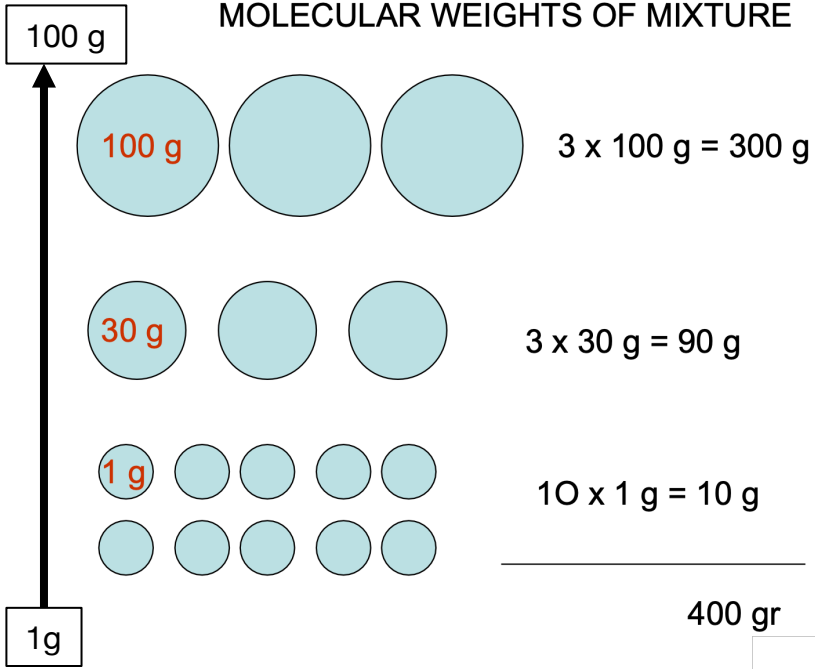
N_i is the number of molecules with weight M_i
x_i is the number fraction (or mole fraction) of polymer with molecular weight M_i.

Weight-Average MW

$$M_w = \frac{\sum M_i^2 N_i}{\sum M_i N_i} = \sum M_i w_i$$

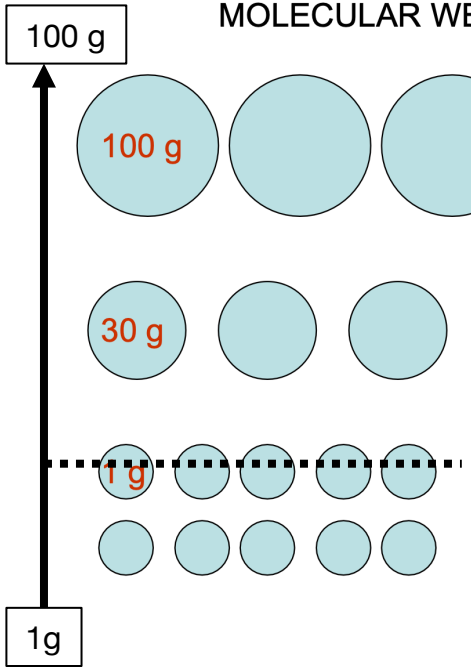
w_i is the weight fraction of polymer with molecular weight M_i.

How MW Calculations Actually Work – An Example



Q: Where do you expect the number-average MW of this mixture to be?

How MW Calculations Actually Work – An Example



MOLECULAR WEIGHTS OF MIXTURE

3 x 100 g = 300 g

3 x 30 g = 90 g

1O x 1 g = 10 g

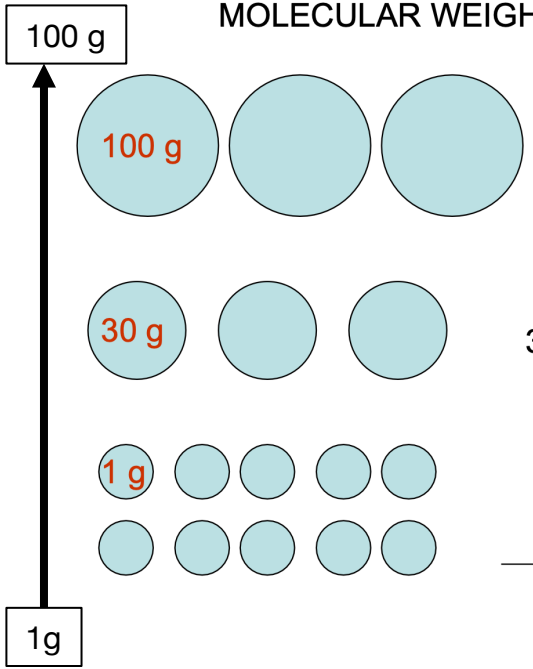
400 gr

Number average = 100 x (3/16) + 30 x (3/16) +1 x (10/16) =

$$\frac{400\ g}{16} = 25\ g/ea$$

$$M_n = \sum M_i x_i$$

How MW Calculations Actually Work – An Example



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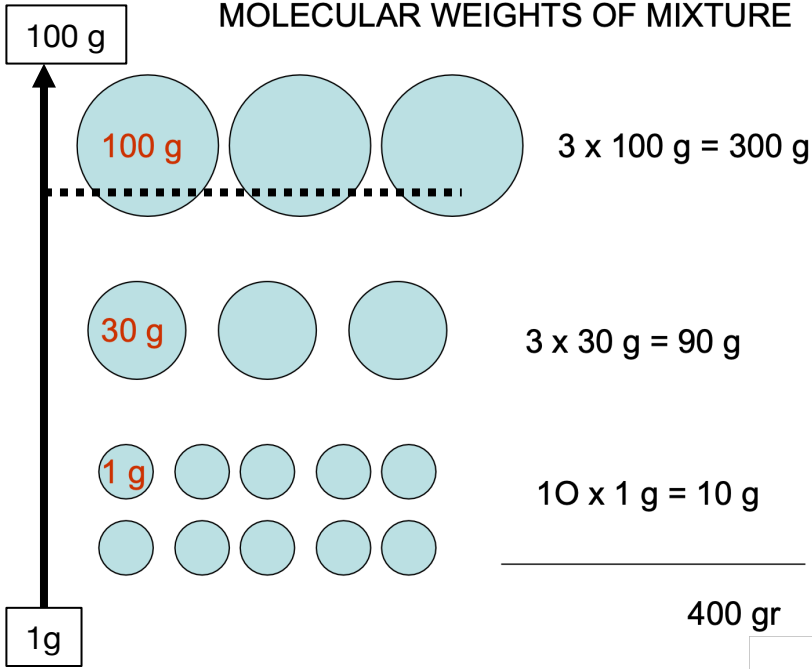
Q: What about weight-average MW?

