Addressing the Challenges: Improving Polymer Characterization by Size Exclusion Chromatography

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Objective

- To show the multiple utilities of size exclusion chromatography (SEC), along with the time and resource saving benefits of implementing a chromatography system with low dead volume, a dual flow refractive index detector and semi-micro columns.

- To demonstrate how the dual flow design of the RI detector in conjunction with semi-micro columns allows for fast and accurate characterization of both synthetic and natural polymers.

- To highlight the multiple applications of single and multi-detector SEC for the analysis of polymers.

Introduction

- Since its inception the main utility of SEC has been to extract quantitative information in the form of molar mass averages and distributions of both synthetic and biopolymers with accuracy and precision.[1]

- Synthetic polymers, as well as most natural polymers, possess a distribution of molar masses. The ability to accurately and precisely characterize the molar mass distribution and averages is essential as the shape and the breadth of a polymer's molar mass distribution will dictate the end-use properties of the polymer, such as hardness, tear strength, impact resistances, wear, etc. One of the most highly used tools for characterizing the molar mass of polymers is size exclusion chromatography.

Introduction

- The molar mass averages and distributions can be obtained via three independent modes depending on the detection methods coupled to the SEC separation: (1) polystyrene relative calibration curve (RI or UV), (2) absolute molar mass (multi-angle light scattering), and (3) universal calibration curve (differential viscometry).

- Here we show the multiple utilities of SEC depending on employed detection methods along with the time and resource saving benefits of implementing a chromatography system with low dead volume, a dual flow refractive index detector and semi-micro columns.

Experimental

Instrumentation: EcoSEC® GPC System (HLC-8320) equipped with a dual flow refractive index detector and UV detector

A dual flow RI detector, such as that in the EcoSEC GPC System, is constructed in such a way that there are two sides:

1. a reference side, consisting of a flowing stream of pure solvent
2. the sample side, containing a flowing stream of analyte in the same solvent as in the reference side

