How switching mobile phases can improve your OMNISEC results

Analysis of ester- and acid-capped PLGA samples in mobile phases of DCM and THF

MOLECULAR SIZE

MOLECULAR STRUCTURE

MOLECULAR WEIGHT

Introduction

Biodegradable, thermoplastic materials polylactic acid and polyglycolic acid have found a wide range of uses, specifically in biomedical applications such as absorbable sutures, and medical implant hardware, as well as food packaging, clothing, and as a feedstock material for 3D printers. The high demand for these materials stems from the fact that they degrade via hydrolysis of the ester linkage into the natural metabolic byproducts lactic acid and glycolic acid. As these compounds are naturally occurring, the body is adept at eliminating them, minimizing the toxicity and other health risks.

In order to fine-tune the degradation rates of the final polymeric products, the two constituent monomers are frequently combined. The result of this copolymerization is poly(lactic-co-glycolic acid) or PLGA. The ratio of the two monomers affects the physical properties of the PLGA samples, including crystallinity and solubility. These factors can present obstacles when using solution-based techniques for material characterization. Another method of controlling the degradation rate is to convert the free carboxylic acid end groups to ester groups, as ester-capped PLGA samples degrade at a slower rate than the acid-capped materials. However, while the shorter degradation times of PLGA samples with the free carboxylic acid group may be desired in some instances, the materials themselves are more reactive. Thus, the end-caps present in a PLGA sample can present another obstacle during characterization.

Gel permeation chromatography (GPC) or, equivalently, size-exclusion chromatography (SEC), is a widely used technique to characterize a wide variety of macromolecules, from bulk manufactured materials to natural polymers and proteins. This technique can be used to measure the molecular weight moments (Mw, Mn), molecular weight distribution (Mw/Mn), intrinsic viscosity (IV) and

hydrodynamic size (R_H) of these macromolecules. Figure 1 shows Malvern's OMNISEC, a complete, all-inclusive GPC/SEC system.

A brief overview of how GPC/SEC works: A solvated sample is carried by a liquid mobile phase through an analytical column full of porous gel particles, where diffusion-controlled separation of the macromolecular components occurs, and is ultimately observed by different detectors as each slice of sample elutes. A common advanced detection GPC/SEC setup includes refractive index (RI), viscometer, and light scattering detectors.

In this Application Note two PLGA samples, one acid-capped and one ester-capped, will be studied in two different sets of GPC/SEC analysis conditions. The results will be compared and optimal analytical conditions will be determined for these samples.

Figure 1: Malvern's OMNISEC advanced detection GPC/SEC system

GPC/SEC Analysis of PLGA

Two commercially available PLGA samples were analyzed by GPC/SEC. One sample was ester-capped, sample PLGA-E, and the other was acid-capped, sample PLGA-A. Both samples were dissolved in dichloromethane (DCM) to prepare sample solutions with concentrations of 5.3 and 3.6 mg/mL, respectively. Initial analysis was done using two T6000M columns with a flow rate of 1 mL/min and injection volumes of 100 µL. The samples were passed through a 0.2 µm PTFE syringe filter as they were transferred to autosampler vials to await injection.

In the following triple detector chromatograms of samples PLGA-E and PLGA-A, the refractive index signal is red, the viscometer signal is blue, and the right angle light scattering signal is green. For these analyses, since the hydrodynamic radius of each sample is less than 8 nm, meaning they're relatively small samples, only data from the right angle light scattering detector will be considered.

GPC/SEC analysis in a mobile phase of DCM

Initial analysis was performed in a mobile phase of DCM because DCM is an established dissolution solvent for PLGA and was used to prepare the samples. The triple detector chromatograms of samples PLGA-E and PLGA-A are shown below in Figures 2 and 3.

