TOSOH

SEC-MALS characterization of copolymers for drug delivery

Your Challenge

- You deal with complex copolymers such as PEG-PGA used as drug carriers for innovative therapies.
- You need to characterize their MW, but no standard is available for conventional calibration.

Our Solution

EcoSEC Elite GPC system and LenS3 MALS detector

- Optimized SEC-MALS solution for MW determination

What was done?

- We compared MW of PEO homopolymers and a PEG-PGA copolymer with conventional calibration and MALS

What was the result?

- Conventional calibration overestimates MW, whereas MALS delivers accurate results

A MALS detector is required to determine the accurate molecular weight of polymers, regardless of their chemistry and structure. This is even more important for biomedical applications.

Your Benefit

Accurate MW determination of critical biomedical drug-delivery polymers

Analysis of drug-delivery vehicles

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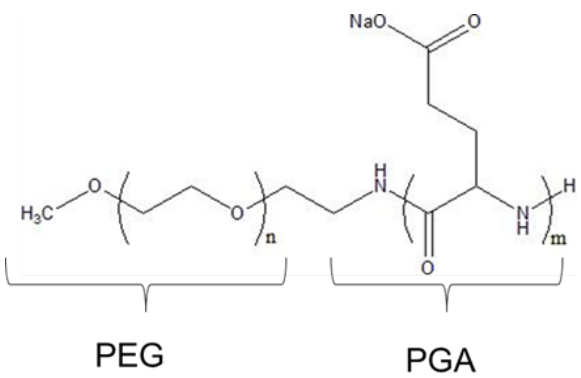
Application Note

SEC-MALS characterization of a poly(ethylene glycol-co-glutamic acid) block copolymer used in drug delivery

Biodegradable polymers are an important class of macromolecules that can be employed as drug-delivery agents to solubilize hydrophobic therapeutic molecules in water. Tailored copolymeric structures such as micelles can carry biologically active molecules to target diseased tissues and specific cells, regulate intracellular trafficking, as well as penetrate the nucleus thanks to their innovative polymeric chemistry. Moreover, polymeric architecture can be controlled to modify certain properties for diagnosis and therapeutics; some of those structures include branched copolymers, graft polymers, dendrimers, polymer-dendron conjugates, and star-shaped polymers.
Polyethylene glycol (PEG) and polyglutamic acid (PGA) are non-toxic and non-immunogenic biodegradable polymers. The combination of water-soluble PEG with a hydrophobic PGA block form an excellent drug carrier. PEG-PGA copolymers (*Figure 1*) and their derivatives are good drug crosslinking agents because of the presence of various reactive end groups. They create a spatial shield around the drug to reduce undesired enzymatic hydrolysis and avoid rapid elimination while allowing the drug to be recognized by cells of the immune system. Amongst possible therapeutic applications, PEG-PGA copolymers have been proposed as anti-cancer drug delivery carriers in the form of micelles or polymer-drug conjugates.

Size exclusion chromatography (SEC) has been widely used to study the molecular characteristics of polymer-drug systems such as molecular weight (MW) and size because these parameters can greatly affect the pharmacokinetics of the polymer within the body. When performing SEC experiments, conventionally, concentration-sensitive detectors such as refractive index (RI) or ultraviolet (UV) detectors are used.

Figure 1. Structure of a PEG-PGA block copolymer.



To obtain accurate MW values in this most common approach, the columns must be calibrated with standards that are chemically and structurally equivalent to the samples being analyzed. Even though such column calibration is the simplest method to determine molecular weight distribution, it has serious limitations because of the lack of variety in polymer standards available. This study presents the accuracy and limitations of conventional calibration and demonstrates how light scattering detection coupled with SEC provides a direct measurement of the molecular weight of a PEG-PGA drug delivery copolymer.

Material and Methods

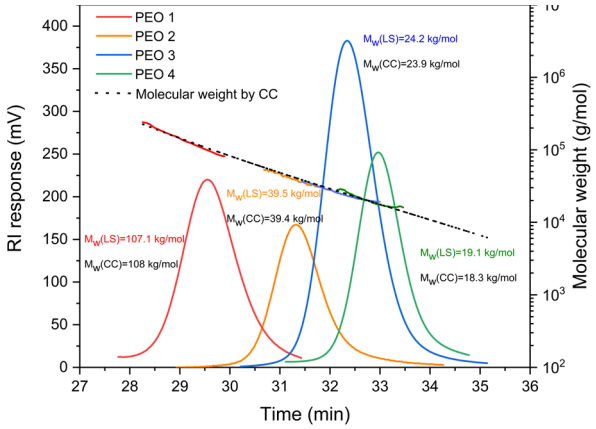
System:	EcoSEC Elite (HLC-8420) GPC system
Columns:	2 x TSKgel GMPW$_{XL}$
Mobile phase:	Water + 0.01M $NaNO_3$ and 0.02% NaN_3
Flow rate:	0.7 mL/min
Detectors:	Refractive index (RI) and LenS$_3$ Multi-Angle Light Scattering (MALS) detector
Samples:	PEO and PEG-PGA block copolymer

Conventional calibration (CC) was performed using PEO standards. Molecular weights by light scattering (LS) were determined from the low angle (LALS, 10°) of the LenS$_3$ MALS detector. The specific refractive indices (dn/dc) were 0.132 mL/g for the PEO samples and 0.142 mL/g for the PEG-PGA copolymer. It should be noted that PEO and PEG are chemically the same polymers, with PEG being the most commonly used name for low MW PEO (<20,000 g/mol).