Experimental Continued

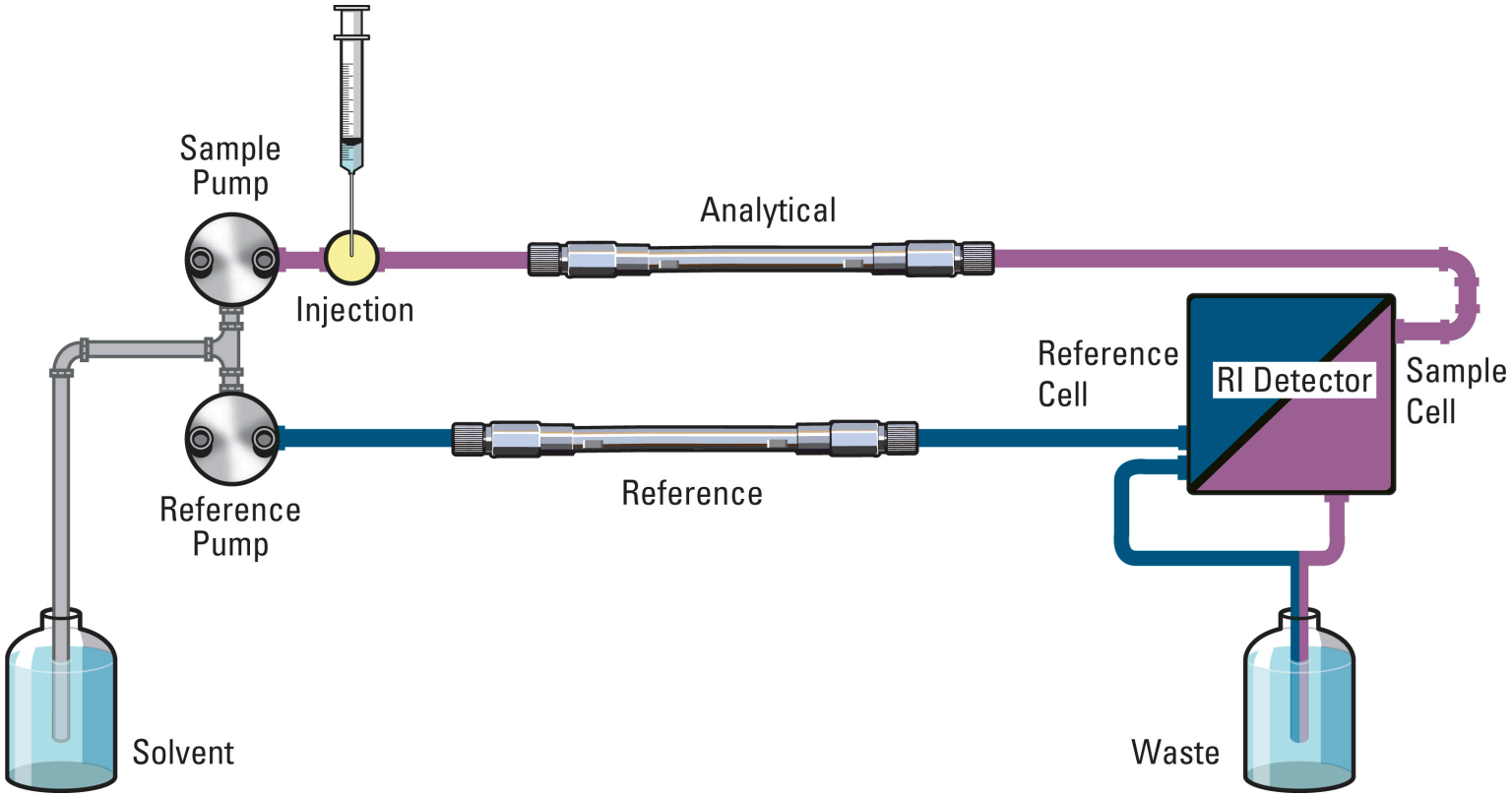


Figure 1: Depiction of the flow paths in the EcoSEC GPC System, showing the dual flow RI detector flow cell when the contents of the reference and sample sides have different refractive indices as each other.

Experimental Continued

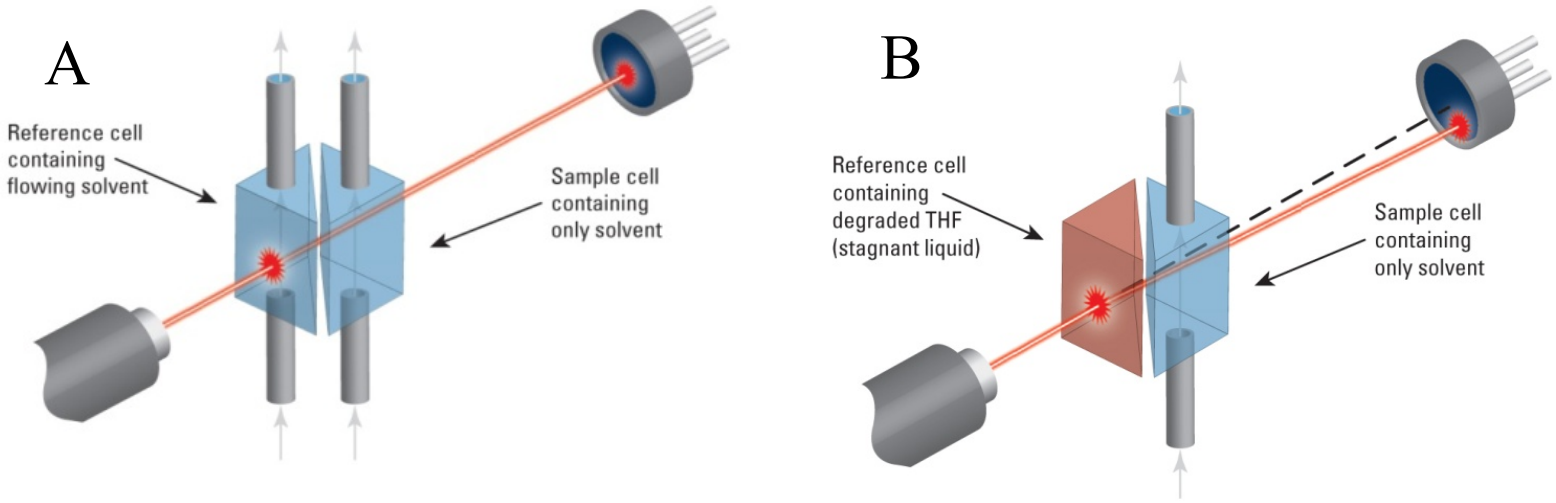


Figure 2: Depiction of a dual flow RI detector flow cell (A) and a conventional RI detector flow cell (B) showing the compensation of the changes in refractive index of the solvent over time.

