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アプリケーションノート

Analysis of Polylactic-co-Glycolic Acid (PLGA) by Gel Permeation Chromatography (GPC) using the Arc™ HPLC System with a Refractive Index (RI) Detector

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Abstract

Polylactic-co-glycolic acid (PLGA) is a biodegradable polymer widely used in medical applications to sustain or control release of drugs. The physical properties of PLGA, including molecular weight and polydispersity, can impact the release behavior of drugs. This study illustrates determination of the molecular weight distribution and polydispersity of PLGA by gel permeation chromatography (GPC). A GPC system composed of an Arc HPLC with a strong solvent compatibility kit and RI detector was used for analysis of PLGA samples using a calibration curve of polystyrene standards. The degradation study of PLGA in 0.5% polyvinyl alcohol (PVA) in water solution showed decrease in molecular weight of the polymer over a 14-day study period.

Benefits

- Reliable GPC analysis of PLGA polymers using the Arc HPLC System with a strong solvent compatibility kit and RI detector
- Efficient processing of GPC data for molecular weight distribution using Empower™ Software with the GPC option
- Easily monitor degradation of PLGA polymer in aqueous media through characterization of molecular

weight and molecular weight distribution

Introduction

Polylactic-co-glycolic acid is a biodegradable functional polymer widely used in pharmaceutical and medical engineering products due its biocompatibility, nontoxicity, and nonimmunogenic properties.[1–3] It plays an important role in controlling the delivery of active pharmaceutical ingredients (APIs) in medical products.[1–2] Usually, a drug is incorporated within the polymer matrix, that acts as a carrier for the drug, and the release profile of the drug relates to the chemical and physical nature of the polymer, including the molecular weight. Polymers with higher molecular weights generally exhibit lower degradation rates, therefore reducing the drug release rate compared to polymers with lower molecular weights.[1–3] PLGA is a copolymer of polylactic acid (PLA) and polyglycolic acid (PGA).[2]

GPC, also referred as size exclusion chromatography (SEC), is a technique commonly used to characterize the molecular weight distribution and polydispersity index (PDI) of polymers. The molecular weight measurements generally include number average molecular weight (Mn), weight average molecular weight (Mw), and z-average molecular weight (Mz). The PDI is a ratio of Mw/Mn and measures the broadness of molecular weight distribution.

In this study, the molecular weight and polydispersity of PLGA samples was determined using the Arc HPLC System equipped with the strong solvent compatibility kit and RI detector. The strong solvent compatibility kit allows the use of strong solvents, such as tetrahydrofuran or dimethylformamide, required for GPC analysis of polymers.[4–5] The molecular weight measurements of PLGA samples were calculated using a relative calibration curve generated with polystyrene standards. The effect of water on the degradation of PLGA was investigated and resulted in reduction of molecular weight of polymer over the duration of the 14-day study. All calculations were performed using Empower Software with the GPC option.[6–7]

Experimental

Tetrahydrofuran (THF) certified with about 0.025% butylated hydroxytoluene purchased from Fisher Chemicals, catalog number: T397–4. Isopropyl alcohol (IPA) purchased from Honeywell, catalog number LC323–4. Polyvinyl alcohol (PVA) purchased from MP Biomedicals, catalog number 151941.

Sample Description

Standard Solutions

ACQUITY™ APC polystyrene middle molecular weight calibration kit, p/n: 186007540 < https://www.waters.com/nextgen/global/shop/standards--reagents/186007540-acquity-apc-polystyrene-middle-mw-calibration-kit.html> . Two standards out of set of three were used in this study:

- Red caps vial: 1.5 mg each of polystyrene at Mp 130 K, 21.5 K, 6.54 K, 1.25 K
- White cap vial: 1.5 mg each of polystyrene at Mp 35.5 K, 9.13 K, 2.28 K, 0.266 K

Polystyrene standard solutions were prepared by adding 1.0 mL of tetrahydrofuran to each vial and allowing them to dissolve for a few hours. Concentration 1.5 mg/mL.

