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Poly(lactide-co-glycolide) (PLGA) Standards

User's Notes/Application Manual

Overview

The PLGA standards are a series of polymers with well characterized molecular weight and other characteristics which can be used in establishing calibration for Gel-Permeation-Chromatography (GPC) systems.

System Setup

All system parameters (columns, mobile phase, temperature, etc.) affect the resultant retention times of eluted samples. In this situation, if a change is made on the system, recalibrating it is suggested. Make sure the system has a suitable gel-permeation chromatograpy column in place and is utilizing an organic solvent that is a good solvent for the sample polymers (typically acetone, THF, or DCM).

PLGA detection

Due to a general lack of conjugated dienes, PLGA has a poor UV-Vis absorbance and UV-Vis detection is not suggested as the signal tends to be weak. In this case, refractive index detection is suggested and **Table 1** below shows the determined dn/dc (refractive index) for different PLGA types in indicated solvents.

Table 1. Batch-mode determined dn/dc values for indicated PLGA types (based on LA%) in indicated solvents.

PLGA Type	**Solvent**	
(% Lactide)	Acetone	THF
50L	0.0977	0.0531
75L	0.0950	0.0486
100L	0.0914	0.0457

Refractive index (dn/dc) determined by batch-mode analysis on T-rEX (Wyatt) in indicated solvent. Data from publication (J. Hadar, J. Garner, S. Skidmore, H. Park, K. Park, Y. K. Jhon, Y. Wang. "Correlation Analysis of Refractive Index (dn/dc) for PLGAs with Different Ratios of Lactide to Glycolide" Scientific Poster presented at 2018 annual meeting of Controlled Release Society)

What's included

Each PLGA standard provided by Akina comes as an exactly measured quantity (~ 50 mg) sealed in an argon-flushed glass vial for direct reconstitution. The traceable lot# indicated on the side of the vial correlates to a specific data sheet (if you do not have the paper copy, this is also available from the website (https://akinainc.com/polyscitech/products/polyvivo/plga_pla_standards.php) by selecting "Data Sheet" for the indicated product. The data sheet provides for a comprehensive characterization of the PLGA material by GPC-4D (universal calibration according to multi-angle light scattering), conventional GPC-ES (against polystyrene standards, provided for an example of data obtained from a conventional system), and NMR for lactide content by HNMR and LA:GA sequencing (blockiness vs randomness).

How to use

Warm PLGA standard vial in desiccator before opening. Make sure to use within 1 month of opening to limit the potential for properties changing due to humidity exposure. Vial provided has exact mass of PLGA standard included in vial listed on side. Add suitable volume of solvent (ideally reconstitute in same solvent used for mobile phase) to dissolve PLGA to a concentration between 1-5 mg/ml solvent, typically 2 mg/ml. Pass PLGA-solution through submicron filter (≤0.45 um) with a filter comprised of a solvent compatible membrane (example: PTFE or PVDF). Inject to the GPC system using the same parameters as applied for the sample(s) to be tested. Input the standards GPC-4D data from the data table in for the GPC software as a 'broad standard' if available. If software does not support this, then input the peak molecular weight data to compare to the peak retention time of the standard. Do not mix the standards together in a singular injection as their peaks may overlap.

Application example

The following example details the use of a set of standards (in this case 75L-H,M,S) on a conventional THF mobile phase GPC-RI system. This is provided only as an example and note that some parameters are system dependent.

Gel-Permeation Chromatography (GPC) calibration example: Water's Breeze-2 System

Sample Preparation

Samples, were dissolved in 0.2 um filtered chromatography grade Tetrahydrofuran (THF) (Fisher Chemical). Each soluble sample was dissolved at a concentration of 2 mg/ml in THF. After dissolution, the samples were passed through a 0.45um PTFE filter to remove particulates and placed directly into a septum capped 2 ml HPLC vial.

Instrument Setup

Gel-permeation performed using a Waters Breeze-2 system operated using Empower software. The system consisted of a model 1515 pump, model 2707 autosampler, and model 2414 refractive index detector. Elution was done with 1 ml/min flow of THF across three columns in sequence. Both columns and detector were temperature controlled at 35 ºC. The first column the samples passed through is a Phenomenex column Phenogel 5µ 50A 300 x 7.8 mm, the second is Phenomenex column 5µ 10E4A 300 x 7.8 mm, and the last one is Aglient Resipore 300 x 7.5 mm 3µm column.

Calibration/Analysis

The peaks were selected and processed using Empower software. The standards were input as broad standards including listing of Number average Molecular Weight (Mn), weight average molecular weight (Mw), and peak average molecular weight (Mp). The software applied recalculation to peak average molecular weight based on

curve obtained data as part of its normal processing method. The curve was set to 5th order polynomial. For this particular system, the void volume (Vo) was set to 16 min. and total volume (Vt) was set to 24 min.

Results

The standards provided discrete peaks. Note that due to the natural polydispersity of PLGA resultant from the chemistry of its synthesis provides for broad peaks (**Fig 1**).