Single Detector Size Exclusion Chromatography

Synthesis Monitoring and Oligomeric Analysis

- The applicability of SEC for synthetic polymers also extends into the realms of synthesis monitoring and oligomeric quantification. Synthesis monitoring using SEC not only allows for separation of polymeric material based on size but also provides information about the reactions, *e.g.,* did the reaction go to completion, is the product uniform in terms of molar mass or size, did a byproduct form, etc.

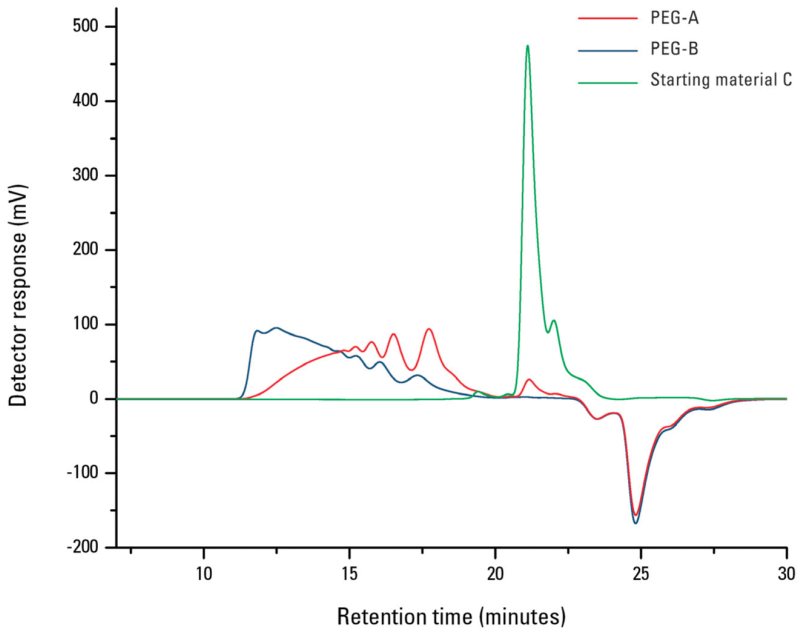
- Oligomeric SEC plays an important role in the quantification of oligomeric content (*i.e.,* low molar mass species) of a polymer sample for the purposes of pre-manufacture notification (PMN) regulations for new chemical substances as well as import and export purposes.[1]

Synthesis Monitoring and Oligomeric Analysis

Synthesis Monitoring

- Two polymers, PEG-A and PEG-B, are composed of the same basic components which vary in molar mass between the two samples. The molar mass difference of the starting material of PEG-A and PEG-B is reflected in the end-products, as PEG-A and PEG-B produce different chromatograms when separated by SEC, Figure 3.

Figure 3: Synthesis Monitoring by SEC of PEG-A, PEG-B, and Starting Material C as Monitored by RI



Column:	TSKgel SuperH3000, 6.0 mm ID × 15 cm × 2
Mobile phase:	THF
Flow rate:	0.30 mL/min
Detector:	RI (EcoSEC GPC System)
Temperature:	35 °C
Injection vol.:	40 µL
Sample:	PEGylated polymers and starting material

Figure 3: Conclusions

- The ability to identify all species within the PEGylated sample is essential in the validation of polymers intended for medical applications.

- The peak shape of the two PEGylated polymers differs; the chromatogram for PEG-B has a greater RI detector response at the earlier elution volume, larger polymer size region of the chromatogram while the opposite is true for PEG-A.