PLGA Sample Solutions

PLGA samples were obtained from Sigma-Aldrich with different ratios of lactide:glycolide (L:G):

- 50:50, molecular weight: 30,000–60,000
- 65:35, molecular weight: 40,000–75,000
- 75:25, molecular weight: 66,000–107,000
- 85:15, molecular weight: 50,000–75,000

PLGA samples were prepared at 5 mg/mL in tetrahydrofuran by allowing them to dissolve for a few hours. Solutions were filtered through 0.45 µm PTFE syringe filters (p/n: 186009315 < https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186009315-wwptfe-acrodisc-mini-045m-13mm-100-pk.html>) using glass syringes prior to GPC analysis.

PLGA Degradation Samples

PLGA with 50:50 and 75:25 lactide:glycolide ratios were used in the degradation study by weighing out approximately 25 mg of polymer into separate scintillation vials. Each vial was incubated in 1 mL of deionized water containing 0.5% PVA at 37 °C. Vials were removed from the incubator on day one, two, six, eight, twelve, and fourteen. After discarding the aqueous media, samples were rinsed with deionized water and dried under vacuum to remove residual water. Finally, samples were dissolved in THF at 5 mg/mL concentration and filtered through PTFE syringe filters (p/n: 186009315 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/186009315-wwptfe-acrodisc-mini-045m-13mm-100-pk.html>) using glass syringes prior to GPC analysis.

Method Conditions

System:	Arc HPLC System with quaternary solvent manager (QSM), flow through needle (FTN) sample manager and strong solvent compatibility kit (p/n: 205002572)
Mobile phase:	Tetrahydrofuran
Separation:	Isocratic
Flow rate:	1.0 mL/min
Columns:	All columns 7.8 x 300 mm with 5 µm, connected in series using a joining tube (p/n: WAT084080) supplied with columns. Column heater/cooler (p/n: 186179100) Styragel™ HR 4, 10,000 Å, molecular weight range: 5,000–600,000, p/n: WAT044225 Styragel HR 2, 500 Å, molecular weight range: 500–20,000, p/n: WAT044237 Styragel HR 1, 100 Å, molecular weight range: 100–5,000, p/n: WAT044234
Column temperature:	35 °C
Detection:	Refractive Index (RI) Sampling rate: 10 pts/sec Polarity: positive Flow cell temperature: 35 °C
Injection volume:	50 µL

Vials:	LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV
Sample temperature:	15 °C
Wash solvents:	Sample manager/purge wash: tetrahydrofuran Seal wash: isopropyl alcohol

Data Management

Chromatography software:	Empower 3 Feature Release 5 Service Release 5 (FR5 SR5). GPC option used for data processing and reporting.

Results and Discussion

For GPC analysis, a bank of three columns with different porosities were selected to cover the molecular weight range of the polystyrene standards and PLGA samples. Varying column porosity ensured adequate resolution of polymers and accurate molecular weight measurements. Columns were connected in series, starting with the largest pore size to the smallest, to minimize back pressure. The standard and sample solutions dissolved in THF solvent were run isocratically using THF as a mobile phase. Data processing and molecular weights calculations were performed using the Empower Software GPC option.

Representative chromatograms of the polystyrene standards and PLGA samples are shown in Figure 1. The retention time corresponds to the elution volume of the polymer. Largest molecular weight eluted first, while the smallest eluted last.