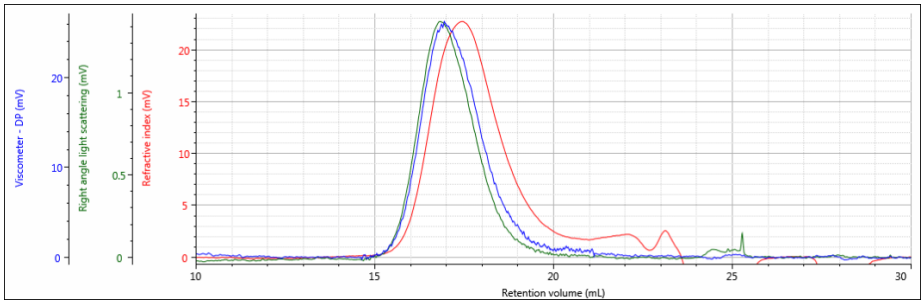

Figure 2: Triple detector chromatogram of sample PLGA-E in a mobile phase of DCM

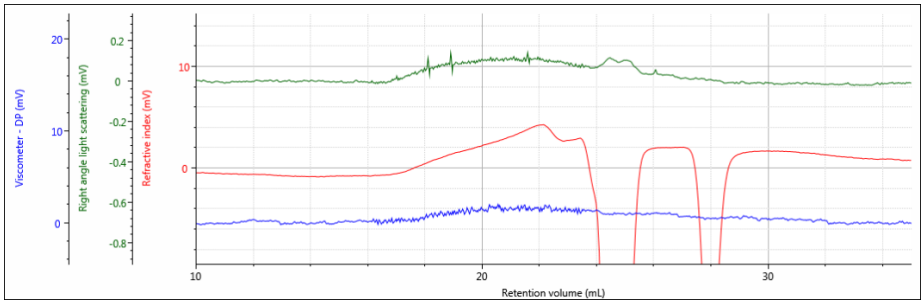

Figure 3: Triple detector chromatogram of sample PLGA-A in a mobile phase of DCM

The most notable observation of the chromatograms of these two samples is that the signal for sample PLGA-A does not look like a Gaussian peak obtained from good chromatography. One potential explanation for this result are that the sample was not truly dissolved and so it was filtered out of solution prior to injection. Another possibility is that the sample is sticking to the column, maybe as a result of the free carboxylic acid end groups. Also, there is a chance that the sample was not polymeric in the first place and so no real sample was actually injected. Since these are commercially available samples that last scenario is unlikely, but should be considered.

The triple detector chromatogram for sample PLGA-E showed good chromatography, but some of the signals were relatively weak. There is only about 1 mV of right angle light scattering data and only 20 mV of RI data. For a sample at a concentration of about 5 mg/mL these detector responses are on the low end of the expected range.

Based on this data, while DCM may be an acceptable mobile phase for sample PLGA-E, it is clear that DCM is not an appropriate mobile phase for sample PLGA-A.

GPC/SEC analysis in a mobile phase of THF

With the hope of improving the data for samples PLGA-E and obtaining usable data for PLGA-A, the mobile phase was switched from DCM to tetrahydrofuran (THF). This switch wasn't completely random; the refractive index of THF is 1.405, which is lower than that of DCM at 1.424. Since the PLGA sample provides a positive peak that means it has a refractive index greater than that of the mobile phase. By using a mobile phase with a lower refractive index the magnitude of the difference between the mobile phase and sample would be increased. This, in turn, will provide a stronger detector response. Another way to describe this relationship is that the dn/dc, or refractive index increment, of PLGA is higher in THF than it is in DCM.

The triple detector chromatograms for samples PLGA-E and PLGA-A are shown below in Figures 4 and 5. It is important to note that the sample solutions injected into a mobile phase of THF for this analysis were the same sample solutions prepared in DCM and analyzed in a mobile phase of DCM; only one preparation of each sample is described in this document. The analytical conditions, including the column set used, remained identical between analyses. The only difference was that the mobile phase was switched from DCM to THF.