How MW Calculations Actually Work – An Example



Number average = 100 x (3/16) +
30 x (3/16) +1 x (10/16) =

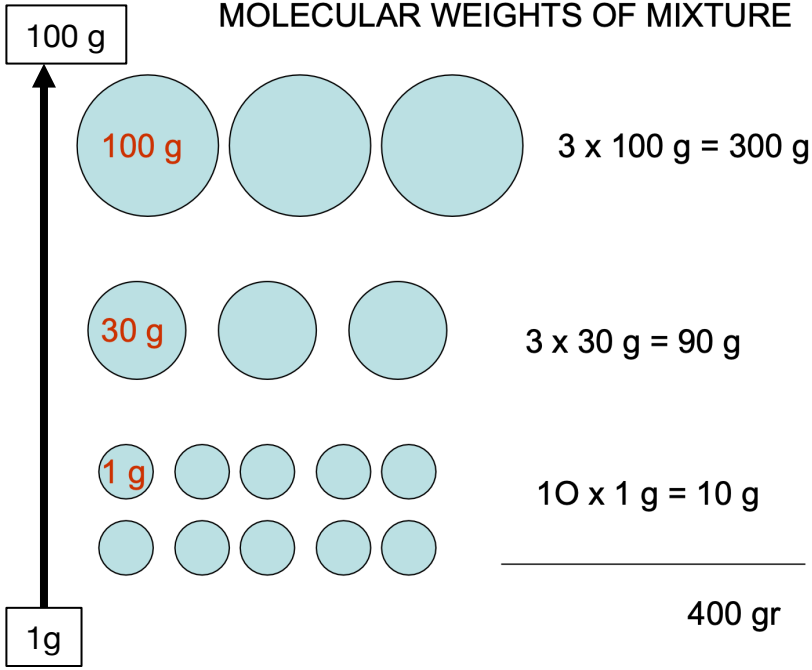
$$\frac{400\ \text{g}}{16} = 25\ \text{g/ea}$$

$$M_n = \sum M_i x_i$$

Weight average = 100 x (300/400) +
30 x (90/400) + 1 x (10/400) = 81.8 g/ea

$$M_w = \sum M_i w_i$$

How MW Calculations Actually Work – An Example



Number average = 100 x (3/16) + 30 x (3/16) +1 x (10/16) =

$$\frac{400\text{ g}}{16} = 25\text{ g/ea}$$

$$M_n = \sum M_i x_i$$

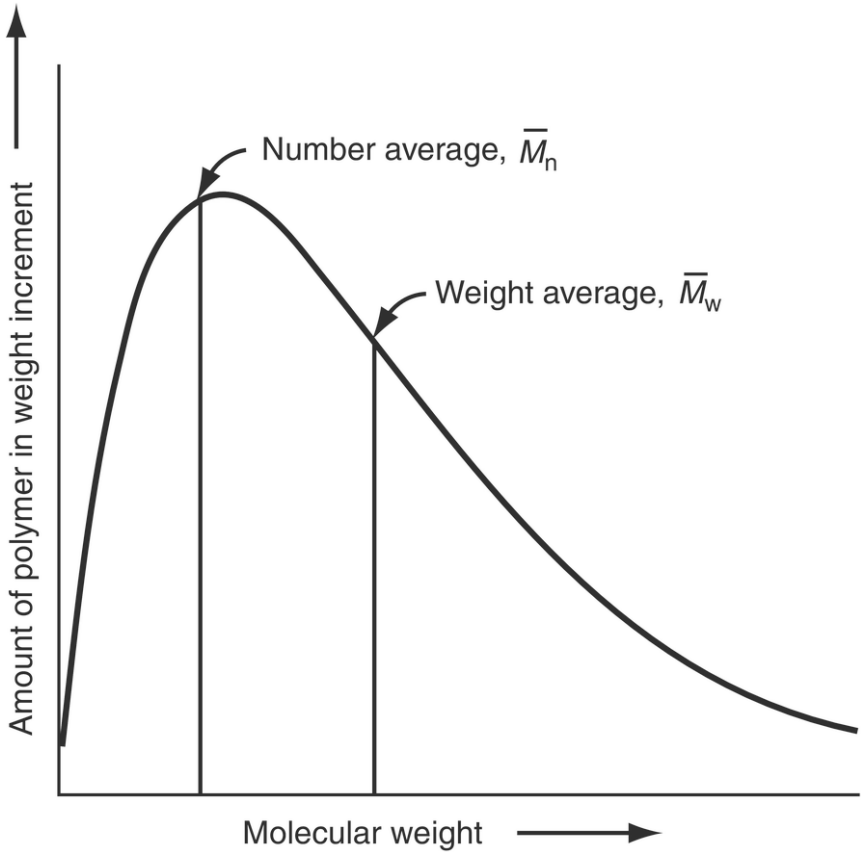
Weight average = 100 x (300/400) + 30 x (90/400) + 1 x (10/400) = 81.8 g/ea

$$M_w = \sum M_i w_i$$

$$\text{POLYDISPERSITY INDEX} = \frac{81.8}{25} = 3.27$$

How MW Calculations Actually Work (Cont.)