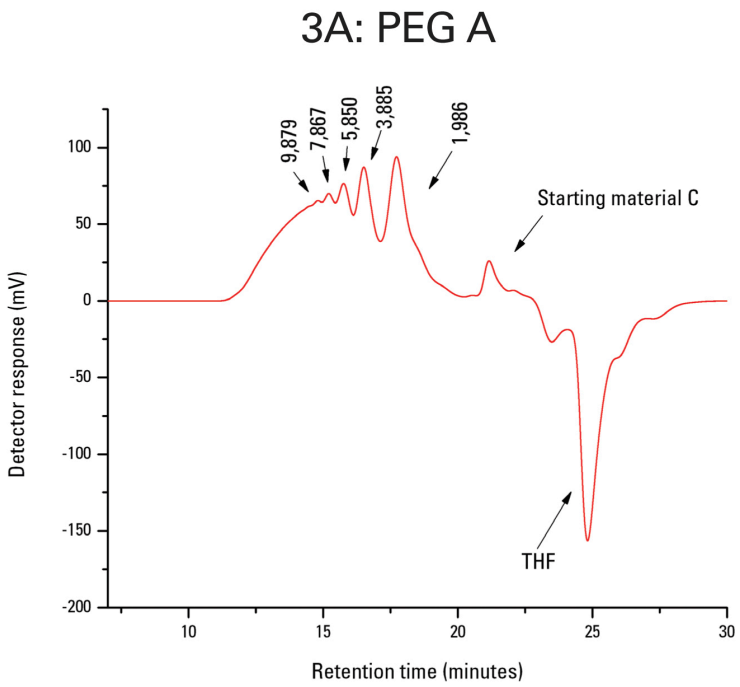
- By comparing the SEC chromatograms of starting material C with that of PEG-A and PEG-B, a fairly substantial amount of starting material C remains in PEG-A while all of starting material C has reacted in PEG-B.

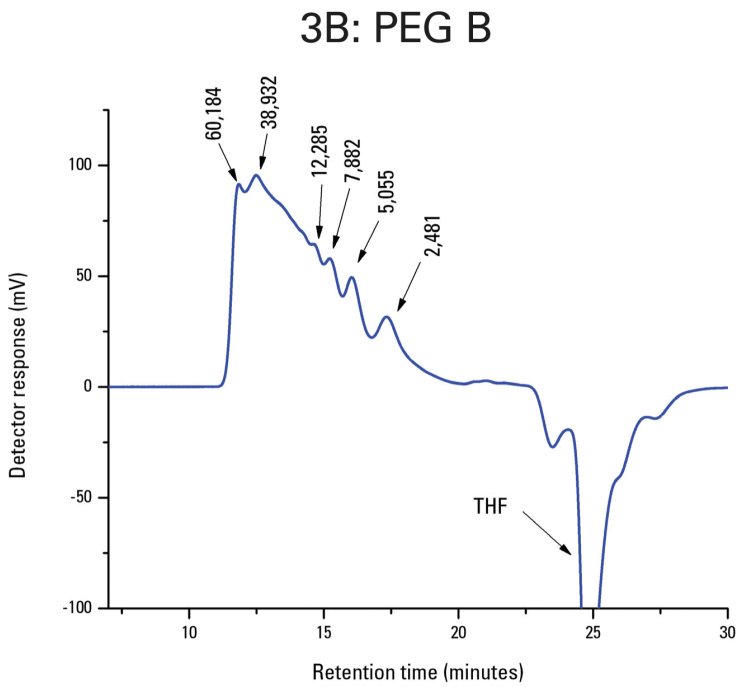
Synthesis Monitoring and Oligomeric Analysis

Oligomeric Analysis

- The characterization of the oligomers present in the PEGylated samples is best achieved by the peak-average molar mass, M_p. The polystyrene relative peak-average molar mass values for each mode in the chromatograms for the PEG-A and PEG-B samples are given in Figures 4A and 4B.

Figures 4A and 4B: SEC Analysis of PEG-A (4A) and PEG-B (4B)





*Numbers on graph represent polystyrene relative peak-average molar mass, M_p, values of each mode.

