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Evaluating Degradation of Drug Delivery Polymers Using Advanced Polymer Chromatography (APC) with Light Scattering and Viscometry

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Abstract

In this study the degradation of a PLA-PEO-PLA drug delivery polymer was characterized. Irradiated samples were seen to have dramatically reduced molecular weight but no changes in their conformation or structure were observed, implying that this degradation was due to polymer scission caused by the radiation. This type of analysis is made possible due to the unique combination of high resolution separation using the ACQUITY APC System with a true multi-detection system, OMNISEC REVEAL, containing not only light scattering detectors but also an online differential viscometer to measure structure.

Benefits

- Determine polymer properties such as molecular weight, dispersity, and branching/conformation
- Improved resolution, reduced sample loading, and faster run times than SEC/GPC methods
- Differentiate molecular structure across the molecular weight distribution
- Determine accurate molar mass

Introduction

Drug delivery is a large and growing research area that involves both synthetic and natural polymers. A number of polymers are being developed for different purposes, such as to conjugate or contain small and large molecule drugs to release at a specified rate. Complexes of polymer and drug are used to customize release rates, achieve targeting, and improve half-life in order to modulate the efficacy of the drug as a therapeutic. Defining the release profile of the drug is a critical parameter which relies in part on the degradation characteristics of the polymer. Polymers such as PLA (polylactic acid), PEO (polyethylene oxide), and PLC (polycaprolactone) are commonly used in these applications due to their biocompatibility and biodegradability.

In order to maximize the effectiveness of these drug delivery polymers, their underlying molecular properties must be well controlled as these will strongly impact their performance. Properties such as molecular weight, dispersity, and branching/conformation will all affect polymer degradation, and thus its drug release and bioavailability. These properties must be measured for both the virgin polymer and subsequent products made from it as the various processes undergone by the polymer will likely affect final properties. For example, any polymer to be used as a drug delivery implant will need to be sterilized by one or more potentially damaging processes before clinical use.

Size-exclusion chromatography (SEC), also known as gel-permeation chromatography (GPC) is a well-known and widely used technique for measuring polymer molecular weight. With SEC a concentration detector is always used, typically a refractive index (RI) detector. Multi-detector SEC measurements expand on this by adding detectors such as light-scattering (LS) or viscometry (IV). LS can measure absolute molecular weight, independent of a molecules shape, structure, chemistry, or conformation. A viscometer allows the measurement of intrinsic viscosity (IV), which is used to study conformation and branching, which can expose structural changes that the polymer may undergo.

Typical analytical SEC measurements can take approximately 25 to 45 minutes and consume 25 to 45 mL of mobile phase. This is time-consuming and can be expensive if working with organic and halogenated solvents. Advanced Polymer Chromatography™ (APC) uses novel SEC column technologies with robust, small particles (<3 µm) to achieve better sample resolution using smaller columns. This increases productivity, while significantly reducing runtime and cost. An additional benefit of the reduced solvent usage is to effectively make APC a 'greener' technology than traditional analytical SEC.

Combining multi-detector SEC and APC has been challenging due to the significance of inter-detector band

broadening (or dispersion). However, recent developments in integrated multi-detector systems allows up to four detectors to be connected to the ACQUITY APC System with only a minimal loss of resolution due to band broadening. Malvern Panalytical recently introduced a version of the OMNISEC REVEAL advanced detector system that through collaboration with Waters, has been optimized for integrated use under APC conditions.

In this application note, Malvern Panalytical's OMNISEC REVEAL multi-detector module was combined with Waters ACQUITY APC System to offer high resolution, rapid multi-detector SEC characterization of a PLA-PEO-PLA drug delivery polymer. The molecular properties of a virgin sample were compared with two samples sterilized by radiation.

Figure 1. ACQUITY Advanced Polymer Chromatography (APC) System and Malvern Panalytical OMNISEC-REVEAL.

Experimental

Sample description

One virgin and two irradiated PLA-PEO-PLA copolymers were prepared in THF at a concentration of nominally 1 mg/mL.

LC conditions

LC system:	ACQUITY APC

Detection:	OMNISEC REVEAL with RI, LS (LS, RALS 90° angle, LALS 7° angle), and IV detectors
Vials:	Waters Vials with pre-slit septa
Column:	ACQUITY APC XT 150 mm, 45 Å, 125 Å, and 450 Å, in series
Column temp.:	40 °C
Sample temp.:	40 °C
Injection volume:	10 to 20 µL
Flow rate:	1.0 mL/min
Mobile phase:	THF (unstabilized)

Data management

ACQUITY APC operation: Standalone ACQUITY Console software

OMNISEC operation, data collection, and processing: Malvern Panalytical OMNISEC software

Methods

The three polymer samples were dissolved in THF and separated using the ACQUITY APC System and ACQUITY APC-XT Columns. The column eluent flowed directly into the OMNISEC REVEAL containing RI, LS, and IV detectors connected in series. The detectors were calibrated using a NIST-traceable polystyrene molecular weight and intrinsic viscosity standard.