Number-average molecular weight is the point in the distribution where the same number of polymer chains are present on each side



Weight-average molecular weight is the point in the distribution where the same mass of polymer chains are present on each side

Argon AS. Structure of solid polymers. In: *The Physics of Deformation and Fracture of Polymers*. Cambridge University Press; 2013:40-76.

Overview of Today's Presentation

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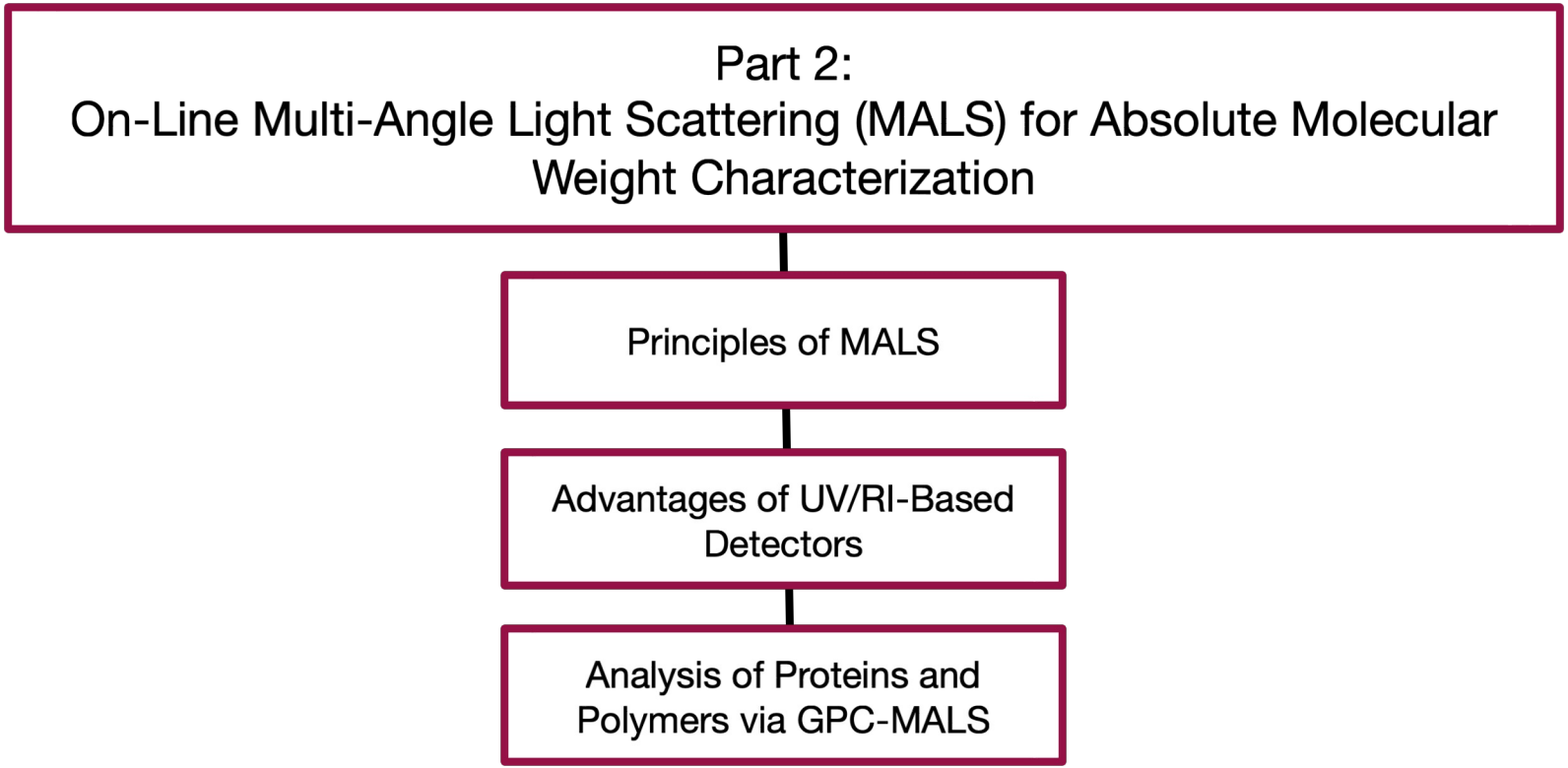
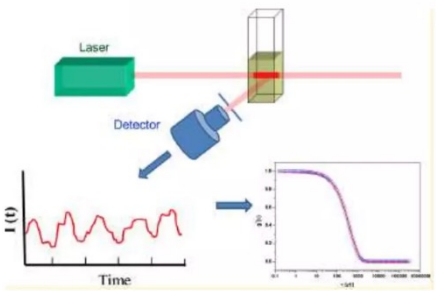
Basics of Gel Permeation Chromatography as a Characterization Technique for Macromolecules

Part 2:
On-Line Multi-Angle Light Scattering (MALS) for Absolute Molecular Weight Characterization

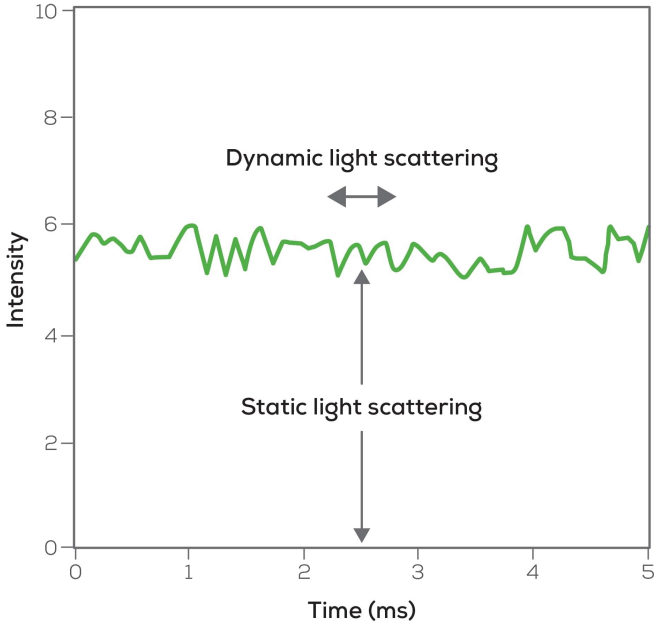

Part 2:
On-Line Multi-Angle Light Scattering (MALS) for Absolute Molecular Weight Characterization
Principles of MALS
Advantages of UV/RI-Based Detectors
Analysis of Proteins and Polymers via GPC-MALS