Figures 4A and 4B: Conclusions

- The values for the peak-average molar mass between the two samples differ significantly. The M_p values of PEG-A range from approximately 2,000 to 10,000 g/mol - indicating that most, if not all, of the species present are oligomeric in nature. Conversely, the M_p values of PEG-B range from approximately 2,500 to 60,000 g/mol - indicating that low and high molar mass species are present.

- Combining the oligomeric content information with the SEC chromatograms provides a more detailed picture about the distribution of the low molar mass species within the two PEGylated samples, information beneficial in the validation and regulation of synthetic polymers.

Dual Detector Size Exclusion Chromatography

Quality Control Procedures

- One of the most common utilities of SEC is the determination of molar mass averages and distributions of polymers using peak position calibration involving polystyrene standards of known molar mass and chemistry for quality control procedures.

- The EcoSEC GPC System is currently used in numerous quality control laboratories to differentiate between “successful” and “unsuccessful” batches of products through monitoring molar mass averages and SEC elution profiles.

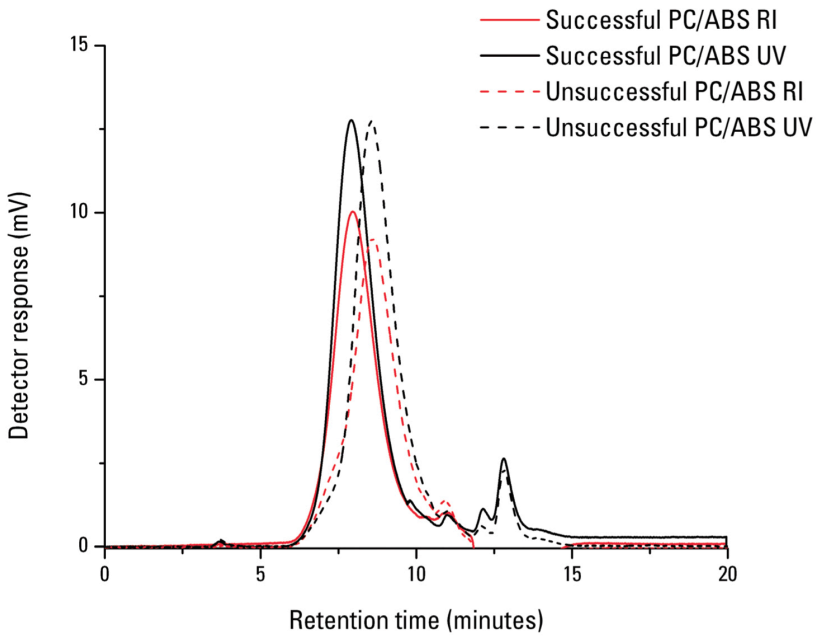
Quality Control Procedures

Failure Analysis: SEC Elution Profile

- The difference between a successful and unsuccessful polymer based material can be determined by observing the difference in SEC chromatograms between the two polymers.

- Figure 5 shows an example of how the EcoSEC GPC System can be used to distinguish between two batches of the same polymer. The sample labeled “successful” is a batch of polymer that performs at or above standards when used in its end-use application while the sample labeled “unsuccessful” has shown to perform below standards in the same end-use application.

Figure 5: SEC Elution Profile of Successful and Unsuccessful Polymers as Monitored by RI and UV



Column:	TSKgel SuperMultiporeHZ-M, 4.6 mm ID × 15 cm × 2
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detectors:	RI (EcoSEC GPC System) UV (EcoSEC GPC System @ 254 nm)
Temperature:	35 °C
Injection vol.:	10 µL
Sample:	polycarbonate based resin

Figure 5: Conclusions

- The shorter retention time of the successful sample indicates that the successful sample is larger in polymeric size than the unsuccessful sample; as the elution order in SEC is that of an “inverse-sieving” technique, large analytes sample a smaller pore volume than smaller analytes resulting in the larger analytes eluting from the column prior to the smaller analytes. Thus, the SEC chromatogram alone provides sufficient indication that the successful and unsuccessful samples are different from one another.