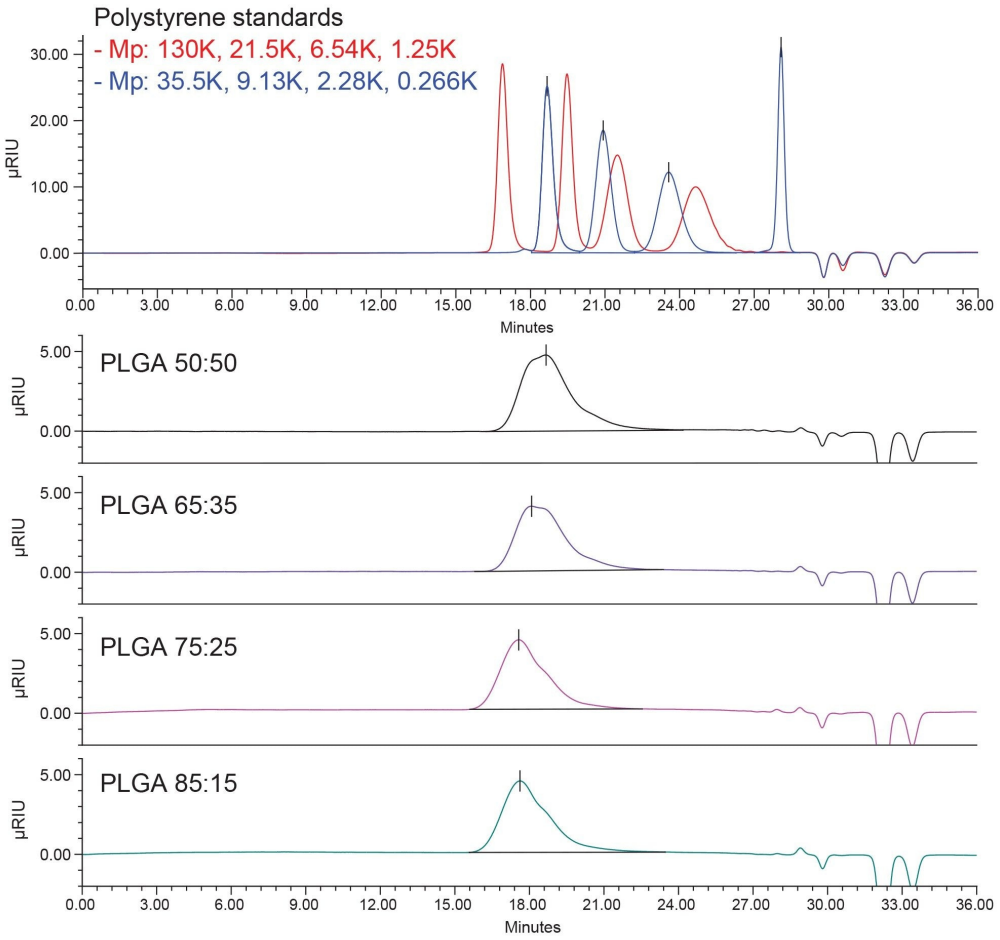

Figure 1. Chromatographic analysis of the polystyrene standards and PLGA samples using an Arc HPLC System with a strong solvent compatibility kit and RI detector.

GPC Calibration

The molecular weight at peak maximum (Mp) of each polystyrene standard were used to create a GPC calibration curve. Empower generated a curve by plotting logarithm of molecular weights (Mp) on the y-axis versus retention time on the x-axis. The method exhibited an acceptable GPC calibration using a third order fit with a correlation coefficient (R^2) of greater than 0.9998 (Figure 2).