Results and discussion

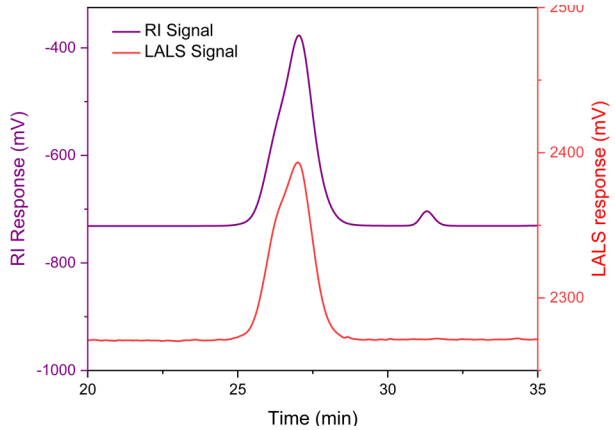
A set of PEO standards with varying molecular weights was analyzed using SEC. The chromatograms from RI and their corresponding molecular weight determined by LS and CC are depicted in *Figure 2*. From the RI elution profiles, PEO samples 1 to 4 elute with increasing retention times, which implies a decreasing molecular size in solution.
The molecular weight traces obtained from LS overlays perfectly with the conventional molecular weight curves. In addition, the weight average molecular weights (M_w) obtained from LS and CC are also presented in *Figure 2*. For each PEO sample, the M_w values obtained by LS and CC are very close. This example perfectly illustrates that if the chemistry of the standards and the samples is the same, then accurate molecular weight can be obtained by CC.

Figure 2. RI chromatograms and molecular weight traces of PEO samples.



The RI and LALS chromatograms of the PEG-PGA sample are presented in *Figure 3*. Despite the relatively low MW of the sample, hence the low scattering intensity, excellent signal-to noise ratio was obtained even at the low angle (LALS, 10°), thus allowing for reliable and accurate MW determination.

Figure 3. RI (top) and LALS (bottom) chromatograms obtained for the PEG-PGA sample.



The RI chromatogram and the molecular weight determined from light scattering and conventional calibration for the PEG-PGA sample are presented in *Figure 4*, showing that the molecular weight distribution obtained by CC is higher than the one obtained by LS. When applying the CC method to determine molecular weight, the size of the PEG-PGA copolymer corresponds to that of a PEO with a higher molecular weight. This indicates that the CC calculation fails in this case, because the chemistry of PGA differs from that of PEO. The PGA block within the PEG-PGA copolymer expands the random coil conformation of the copolymer in solution compared to a PEO (or PEG) homopolymer. As a result, the hydrodynamic size of the copolymer increases, which leads to an earlier elution of the sample. On average, the relative M_w determined from CC is 9.2 kg/mol, whereas LS provided a true M_w of 7.2 kg/mol.

Figure 4. RI chromatogram and molecular weight traces obtained for PEG-PGA by light scattering (LS) and conventional calibration (CC).

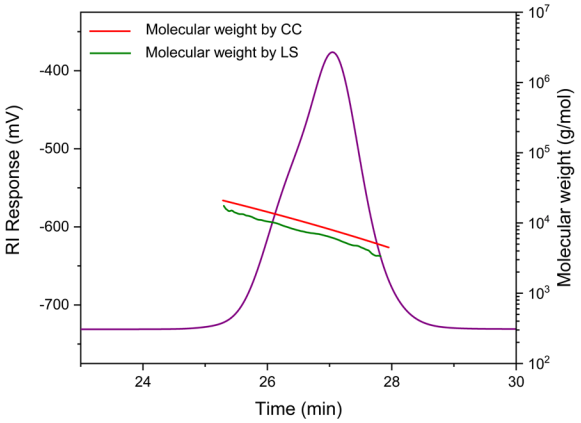


Table 1. Molecular weight of PEG-PGA sample from LS and CC.

	Time (min)	M_n (g/mol)		M_w (g/mol)		M_z (g/mol)		PDI	
		LS	CC	LS	CC	LS	CC	LS	CC
	27.05	7,167	8,036	7,215	9,241	7,493	10,511	1.01	1.15
CV* (%)	0.02	0.57	0.34	0.38	0.77	4.1	1.5	0.17	0.41

* coefficient of variation calculated from three injections

Conclusion

A series of PEO homopolymers and a PEG-PGA copolymer were analyzed and compared using SEC with conventional calibration and MALS. The use of conventional calibration provides accurate molecular weight only if the structure and the chemistry of the calibration standards and the analyzed samples are identical. The PGA block in the PEG-PGA copolymer modifies the conformation of the PEO chain, resulting in an overestimated molecular weight distribution when using a PEO calibration. The use of a light scattering detector is required to determine the true molecular weight of polymers, regardless of their chemistry and structure. This is even more important in biomedical applications since molecular weight is a critical parameter in the design of efficient polymeric drug carriers.