SLS vs DLS

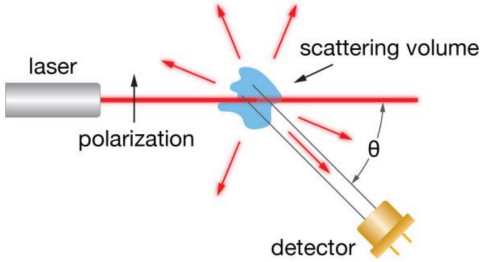
Dynamic Light Scattering (DLS):



Measurement of fluctuations in scattered light intensity

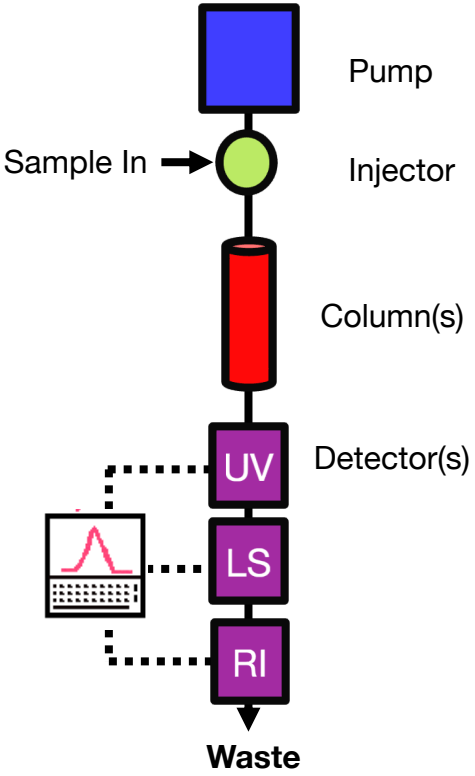


Static Light Scattering (SLS):

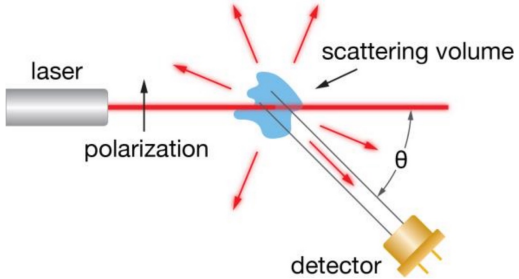


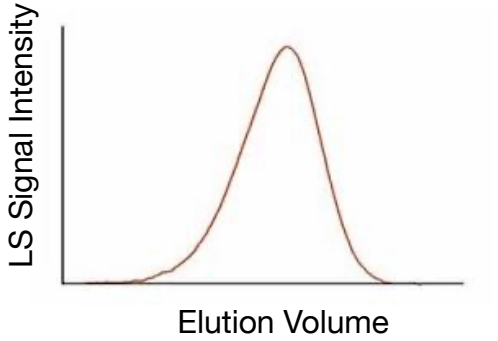
Measurement of average light scattered intensity

On-Line MALS

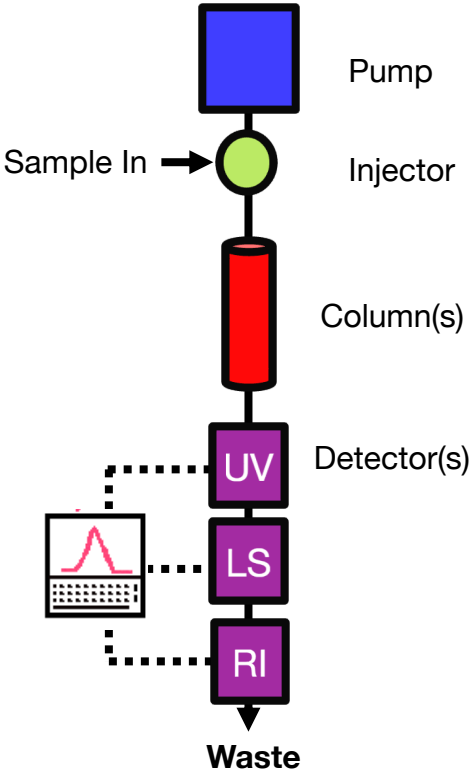


- MALS: Multi-Angle (Static) Light Scattering
  - Measures SLS signal at multiple angles relative to beam path
- On-Line MALS detector provides light scatting intensity chromatographs alongside UV and RI chromatographs





On-Line MALS



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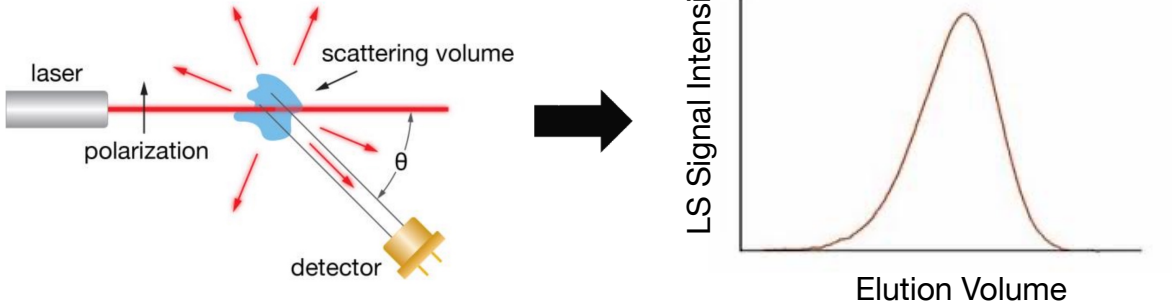


Q: What advantage does MALS provide over other detectors?