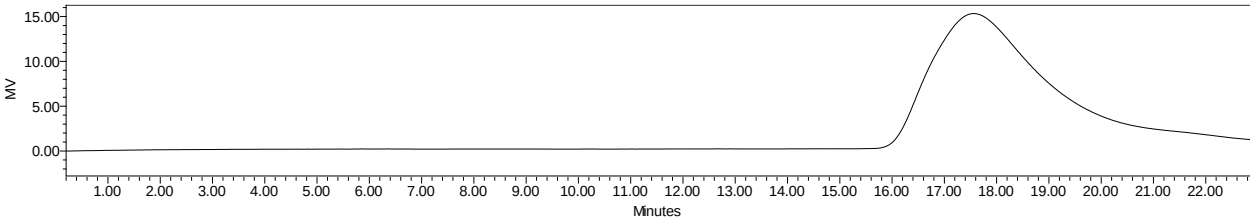

Figure 1. Example Chromatograph (PLGA-75L-H, Lot# 180313RAI-B) on Waters Breeze-2 system.

With the broad standard input the three standards run (PLGA-75L-H, Lot# 180313RAI-B; PLGA-75L-M, Lot# 180323RAI-A; PLGA-75L-S, Lot# 180410RAI-A) provided for a 9-point calibration curve (**Table 2**).

Table 2. Calibration curve data from GPC software (Empower software).

	Retention Time	Elution Volume	Mol Wt	Log Mol Wt	Calculated Weight	% Residual
1	17.557	17.557	105109	5.021638	104994	0.109
2	17.736	17.736	96330	4.983762	96528	-0.205
3	18.218	18.218	76170	4.881784	76052	0.155
4	19.516	19.516	25370	4.404320	25635	-1.032
5	19.675	19.675	22924	4.360297	22618	1.353
6	20.033	20.033	17830	4.251151	17879	-0.271
7	20.106	20.106	17117	4.233425	17171	-0.314
8	20.210	20.210	16290	4.211921	16256	0.208
9	20.712	20.712	12180	4.085647	12178	0.015

This data was used to calculate a standard curve according to the following parameters (**Fig 2**).

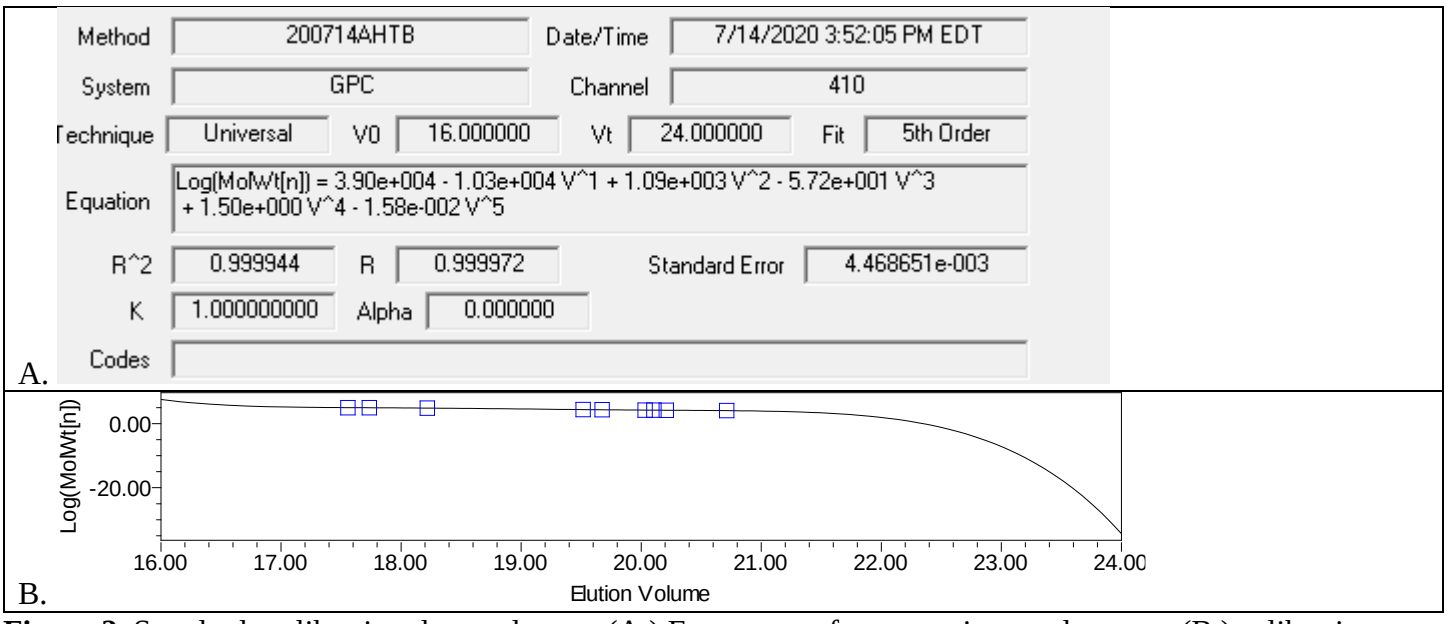

Figure 2. Standards calibration data and curve (A.) Empower software settings and outputs (B.) calibration curve.

This provides one example of the use of the standards for calibration of a system. Note that each system will have different parameters based on columns, mobile phase, and other configuration design properties.