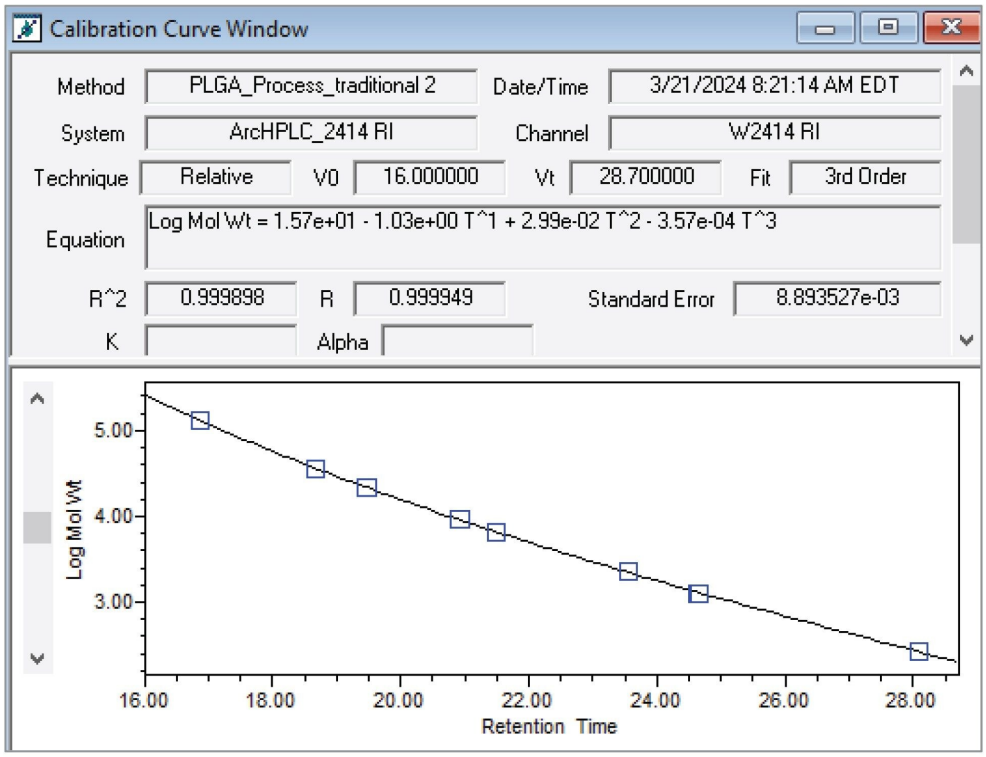

Figure 2. GPC calibration curve generated using polystyrene standards acquired using an Arc HPLC System with a strong solvent compatibility kit and RI detector.

Analysis of PLGA Samples

The molecular weight measurements of PLGA samples with lactide:glycolide ratios of 85:15, 75:25, 65:35, and 50:50 were calculated using a relative calibration curve produced using polystyrene standards. The calculated Mw value ranged from 39,722 to 74,688 Daltons with polydispersity index of 1.6 to 1.7 (Figure 3).

GPC_Molecular Weight
Result Set Id: 4437 Calibration Id: 4444
Processed Channel Descr.: W2414 RI

	Sample Name	RT	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	Mz (Daltons)	Mz+1 (Daltons)	Polydispersity
1	PLGA 50:50	18.671	36233	39722	24600	54240	67864	1.6
2	PLGA 65:35	18.087	53826	44637	25843	62129	78186	1.7
3	PLGA 75:25	17.573	77287	74688	46507	103389	132013	1.6
4	PLGA 85:15	17.622	74614	70809	43322	98363	125756	1.6

Figure 3. Molecular weights of PLGA samples generated using an Arc HPLC System with a strong solvent compatibility kit and RI detector.

Degradation Study of PLGA

In this study, a degradation of PLGA 50:50 and PLGA 75:25 incubated in aqueous media at 37 °C for two weeks was investigated. An aqueous media containing 0.5% PVA in water was used to incubate PLGA samples based on a previously published work, which suggested that the PLGA polymers are prone to degradation through interaction of hydrophilic groups within the polymer with water.[3] These interactions may change the polymer's properties, which can impact the performance of drug delivery systems.[3]

In this study, the molecular weight of samples pulled at different time intervals was calculated using the polystyrene standards (Mp: 266–130,000 Da) prepared at 1 mg/mL in THF. The results showed a decrease in Mw of the incubated PLGA samples over the study duration of 14 days (Figure 4).