Signal Dependences UV, RI, and LS

UV

$$I \propto C, \varepsilon$$

C = Concentration
ε = Ext. Coefficient

RI

$$I \propto C, dn/dc$$

C = Concentration
dn/dc = Diff. RI vs Conc

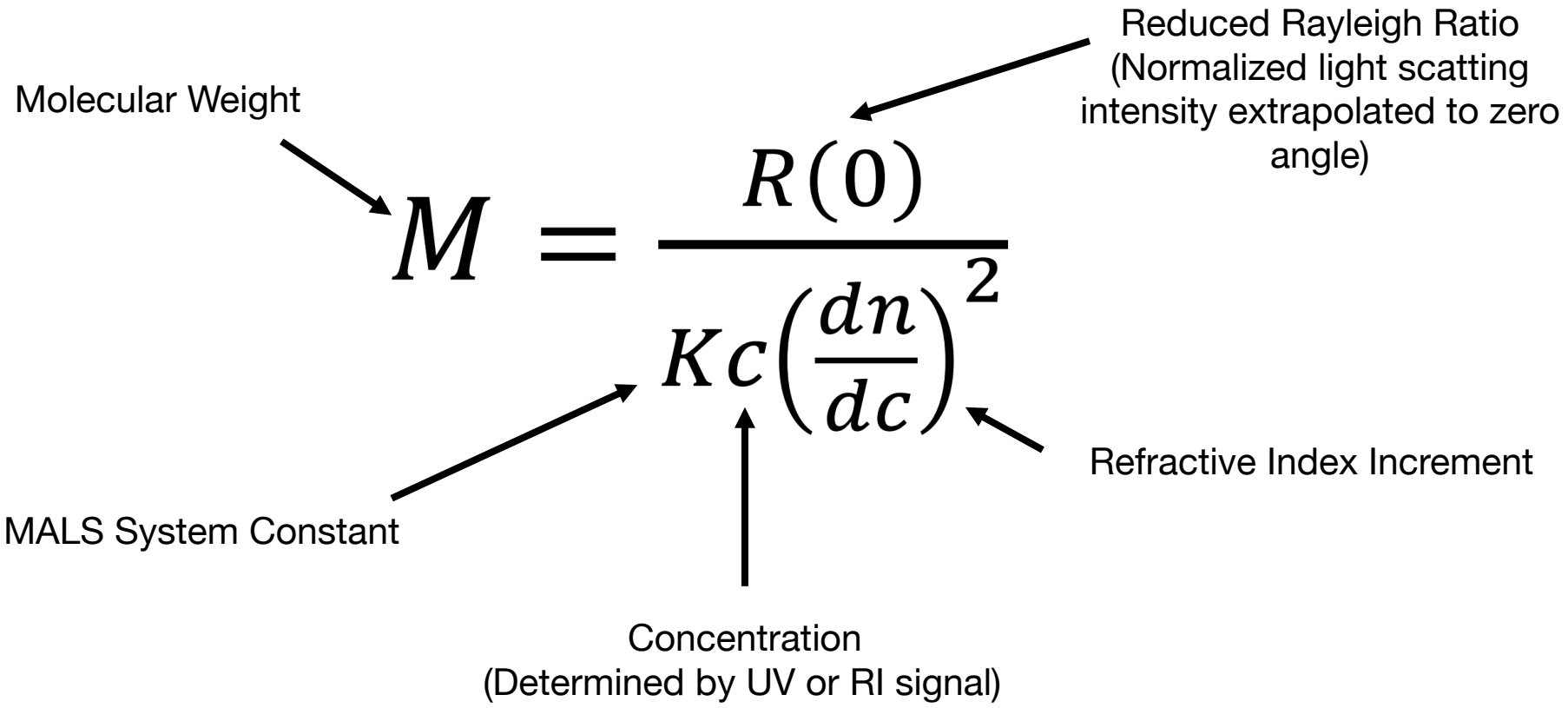
LS

$$I \propto C, n_o, (dn/dc)^2, M$$

C = Concentration
n_o = Refractive Index of Buffer
dn/dc = Diff. RI vs Conc
M = Molecular Weight

Light scattering intensity is proportional to macromolecule molecular weight!

Obtaining MW from MALS Data

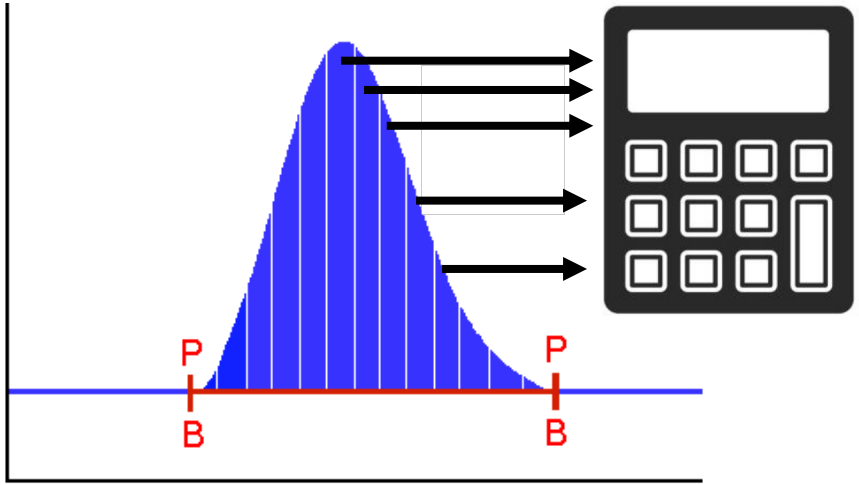


Key Principles of GPC-MALS

- In GPC-MALS, the GPC column is used solely to separate the various species in solution so that they enter the MALS and concentration detector cells individually.
- The actual retention time has no significance for the analysis except as far as how well the macromolecules are resolved.
- Since the instruments are calibrated independently of the column and do not rely on reference standards, GPC-MALS yields *absolute molecular weight*.