Results and Discussion

Figure 2 shows a comparison of analytical SEC with the ACQUITY APC System. The time and mobile phase

saving per injection is clearly visible.

Figure 2 shows the multi-detector chromatograms for the three samples, the virgin and two irradiated PLA-PEO-PLA polymers. The chromatograms and separations are of high quality and differences between the samples are clearly visible.

Table 1 shows the numerical results from these analyses. The differences between the samples are very clear and the degradation in the irradiated samples is clearly significant. The molecular weight of the polymer has reduced by approximately two-thirds or more. The intrinsic viscosity and hydrodynamic radii of the polymer samples are also significantly reduced.

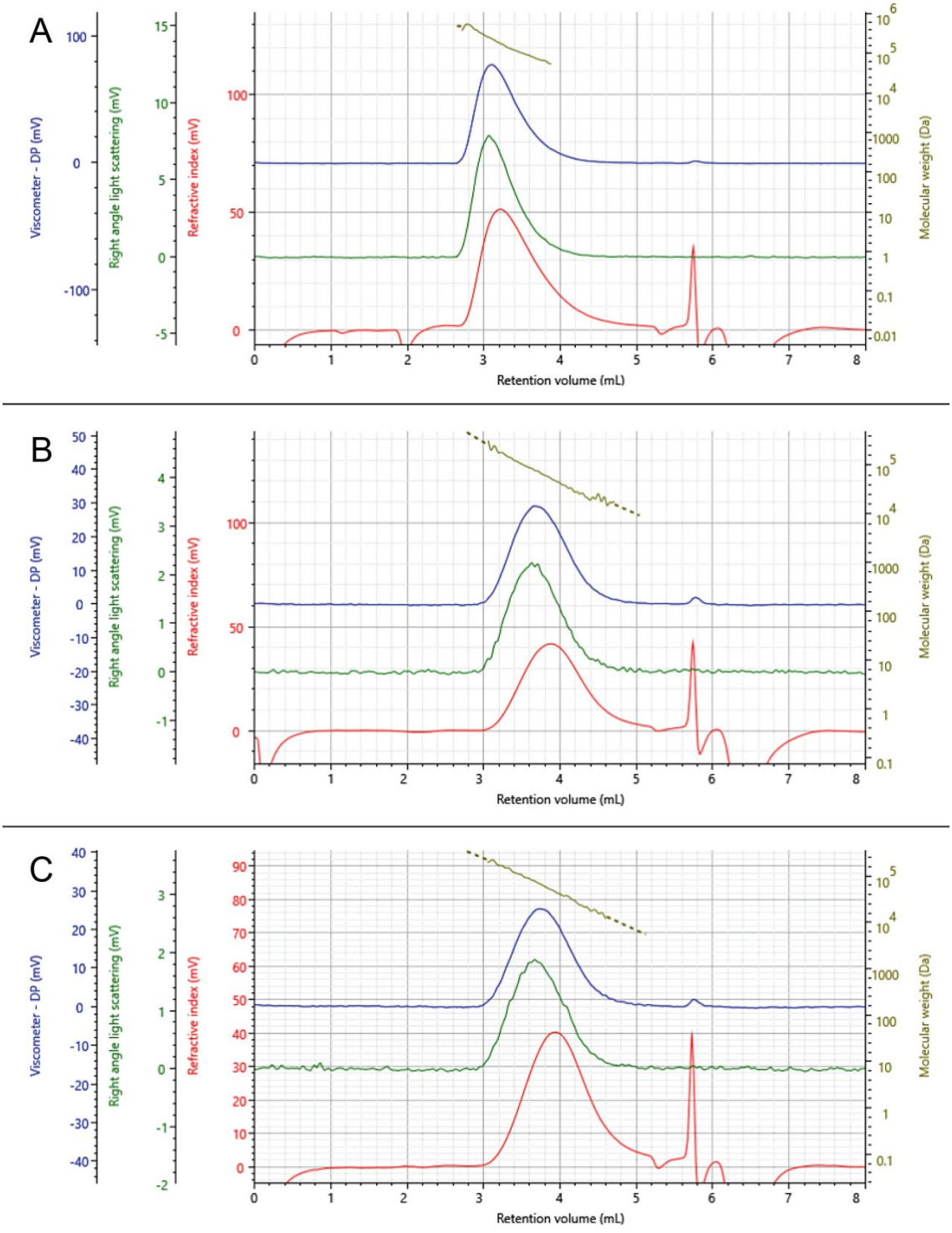

Figure 2.

Overlaid chromatograms of the virgin polymer showing the UPLC (red) and analytical SEC (purple).

	Virgin		Irradiated 1		Irradiated 2	
	Mean	%RSD	Mean	%RSD	Mean	%RSD
RV (mL)	3.208	0.4	3.883	0.0	3.9	1.5
Mn (g/mol)	146,400	6.6	41,970	4.0	30,490	2.3
Mw (g/mol)	184,400	1.2	65,280	5.3	54,920	3.1
Mw/Mn	1.263	5.7	1.555	2.7	1.802	5.4
IVw (dL/g)	1.54	6.7	0.74	6.5	0.65	3.5
Rh(η)w (nm)	16.14	2.9	8.83	4.0	7.93	2.2

Table 1. Molecular weight and intrinsic viscosity data for the three polymer samples.