Quality Control Procedures

Failure Analysis: Molar Mass Distribution

- Variations in the molar mass averages is an important characteristic of any product as the molar mass averages dictate end-use properties such as tensile strength, elongation, brittleness, hardness, toughness, etc.

- The difference in the molar mass averages between the successful and unsuccessful samples can also be observed in the form of molar mass distributions, *MMD*, and are shown in Figure 6.

Figure 6: Overlay of Cumulative and Differential Molar Mass Distributions of Successful and Unsuccessful Polymers

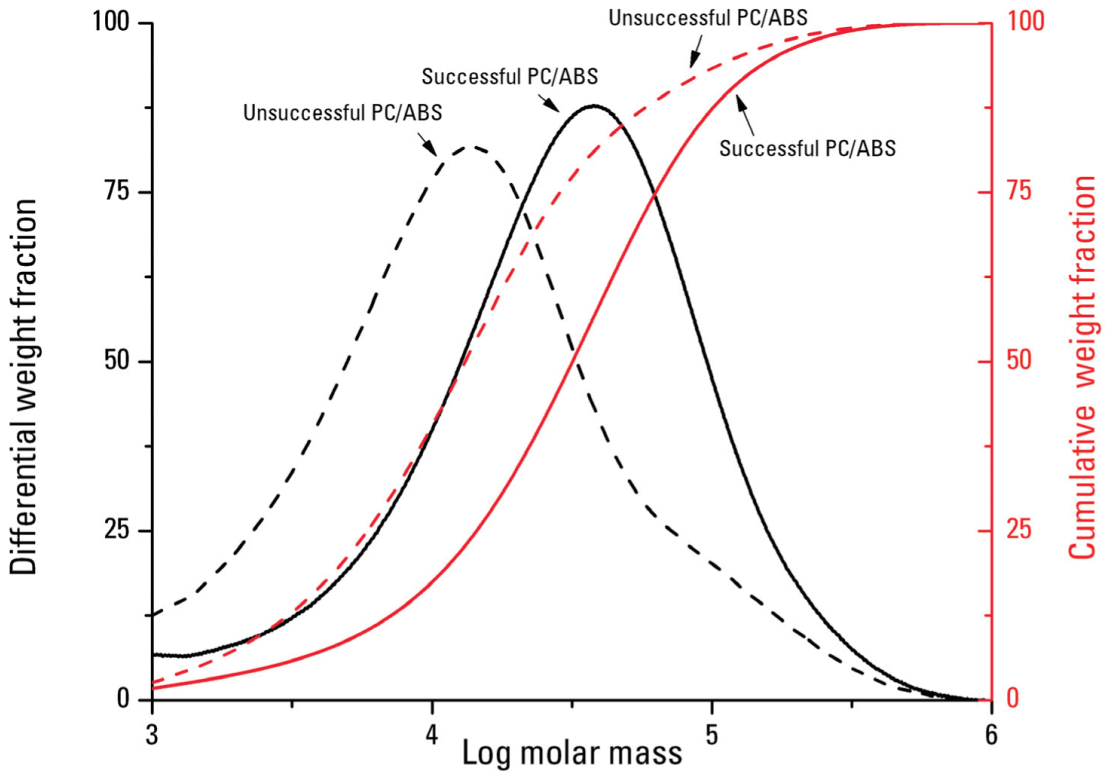


Figure 6: Conclusions

- The successful sample extends significantly further in the high molar mass direction than the unsuccessful sample, while the unsuccessful sample contains a considerably higher quantity of low molar mass species than the successful sample. The molar mass averages and distributions between the two samples differ enough to result in a successful and an unsuccessful end-use product.

Multi-Detector Size Exclusion Chromatography

Polymer Characterization

- Detailed characterization of the physiochemical properties of natural and synthetic polymers can be determined by utilizing the EcoSEC GPC System with an internal dual flow RI detector and UV detector coupled to a multiplicity of physical detection methods, namely multi-angle light scattering (MALS), quasi-elastic light scattering (QELS), and differential viscometry (VISC).