https://www.wyatt.com/files/literature/white-papers/WP1615-SEC-MALS-for-absolute-biophysical-characterization.pdf

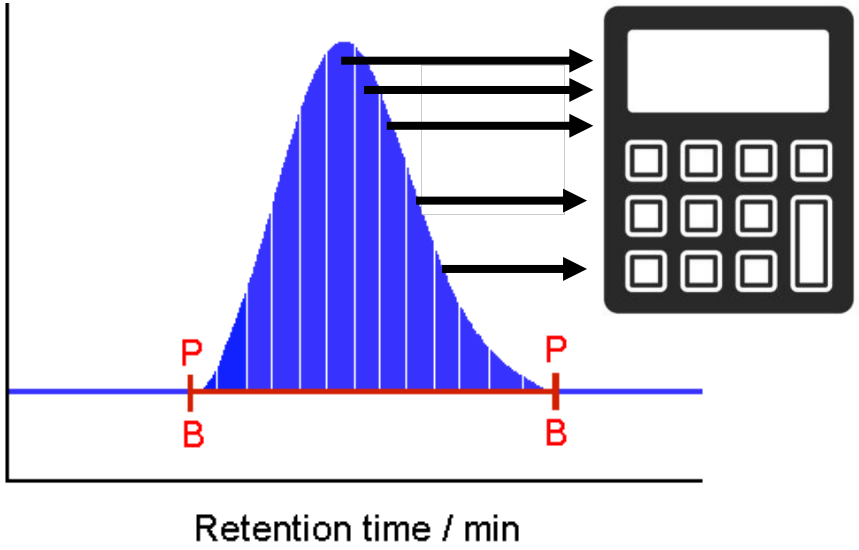
Key Principles of GPC-MALS (Cont.)



$$M = \frac{R(0)}{Kc\left(\frac{dn}{dc}\right)^2}$$

GPC-MALS reports an absolute MW for each of these volume slices. Therefore, can also calculate Mn, Mw, PDI as before!

Key Principles of GPC-MALS (Cont.)



$$M = \frac{R(0)}{Kc\left(\frac{dn}{dc}\right)^2}$$

Measured during GPC run

Known

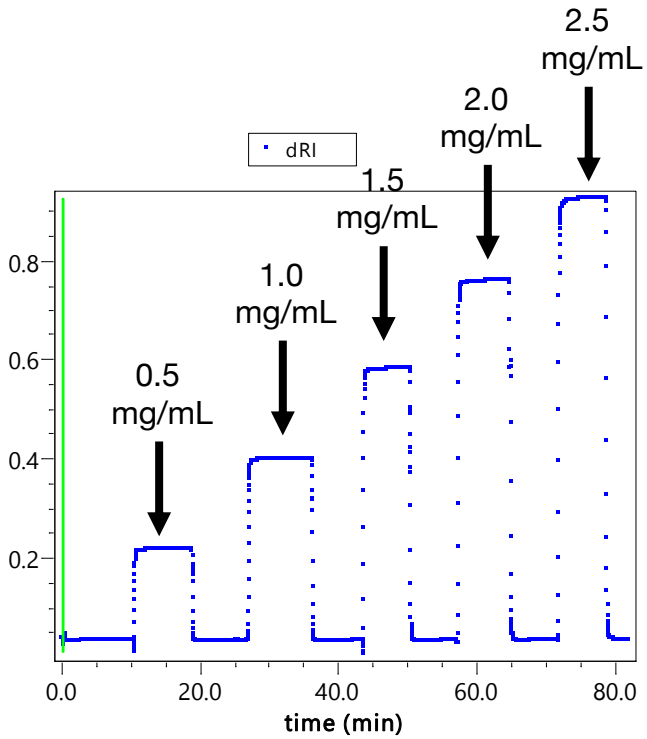
Measured during GPC run

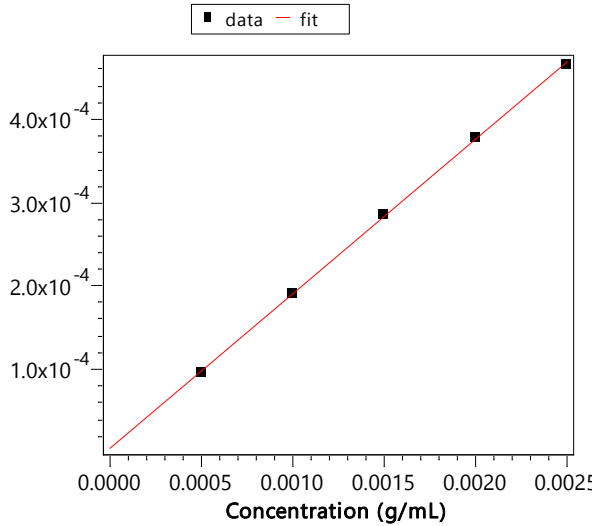
Inherent to sample, need to measure

GPC-MALS reports an absolute MW for each of these volume slices. Therefore, can also calculate Mn, Mw, PDI as before!

