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White-Paper: GPC-external Standard-GPC-4D correlation

December 19, 2022

Background

Poly(lactide) and Poly(lactide-co-glycolide) have multiple methods for determination of molecular weight. The method applied can have an impact on the resultant molecular weight determination. In this whitepaper, the results for a series of controlled polymer standards at known LA:GA ratios are determined utilizing both conventional gel-permeation chromatography (GPC)-external standard (GPC-ES) using commercial polystyrene standards and universal calibration by GPC-quaternary detection (multi-angle light scattering, dynamic light scattering, refractive index, viscometer). The resultant values for number average molecular weight (Mn) are compared between the two methods.

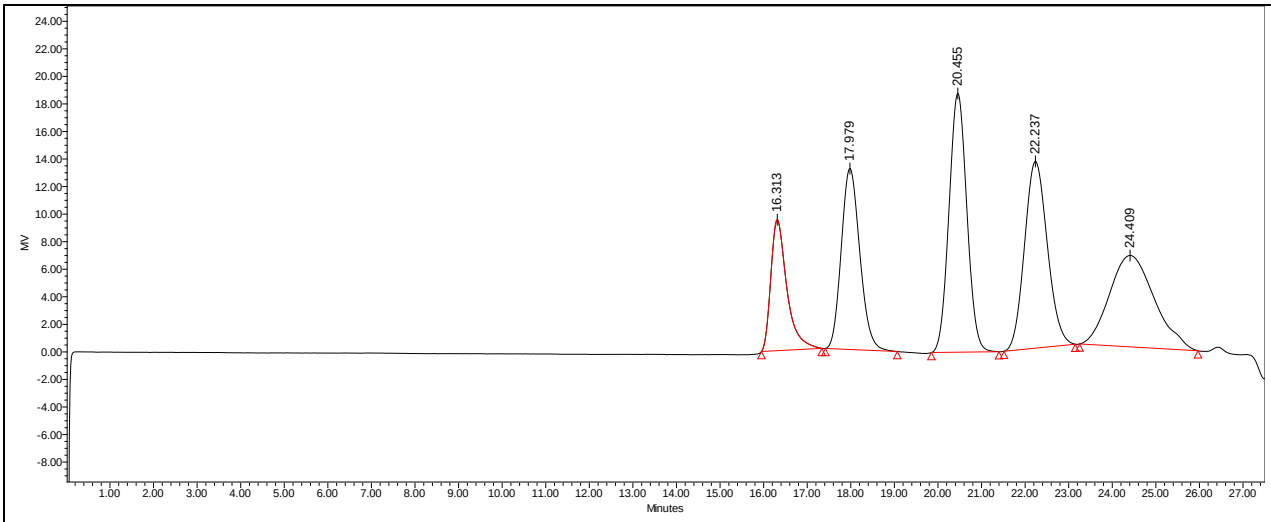
Method

Polymer standards of PLA and PLGA with decanol ester endcap (https://akinainc.com/polyscitech/products/polyvivo/plga_pla_standards.php) were samples separately and run, in parallel, on two instruments for molecular weight determination.