Polymer Characterization

Natural Polymers

- The characterization of the physicochemical properties of sugar beet pectin (SBP) is of interest to the USDA, as it has the potential to be used in the production of industrial products, *e.g.,* as an emulsifying agent in food systems.

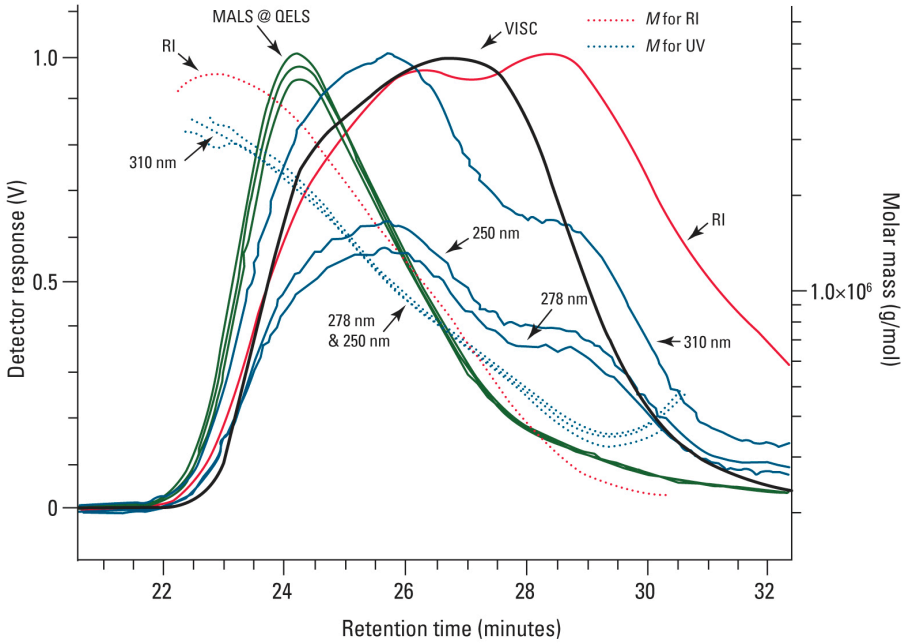
Table 1: Physical Properties of SBP

- The weight-average molar mass for the SBP was determined to be approximately 1.1×10^6 g/mol and shown to vary less than 7% amongst the four concentration-sensitive detectors (UV @ 310, 278, and 250 nm and RI). The addition of the external detectors to the EcoSEC GPC System allows for the determination of polymeric size (R_G and R_H) and intrinsic viscosity, also given in Table 1.

	Detection Method			
	RI	UV @ 250 nm	UV @ 278 nm	UV @ 310 nm
M_W (g/mol)[a]	1.098 x 10^6	1.097 x 10^6	1.147 x 10^6	1.187 x 10^6
	(0.003 x 10^6)[b]	(0.004 x 10^6)	(0.004 x 10^6)	(0.002 x 10^6)
$R_{G,z}$ (nm)[a]	43 (1)	43 (1)	45 (1)	42 (1)
$R_{H,z}$ (nm)[c]	53 (1)	43 (1)	43 (1)	44 (1)
$[\eta]_W$ (dL/g)	3.5 (0.1)	3.4 (0.1)	3.5 (0.1)	3.5 (0.1)

[a] with MALS; [b] Standard Deviation; [c] with QELS

Figure 7: SEC Elution Profile of Sugar Beet Pectin



Column:	TSKgel GMPW$_{XL}$, 7.8 mm ID × 30 cm × 2
Mobile phase:	H_2O with 0.05 mol/L $NaNO_3$ and 0.1% NaN_3
Flow rate:	0.7 mL/min
Detectors:	RI (EcoSEC GPC System), UV (EcoSEC GPC System @ 310, 278, and 250 nm), MALS (Wyatt Dawn® HELEOS® II), QELS (Wyatt QELS™), VISC (Wyatt ViscoStar®)
Temperature:	35 °C
Injection vol.:	200 µL
Sample:	sugar beet pectin