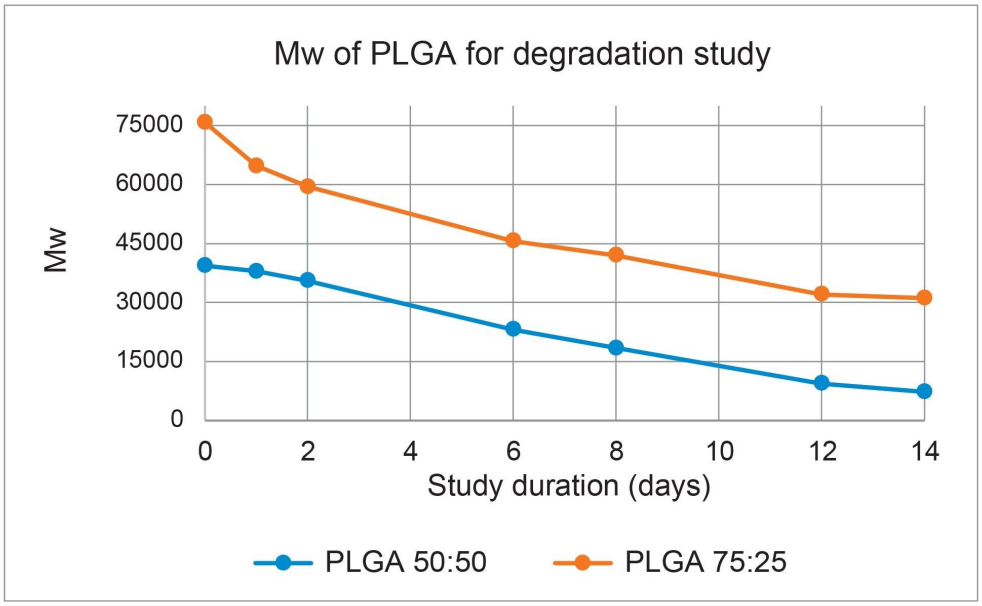

Figure 4. Weight average molecular weights (Mw) of PLGA polymers incubated in 0.5% PVA in water solution at 37 °C.

The GPC results for PLGA samples with 50:50 and 75:25 L:G ratios acquired at the start of the study (day zero) were compared with days eight and fourteen incubation intervals to assess for molecular weight changes over time. The chromatographic separation showed that incubated samples with smaller molecular weight eluted later compared to day zero samples (Figure 5). Additionally, the molecular weight distribution was evaluated using distribution plots. Empower generated distribution plots by plotting dwt/d(log M) and cumulative percent (%) molecular weight distribution versus the slide log molecular weight (Figure 6). The plots showed that the incubated PLGA 50:50 samples exhibited larger molecular weight distribution compared to day zero samples (Figure 6A). For the PLGA 50:50, day 14 incubation sample had the largest polydispersity index (PDI) of 2.4 compared to 1.8 and 1.6 for day eight and day zero samples, respectively. The PLGA 75:25 samples exhibited a similar polydispersity index over the course of the study (Figure 6B).

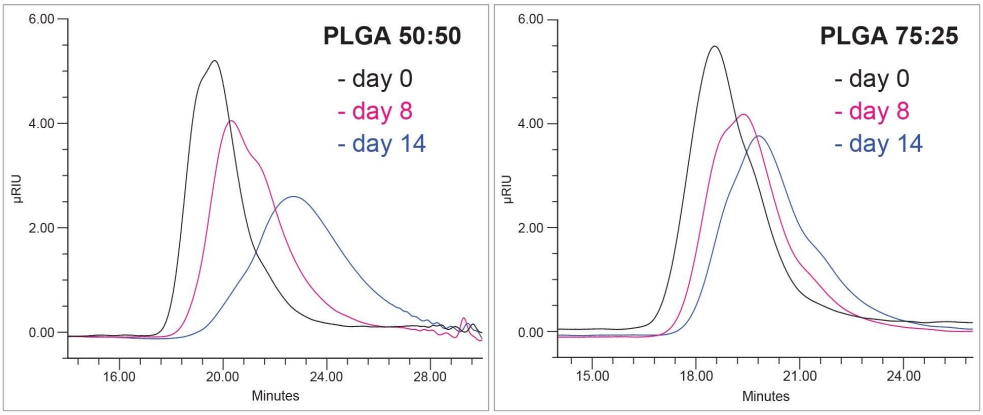

Figure 5. Chromatographic elution of PLGA samples from day zero, days eight and 14 incubation in 0.5% PVA in water solution at 37 °C.