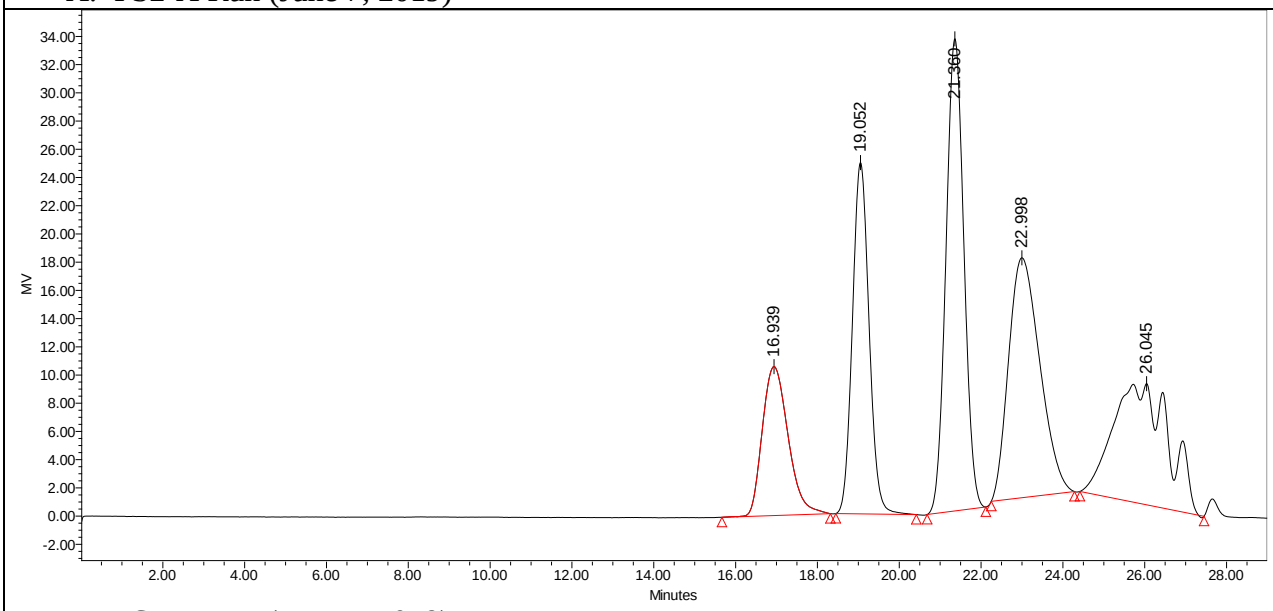
GPC-ES

GPC-ES assay was conducted as previously described in literature [1 – 3]. Samples were analyzed using GPC against polystyrene standards (external standard). The GPC system consisted of Waters 1515 Isocratic HPLC pump connected to Waters 2707 Autosampler and Waters 2414 Refractive Index Detector. GPC analysis performed by injecting 100µL of ~ 2.0 mg per mL polymer solution dissolved in 2.0 µm filtered THF. A run time of 60 minutes was set with the flow rate of 1mL THF/min, and separation performed by a series of three GPC columns. The first one the samples passed through is a Phenomenex column Phenogel 5µ 50A 300 x 7.8 mm, the second is Phenomenex column Phenogel 5µ 10E4A 300x7.8, and the last one is Aglient Resipore 300 x 7.5 mm 3µm column. These samples were tested against Agilent Technologies EasiCal PS2 polystyrene standards lot number PL2010-0601. These standards were prepared according to manufacturer instructions using 0.2 µm filtered THF. Both columns and detector are temperature controlled at 35 ºC.

GPC-ES was calibrated against polystyrene. This process was performed at the beginning of each run sequence to have a fresh calibration for the subsequent samples. Figure 1. shows the resultant chromatographs from a representative set of polystyrene standards with peak r.t. marked.



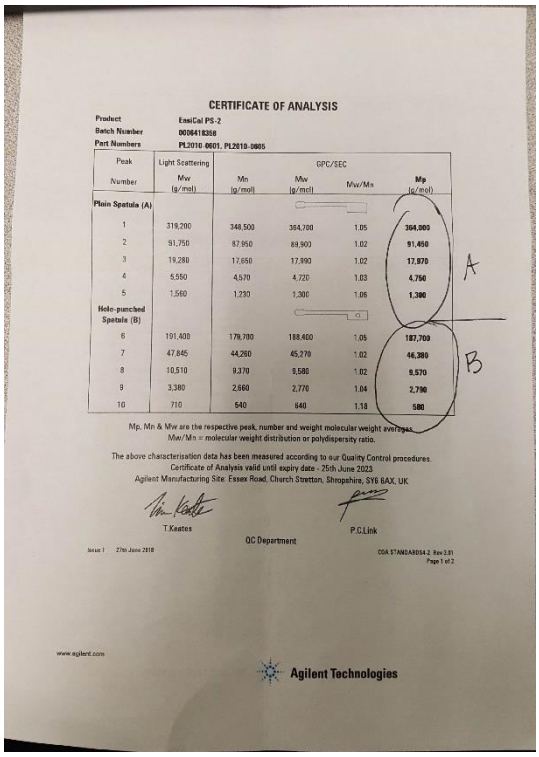
A. PS2-A Run (June 7, 2019)



B. PS2-B Run (June 7, 2019)

Figure 1. GPC chromatograms polystyrene standards.

The calibration data was correlated to molecular weight using the MFG provided analytical information for the polystyrene standards (**Fig 2**).



