Cambridge Polymer Group

GPC/SEC Analysis of PLGA Used for Drug Delivery

Summary

Poly(lactide-co-glycolide) (PLGA) has emerged as a versatile polymer in the medical device industry, primarily due to its highly tunable degradation rate, varying from months to years. This tunability allows PLGA to be used in various applications, with particular interest in drug delivery systems. While the degradation rate is tied to the block copolymer's chemical composition, it is also influenced by the polymer's structural characteristics.

In this study, we present findings from gel-permeation chromatography (GPC) analysis of a drug delivery material, employing triple detection techniques. This approach allows for the determination of crucial parameters, including absolute molecular weights, molecular weight distribution, Mark-Houwink parameters, and extent of polymer chain branching. Additionally, we offer a concise overview of the capabilities and applications of triple detection GPC, highlighting its significance in polymer characterization.

Introduction

PLGA has a long history of being used in long-lasting injectable formulations. Molecular weight analysis of PLGA is traditionally performed by GPC. However, conventional GPC using a conventional refractive index detector and comparing the elution times of the test materials against polymer molecular weight standards is not adequate for characterization of unique PLGA polymers (and more broadly branched polymers in general). The Food and Drug Administration requires more detailed characterization of polymers used in drug delivery for new formulations as well as in generic formulations to show bioequivalence, including qualitative/quantitative (Q1/Q2 sameness) to their Reference Listed Drug (RLD).

Triple detection is, as the name implies, an extension of conventional GPC that utilizes three different detectors, namely refractive index detector, viscometer detector, and light scattering detector. This approach allows the density of the polymer coil to be inferred independently of the elution time. In this application note, we have used triple detection on a group of PLGA samples to determine absolute molecular weight, intrinsic viscosity, and polymer branching profile.

Experimental

GPC testing was performed using an Agilent PL-220 GPC equipped with a thermostatted column oven, a refractive index detector, a viscometer and a light scattering detector. Three Agilent PLgel Mixed-B columns (10 µm, 300 mm X 7.5 mm) connected in series were used for separation. Conventionally the time which a polymer takes to pass through the column (known as the elution time) can be used to infer the molecular weight by comparing the time to that of a known standard

polymer. However, the rate of elution measures the relative overall size of the polymer, and this size is dependent on the exact composition and structure of the polymer. For this reason, orthogonal detectors that can infer something about the density of the polymer yield information about the structure.

The Mark-Houwink equation gives a relation between intrinsic viscosity ([η]) and molecular weight (M):

$$[\eta] = KM^{\alpha}$$

Plotting the log of this relation gives a straight line with intercept of log (K) and a slope of alpha (α). This is a useful relationship because the values of K and α give information about the conformation of the polymer in solution. For most polymers, the value of α ranges between 0.5 and 0.8, with a lower value indicating a more compact, dense structure approximating a "hard sphere". A random coil polymer in a good solvent would be expected to have values in the range of 0.7-0.8 with increasing values indicating larger, more solvated structures. The limiting value of 2.0 implies a structure that approximates a "rigid rod" where the polymer is stretched out in one direction. Determination of branching is therefore based on measuring the intrinsic viscosities of a linear (reference sample) and branched sample of the same molecular weight where the branched sample is more compact and therefore exhibits a lower viscosity compared to the linear reference.

The branching ratio (g') is determined from the relative intrinsic viscosities in the following equation:

$$g'(M) = \frac{IV_{branched}}{IV_{linear}}$$

Where IV branched and IV linear are the intrinsic viscosities of the branched and linear PLGA having the same molecular weight M.

The g was calculated based on the relation:

$$g' = ge$$

Where "e" is a "drainage factor" that describes how the solvent interacts with the polymer and is specific for each PLGA. The "e" factor was selected to equal 0.75 based on a PLGA reference[1].

The branching value or functionality value of PLGA, F, was then calculated fitting g into the star branched regular model that assumes monodisperse length of branches in the star using the equation:

$$g = (3F\text{-}2)/F^2$$

Results

In Figure 1, we present the comparison between the reference linear material and the test articles in terms of a molecular weight distribution. The linear sample exhibited higher average molecular weight moments (M_p, M_n, M_w) and a narrower distribution (sharper peak) compared to the other samples. It is important to note however that based on this plot one would infer that the molecular weight distribution was different but could state nothing about the relative structures of the polymers.