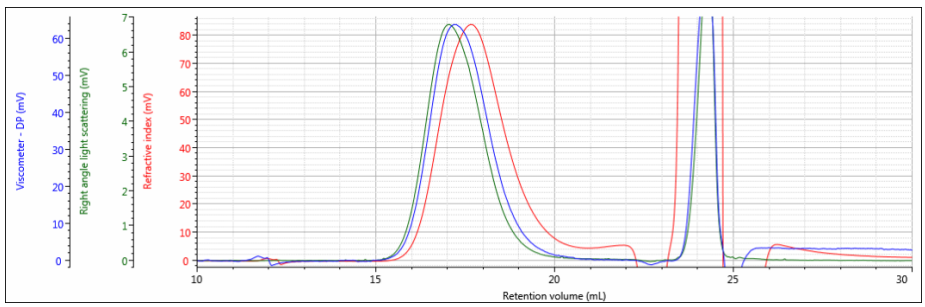

Figure 4: Triple detector chromatogram of sample PLGA-E in a mobile phase of THF

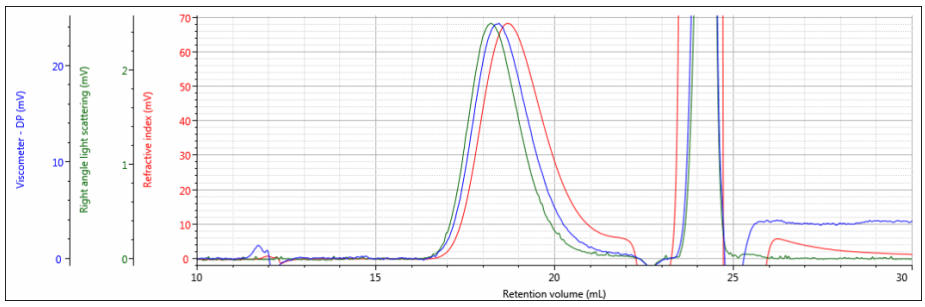

Figure 5: Triple detector chromatogram of sample PLGA-A in a mobile phase of THF

In both chromatograms there is a large peak that elutes from 23-25 mL and extends beyond the vertical range of the figures. This is from the DCM eluting in the mobile phase of THF. The high concentration of the dissolution solvent creates a strong response in all detectors. However, since the dissolution solvent is a

small molecule, the peak is resolved from that of the sample and does not affect the calculated results.

As can be seen from Figures 4 and 5, the chromatography and detector responses improved for both samples PLGA-E and PLGA-A when a mobile phase of THF was employed. In the case of sample PLGA-E, which provided good but not great data in a mobile phase of DCM, the peak shapes remain similar in both mobile phases, however the detector signal amplitudes increased. The RI signal increased from 22 mV in a mobile phase of DCM to 84 mV in a mobile phase of THF and the right angle light scattering signal increased from 1.4 mV in DCM to 6.8 mV in THF. This increase in RI and right angle light scattering signals is attributed to the increased refractive index difference, or dn/dc value, between the sample and mobile phase.

The difference between the chromatograms of sample PLGA-A in a mobile phase of DCM and a mobile phase of THF was even more significant. In a mobile phase of DCM, sample PLGA-A did not elute from the column properly, for a number of potential reasons mentioned previously: incomplete dissolution, sample adhering to the column, or no sample present at all. Analysis of sample PLGA-A in a mobile phase of THF provided evidence that eliminated two of those possibilities. As Figure 5 illustrates, there is clearly sample present and it is more than sufficiently soluble. The RI signal and right angle light scattering signal increased from nothing to 68 mV and 2.4 mV, respectively. The mobile phase of THF provided two benefits in the case of sample PLGA-A: 1) it helped sample PLGA-A to elute from the column set properly, and 2) it provides a higher dn/dc value, and thus stronger RI and light scattering detector responses.

Figures 6 and 7 provide a visual summary of the two analyses of samples PLGA-E and PLGA-A. Figure 6 shows the RI signals for samples PLGA-E and PLGA-A in both mobile phases, and Figure 7 shows the right angle light scattering signal for samples PLGA-E and PLGA-A in both mobile phases. The improvement in detector response for each sample is striking; both detector signals for sample PLGA-E in a mobile phase of THF are roughly four times that of the respective signal in a mobile phase of DCM. And sample PLGA-A, unobservable in a mobile phase of DCM, yields strong detector responses in a mobile phase of THF. Again, the single factor responsible for this stark improvement in data between the two analyses for a given sample is the mobile phase.

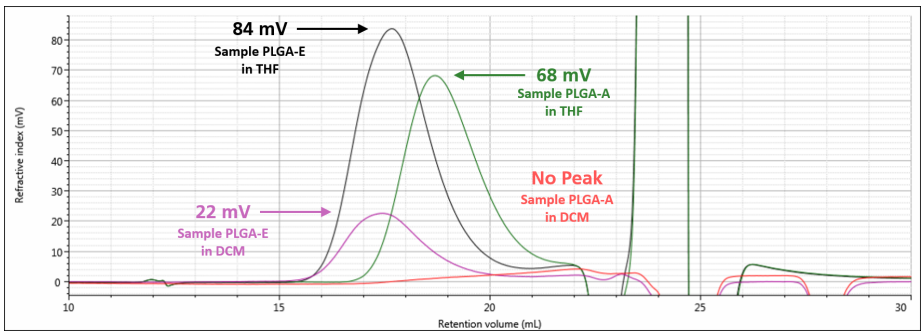

Figure 6: Overlay of refractive index chromatograms in DCM for samples PLGA-E (purple) and PLGA-A (red) and in THF for samples PLGA-E (black) and PLGA-A (green)