An Example: Determining dn/dc for BSA

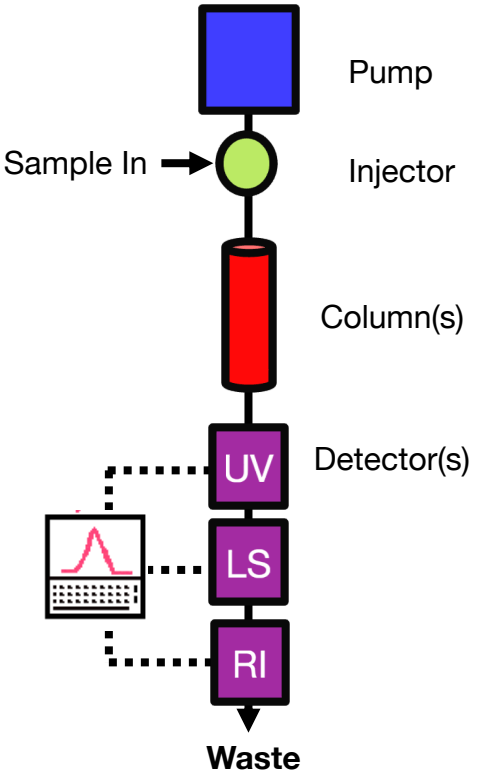
Direction injection of sample into refractometer with syringe pump

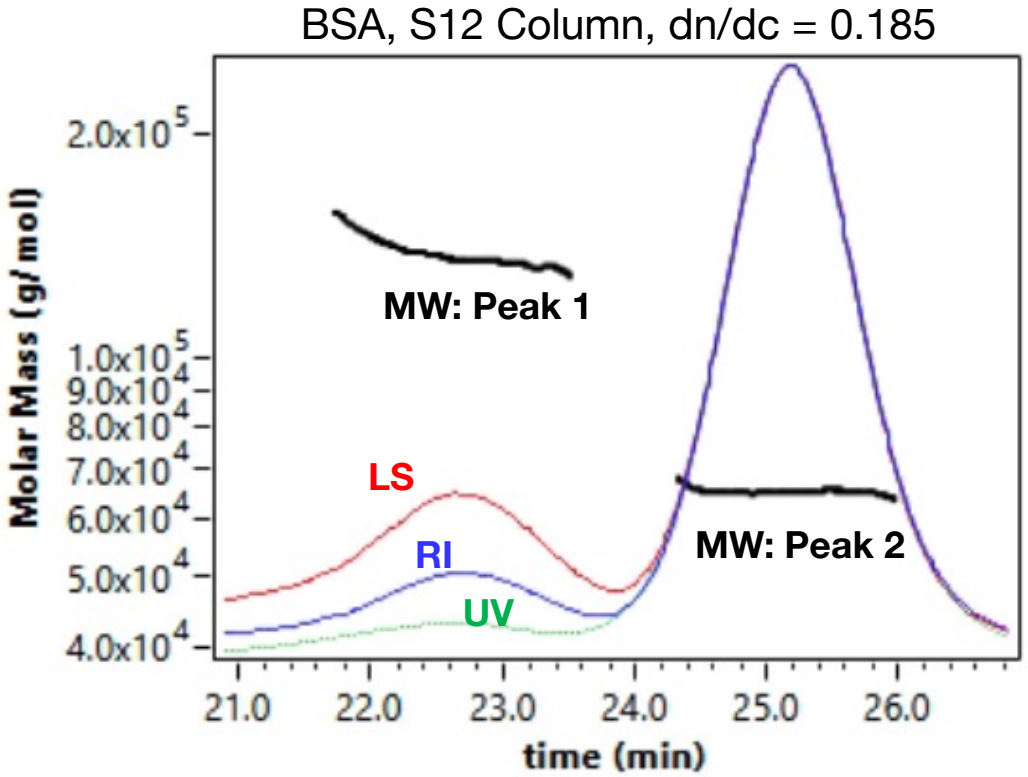




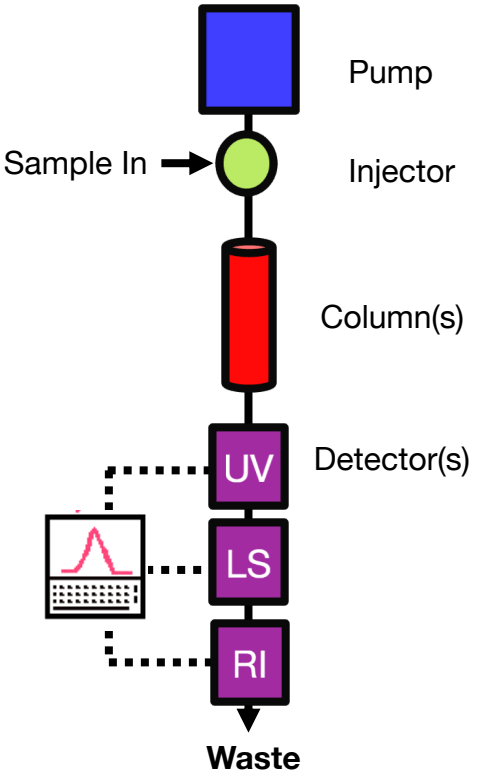
dn/dc = 0.1851 ± 0.002 mL/g
R^2 = 0.9996