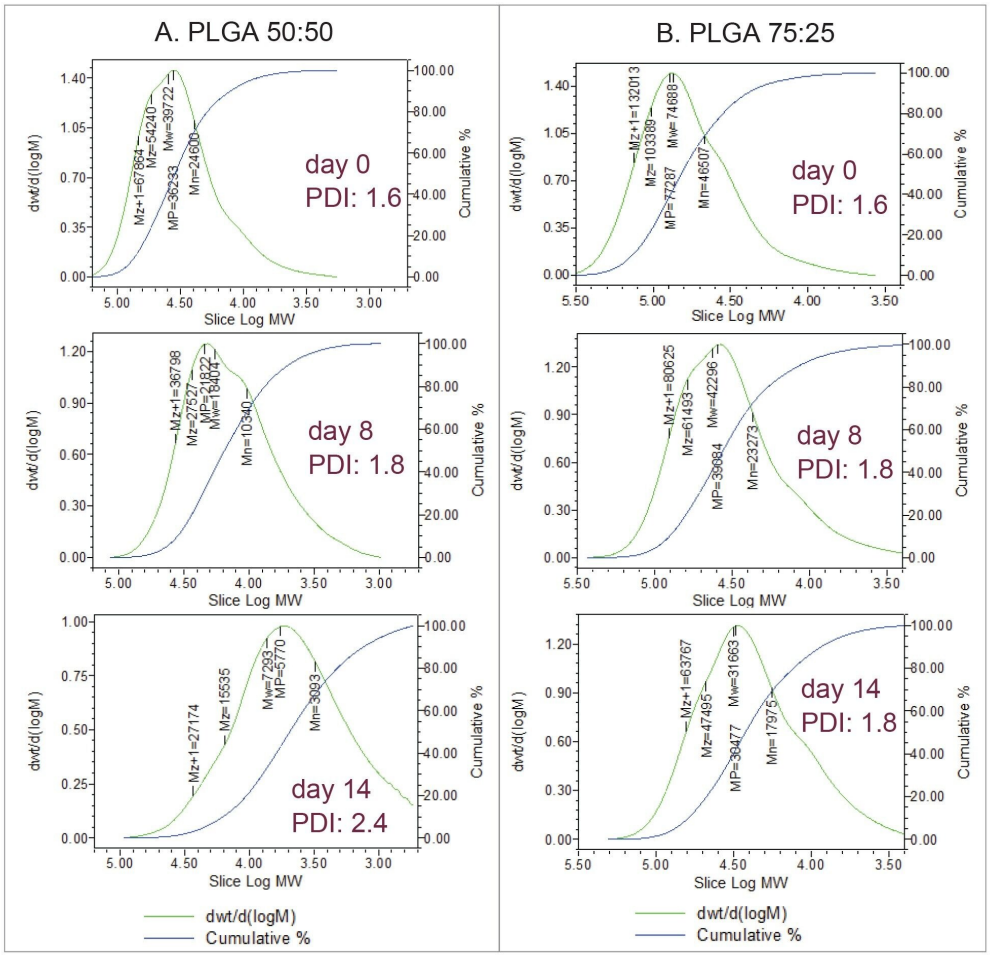

Figure 6. Molecular weight distribution plots for PLGA 50:50 (A) and PLGA 75:25 (B) degradation study by incubation in 0.5% PVA in water solution at 37 °C. PDI: polydispersity index.

Conclusion

The molecular weight distribution of PLGA polymers with different ratios of lactide:glycolide was calculated using a GPC system composed of an Arc HPLC System with a strong solvent compatibility kit. The molecular weights of the PLGA samples were calculated using a relative molecular weight calibration curve generated using polystyrene standards. The degradation study showed that the presence of water with 0.5% PVA reduces

the PLGA molecular weight. The Empower Software GPC option enabled quick data analysis to effectively characterize polymer molecular weight and molecular weight distribution.

References

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