Figure 7: Conclusions

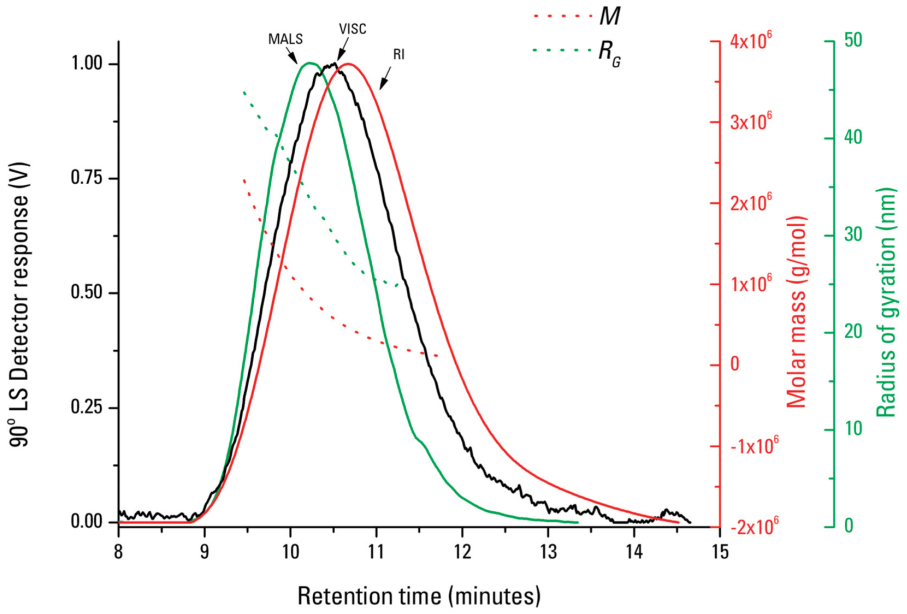
- The SEC elution profile of the SBP, as measured by the four concentration-sensitive detectors, displays a distinct bimodal distribution. Evidence of molar mass polydispersity of SBP is seen, as the molar mass of the SBP decreases by an order-of-magnitude with increasing elution volume. The molar mass of the SBP is higher at lower elution volumes and lower at larger elution volumes via the RI detector than via the UV detector, an indication that the particles with higher molar masses have fewer UV absorbing molecules associated with them than their lower molar mass counterparts.

Polymer Characterization

Synthetic Polymers

- Natural and synthetic rubbers are key components to many applications, *e.g.* clothing, vehicles, toys, fire arms, etc. Currently synthetic rubbers constitute 75% of all rubber consumption worldwide. Due to the high consumption of synthetic rubber and synthetic rubber products, it is critical to know the molar mass averages and distributions, as well as information regarding polymeric size, as these physicochemical properties directly affect the end-use of performance of a given product.

Figure 8: SEC Elution Profile of Synthetic Rubber



Column:	TSKgel SuperMultiporeHZ-H, 4.6 mm ID × 15 cm × 3
Mobile phase:	THF
Flow rate:	0.35 mL/min
Detectors:	RI (EcoSEC GPC System), MALS (PSS SLA 7000 Light Scattering Photometer), VISC (PSS ETA-2010 Viscosity detector)
Temperature:	35 °C
Injection vol.:	25 µL
Sample:	synthetic rubber

Figure 8: Conclusions

- The chromatogram of the synthetic rubber, as monitored by the individual detectors from the triple-detector GPC set-up, shows both the molar mass and size polydispersity of the synthetic rubber as the molar mass and radius of gyration, R_G, both decrease with increasing elution volume.

Conclusions

- The utilities of SEC are numerous as the size base mechanism of the technique and the ability to determine molar mass averages and distributions allows for various experimental goals. To name a few, single-, dual-, and multi-detector SEC can be successfully used for synthesis monitoring, oligomeric analysis, failure analysis, and the characterization of the physiochemical properties of polymers.

- Through multiple applications it has been demonstrated how a low dead volume SEC system equipped with a dual flow refractive index detector and semi-micro columns will save time and resources when analyzing polymers.

References

[1] Striegel, A.M.; Yau, W.W.; Kirkland, J.J.; Bly, D.D. *Modern Size Exclusion Liquid Chromatography, 2nd edition;* Wiley: New York, 2009.