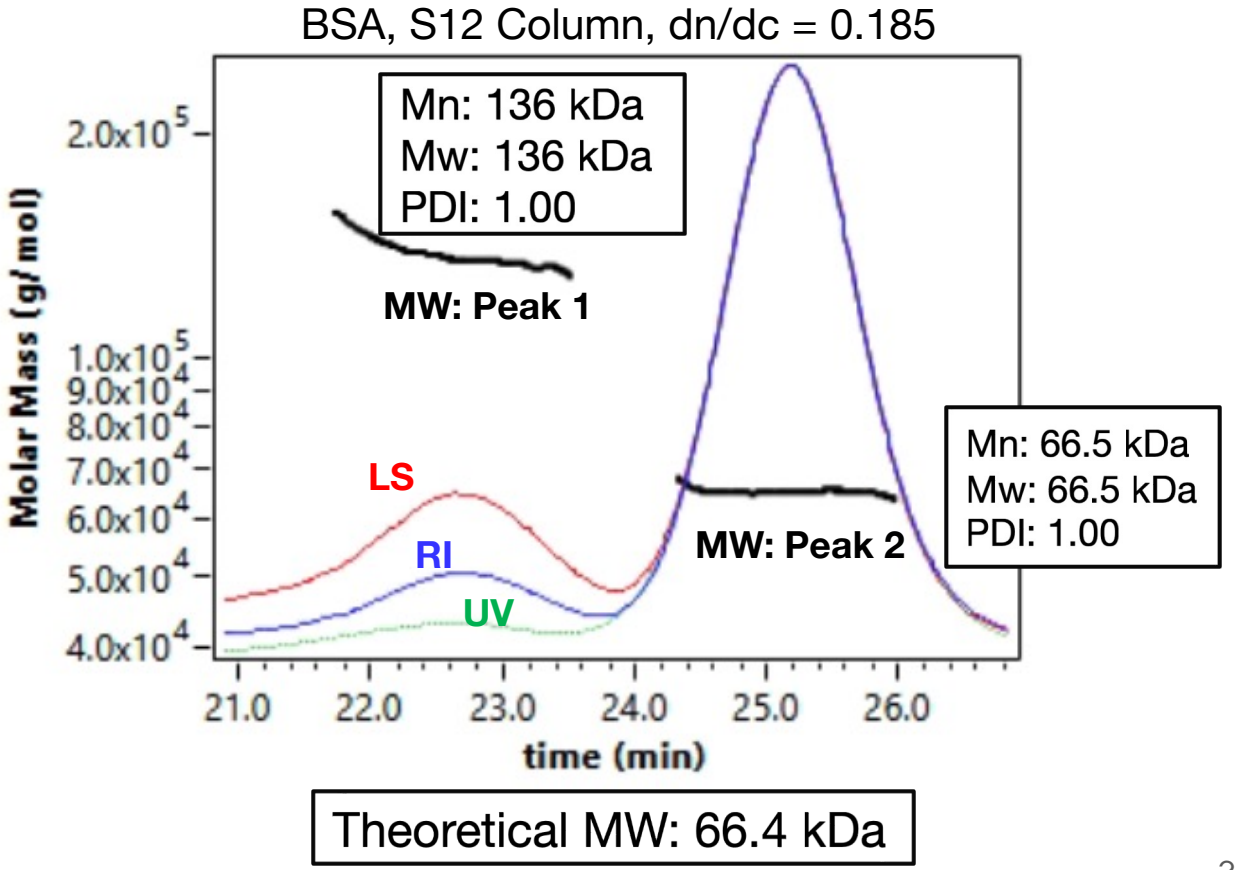
An Example: Determining Absolute MW of BSA



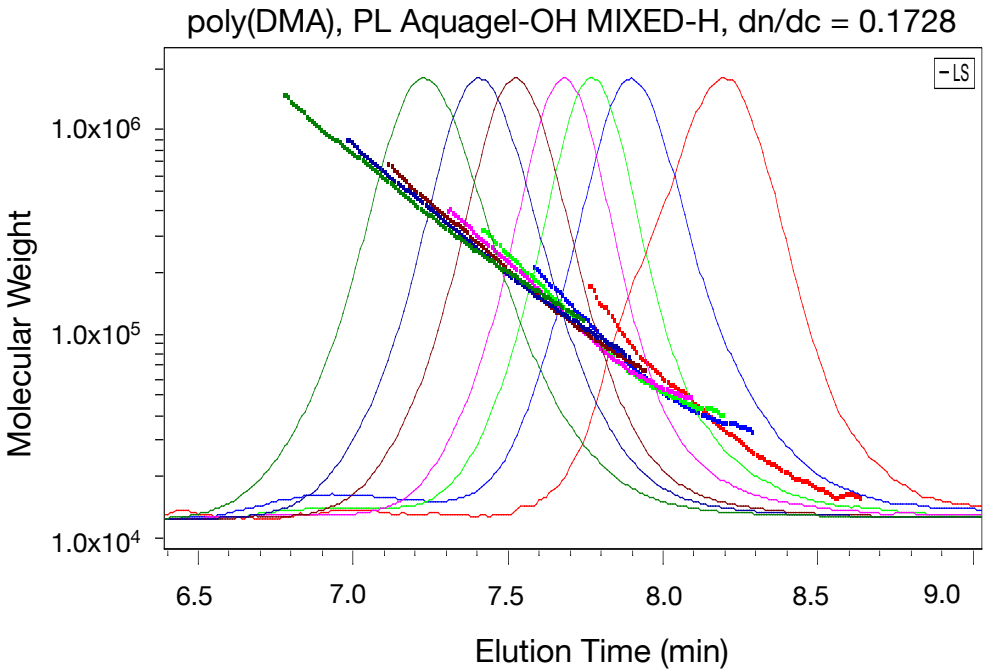


An Example: Determining Absolute MW of BSA





Last Example: Absolute MW of polyDMA Ladder



Target DP	Theo MW (kDa)	SEC-MALS Characterization Data		
		Mn (kDa)	Mw (kDa)	PDI
250	**24.8**	26.1	32.8	1.26
500	**49.6**	52.2	62.2	1.19
750	**74.3**	69.2	85.7	1.24
1000	**99.1**	87.8	110	1.26
1500	**149**	132	167	1.27
2000	**198**	166	216	1.30
3000	**297**	250	332	1.33

Learning Objectives

- Develop foundational understanding of gel permeation chromatography as a analytical technique to assess macromolecule physiochemical properties
- Review practical considerations for determining molecular weight of polymers via GPC to make well-informed decisions for experimental setup (i.e., column selection) and analysis (i.e., standard selection)
- Understand roles and tradeoffs of various detectors in multimodal GPC