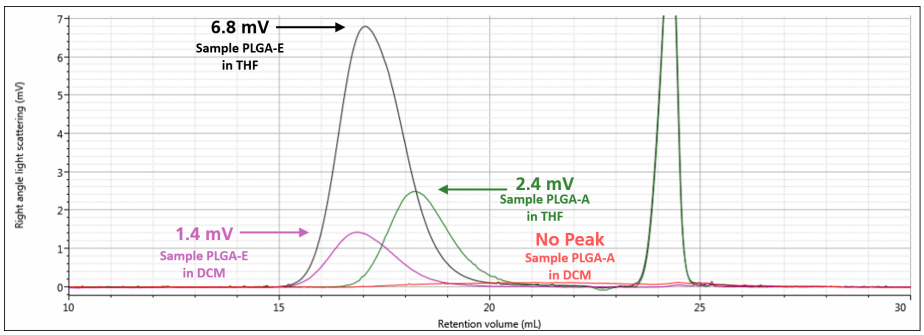

Figure 7: Overlay of right angle light scattering chromatograms in DCM for samples PLGA-E (purple) and PLGA-A (red) and in THF for samples PLGA-E (black) and PLGA-A (green)

Table 1 presents the molecular characterization data for samples PLGA-E and PLGA-A in mobile phases of DCM and THF. It is important to note that even though the detector response was not as strong in a mobile phase of DCM, the molecular data calculated for sample PLGA-E is comparable to that obtained in a mobile phase of THF. This is primarily due to the sensitivity of the OMNISEC system, which can measure low molecular weight materials with low dn/dc values, as demonstrated in a previous Application Note. No data was calculated for sample PLGA-A in a mobile phase of DCM.

Sample (mobile phase)	Mz	Mw	Mn	Mw/Mn	IV (dL/g)	Rh (nm)	dn/dc
PLGA-E (DCM)	151,700	104,300	58,210	1.79	0.325	7.80	0.02
PLGA-E (THF)	157,800	103,700	64,170	1.62	0.319	7.73	0.03
PLGA-A (DCM)	—	—	—	—	—	—	0.02
PLGA-A (THF)	67,890	44,580	26,300	1.70	0.148	4.52	0.03

Table 1. Molecular characterization data for samples PLGA-E and PLGA-A in mobile phases of DCM and THF; Mz, Mw, Mn in Da

A potential reason for why sample PLGA-A elutes in a mobile phase of THF but not DCM might have to do with the reactivity of the carboxylic acid end groups present. Sample PLGA-E, with ester end groups, eluted in a regular manner regardless of mobile phase, but the more reactive carboxylic acid end groups may have caused sample PLGA-A to adhere to the column set. Switching the mobile phase to THF may have improved the chromatography because THF is a hydrogen

bond acceptor. The reactive part of the carboxylic acid end group is the labile proton, which is available to participate in hydrogen bonds. In a mobile phase of DCM, which is not a hydrogen bond acceptor, this reactive portion of the molecule was free to interact with the stationary phase of the column set. In a mobile phase of THF, however, the mobile phase molecules could form hydrogen bonds with the free carboxylic acid end groups effectively reducing their reactivity and "shielding" them from potential interactions with the column set. So in addition to providing a higher dn/dc value for PLGA samples in general, THF in this case serves as a better mobile phase for both acid- and ester-capped PLGA samples.

Conclusions

Malvern's OMNISEC Triple Detection GPC/SEC System provides outstanding chromatography data for the analysis of both acid- and ester-capped PLGA samples. The analysis of the two types of PLGA samples was demonstrated in mobile phases of DCM and THF. While DCM was used as a dissolution solvent, the optimal mobile phase for the analysis of these PLGA samples was found to be THF for two reasons: 1) the dn/dc value of PLGA in THF is greater than that in DCM, and 2) a mobile phase of THF is capable of eluting both ester- and acid-capped PLGA samples from the column set, possibly by forming hydrogen bond pairs with the reactive end groups in the acid-capped PLGA samples.

It should be noted that the analysis conditions described in this document might not apply to all PLGA samples. In general, PLGA and related poly(lactic acid) and poly(glycolic acid) samples possess low dn/dc values, therefore the light scattering sensitivity of the OMNISEC system is required to obtain reliable data. When working with different PLGA or similar samples, the experiments outlined in this document should serve as a guide to determine the ideal run parameters for your OMNISEC system for each unique sample.

Since the molecular weight and composition of PLGA samples dictates the physical properties of the end products, accurate measurement of these parameters is critical for researchers and manufacturers. These accurate measurements are dependent on the quality of the method, including the system hardware and specific analytical conditions, such as mobile phase. With these tools and knowledge in place, scientists can better control the molecular properties that are crucial to producing application specific materials that are depended upon in biomedical situations, food packaging, clothing, 3D printing, and other areas of life.

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