[1] J. Hadar, S. Skidmore, J. Garner, "Characterization of branched poly(lactide-co-glycolide) polymers used in injectable, long-lasting formulations", *J. Control Release*, 304(2019) 75-89

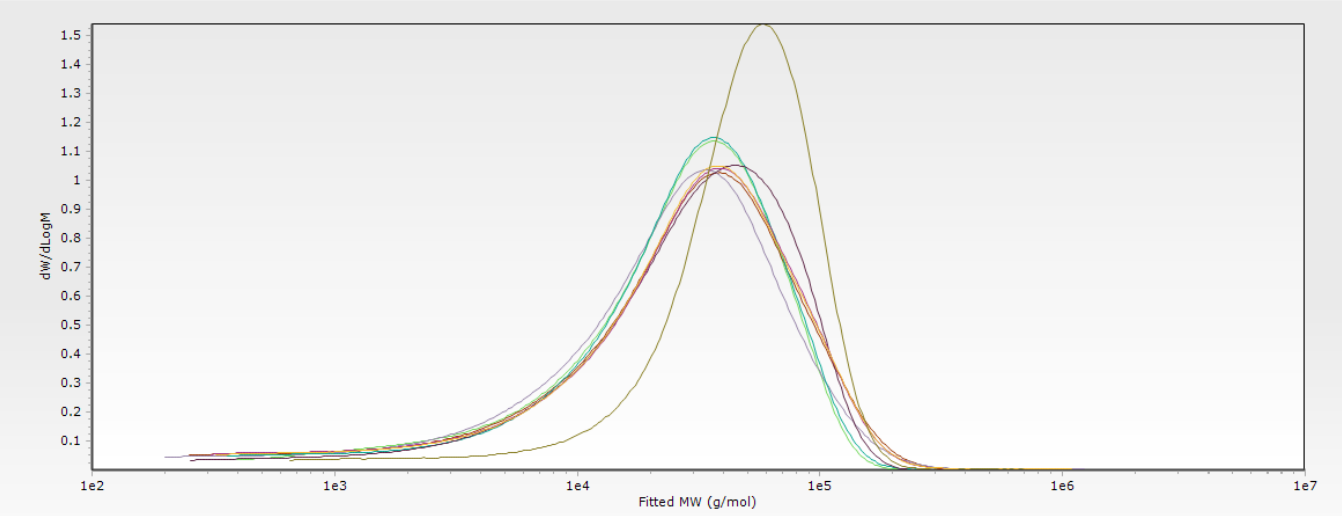

Figure 1: Molecular weight distribution of PLGA samples. Reference linear sample is shown in khaki green; most branched sample is shown orange.

Structural differences between the polymers are clearer when the Mark-Houwink plots are examined, representing intrinsic viscosity as a function of molecular weight, as provided in Figure 2. The intrinsic viscosities of the linear reference sample were higher than viscosities of all other samples having the same molecular weight, indicating that the samples are branched and have more compact structure than their linear counterpart. Branching means that space that would otherwise not be filled by the random linear polymer chain can be occupied by side-shoots of the main chain, thus making the chain denser for the same overall dimension. The highest alpha value of 0.58 was obtained for the linear sample, characteristic of a random coil in a good solvent. The alpha values for the rest of the samples were determined to be in the range from 0.47 to 0.37, suggestive of "hard sphere", indicating dense polymer chains. The most branched sample (orange) had an alpha value of 0.37.

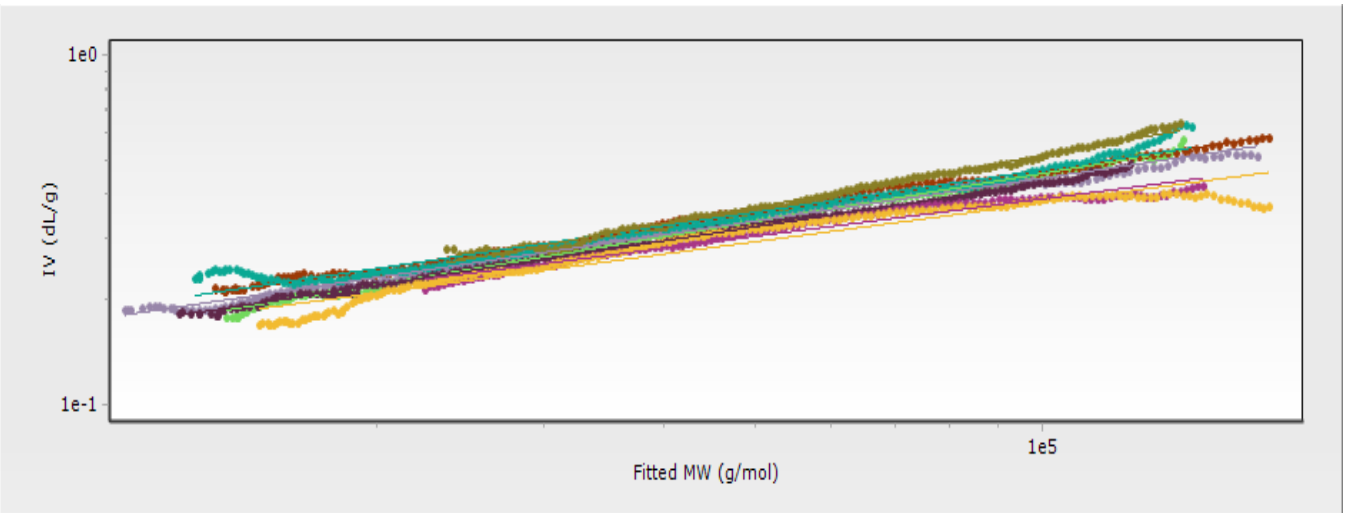

Figure 2: Mark-Houwink plot overlay. Reference linear sample is shown in khaki green; most branched sample is shown orange.