Figure 3 shows Mark-Houwink plots for the three polymer samples. A Mark-Houwink plot shows intrinsic viscosity as a function of molecular weight. Any polymer with a distribution of molecular weights is represented as a line on the plot. A change in the gradient of the line is related to the way chains are added to the polymer backbone, a branched or cross-linked polymer is expected to have a lower gradient. A shift in the intercept with the Y-axis represents differences in the density of the backbone structure of the polymer; typically this is seen when different polymer chemistries are compared. In this case, the Mark-Houwink plots for the three samples overlay suggest no structural differences between them. The conclusion that can be drawn from this is that the polymer degradation is a result of simple polymer scission and not a more complicated mechanism.

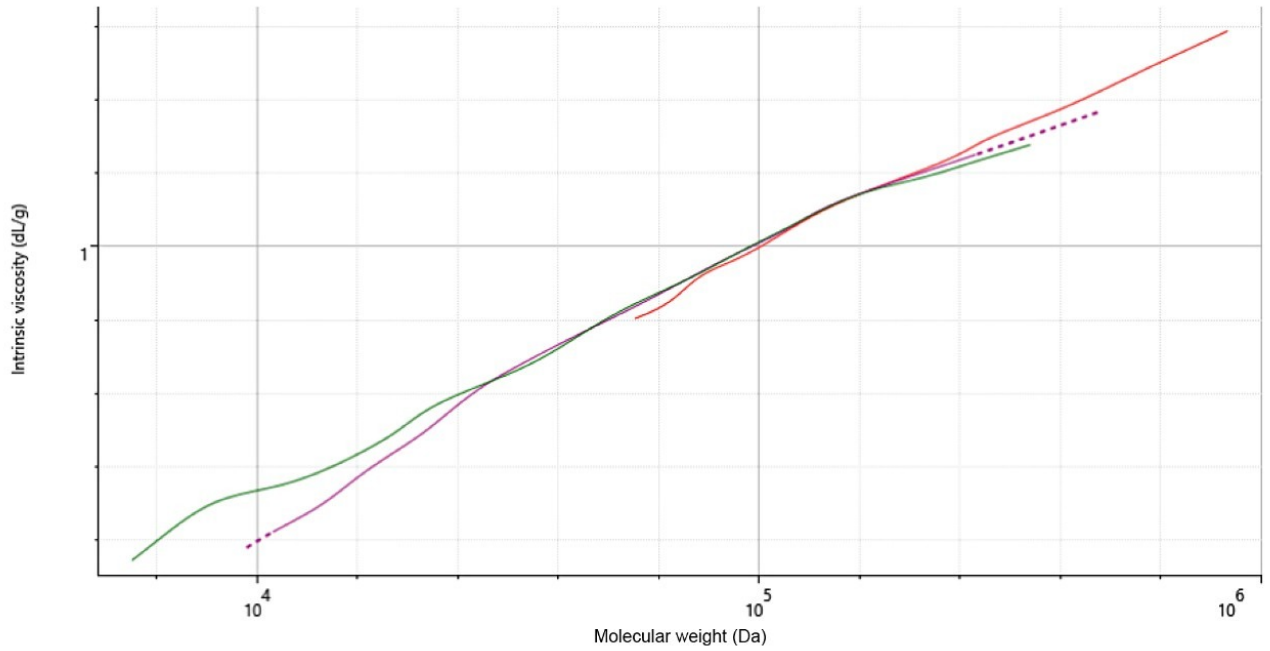

Figure 3. Overlaid Mark-Houwink plots for the three polymers showing virgin (red), irradiated 1 (purple), and irradiated 2 (green) samples.

Conclusion

The combination of the ACQUITY APC System with the OMNISEC-REVEAL multi-detector system provides high quality, high-resolution and rapid sample analysis. The ACQUITY APC System performs measurements with improved resolution, reduced sample loading, and with faster run times. This increases productivity, reduces the cost of these kinds of studies, and can be considered 'greener' thanks to reduced solvent usage. When combined with multi-detector systems, a higher level of characterization can be achieved, including the measurement of absolute molecular weight, independent of a polymer's chemistry or structure, as well as a the ability to make a direct measurement of any structural changes that occur.

In this study the degradation of a PLA-PEO-PLA drug delivery polymer was characterized. Irradiated samples were seen to have dramatically reduced molecular weight but no changes in their conformation or structure were observed, implying that this degradation was due to polymer scission caused by the radiation. This type of analysis is made possible due to the unique combination of high resolution separation using the ACQUITY APC System with a true multi-detection system, OMNISEC REVEAL, containing not only light scattering detectors but also an online differential viscometer to measure structure.

Acknowledgements

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