The branching functionality distribution per molecule as a function of molecular weight is shown in Figure 3. All samples had branching functionality of more than two (F=2 corresponds to the linear sample) which increased with increasing molecular weight. The most branched sample (orange) had functionality of ~6, which is consistent with its lowest alpha value.

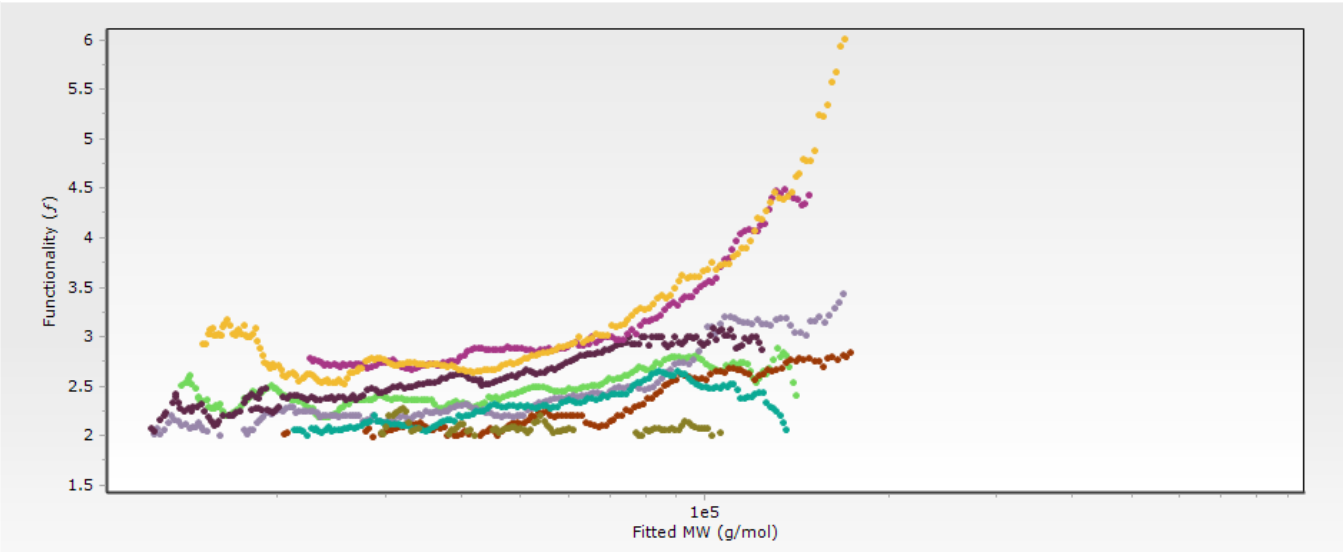

Figure 3: Branching functionality (F) distribution plot overlay for all samples. Reference linear sample is shown in khaki green; most branched sample is shown in orange.

Conclusions

The data presented in this study demonstrate the remarkable sensitivity and precision of triple detection GPC. While specific structural information may still be challenging to infer directly, this technique offers a unique advantage in unambiguously identifying the presence of branching - a capability that conventional GPC lacks. This ability to detect branching is crucial for a comprehensive understanding of polymer properties and behavior.

Broader Applications

Although this case study focused on PLGA, the implications of branching extend far beyond this specific polymer:

1. **Post-polymerization reactions**: Branching can significantly impact polymers that undergo reactions after initial polymerization.
2. **Weakly crosslinked polymers**: Materials such as hyaluronic acid can exhibit branching that is undetectable through conventional GPC methods.
3. **Post-sterilization effects**: Polymers like polyethylene that undergo radiation sterilization may develop branching structures.

Impact on Material Properties

The presence of branching, even when subtle, can profoundly influence various polymer characteristics, including diffusion behavior, viscosity, and overall material performance. These properties are particularly sensitive to branching, underscoring the importance of accurate detection and characterization.

In conclusion, triple detection GPC emerges as a powerful analytical tool, offering insights into polymer structure and behavior that were previously unattainable. This technique's ability to reveal subtle yet impactful structural features makes it invaluable for polymer science and its diverse applications in materials engineering and biomedical research.

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