CERTIFICATE OF ANALYSIS

Product: EasiCal PS-2
Batch Number: 0010413358
Part Numbers: PL2010-0601, PL2010-0605

Peak Number	Light Scattering Mw (g/mol)	GPC/SEC Mn (g/mol)	Mw (g/mol)	Mw/Mn	Mp (g/mol)
Plain Spatula (A)					
1	379,200	348,500	384,700	1.05	364,000
2	91,750	87,950	89,900	1.02	91,450
3	19,280	17,650	17,990	1.02	17,970
4	5,550	4,570	4,720	1.03	4,750
5	1,560	1,230	1,300	1.06	1,300
Holo-punched Spatula (B)					
6	191,400	179,700	188,400	1.05	187,700
7	47,845	44,260	45,270	1.02	46,380
8	10,510	9,370	9,580	1.02	9,570
9	3,380	2,660	2,770	1.04	2,790
10	710	540	640	1.18	580

Mp, Mn & Mw are the respective peak, number and weight molecular weight averages.
Mw/Mn = molecular weight distribution or polydispersity ratio.

The above characterisation data has been measured according to our Quality Control procedures.
Certificate of Analysis valid until expiry date - 25th June 2023
Agilent Manufacturing Site: Essex Road, Church Stretton, Shropshire, SY6 6AX, UK

T.Keates — QC Department — P.C.Link

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Figure 2. Manufacturer provided standards information (note Mp for PS2-A and PS2-B circled).

This correlation was used in Empower software to generate a calibration curve as detailed in Figure 3 and Table 4 below.

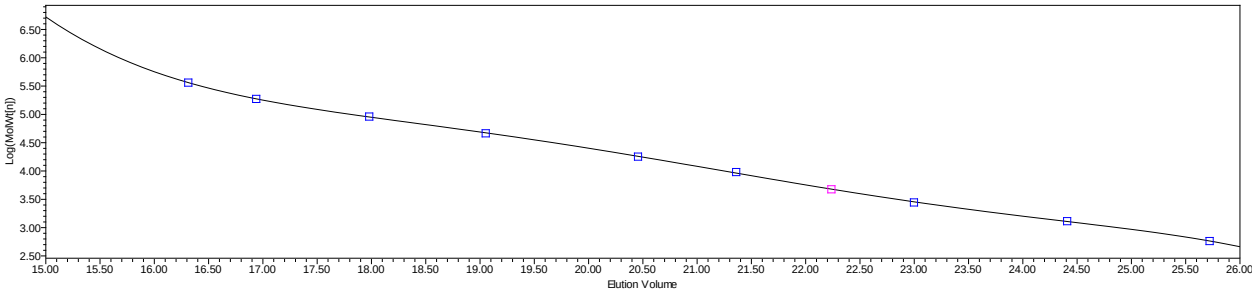


Figure 3. GPC calibration curve data.

Table 1. GPC Calibration curve data.

	Retention Time	Elution Volume	Mol Wt	Log Mol Wt	Hydrodynamic Volume	Log(MolWt[n])	Calculated Weight	% Residual
1	16.313	16.313	364000	5.561101	364000	5.561101	364236	-0.065
2	16.939	16.939	187700	5.273464	187700	5.273464	188230	-0.282
3	17.979	17.979	91450	4.961184	91450	4.961184	90014	1.596
4	19.052	19.052	46380	4.666331	46380	4.666331	47273	-1.890
5	20.455	20.455	17970	4.254548	17970	4.254548	18191	-1.215
6	21.360	21.360	9570	3.980912	9570	3.980912	9192	4.117
7	22.237	22.237	4750	3.676694	4750	3.676694	4789	-0.810
8	22.997	22.997	2790	3.445604	2790	3.445604	2858	-2.382
9	24.408	24.408	1300	3.113943	1300	3.113943	1282	1.376
10	25.720	25.720	580	2.763428	580	2.763428	582	-0.283

Table 2. Calibration Curve Mathematical Parameters.

Processing Method	Date Calibrated	R	R^2	Standard Error	v0	vt	Equation	Fit Type	RSD

190610AHT	6/10/2019 9:14:48 AM EDT	0.999961	0.999923	1.24E-02	15	26	Log(MolWt[n]) = 9.09e+002 - 2.17e+002 V^1 + 2.07e+001 V^2 - 9.82e-001 V^3 + 2.32e-002 V^4 - 2.17e-004 V^5	Fifth (5th Order)	22.511441

Later testing using calibration curve as follows.

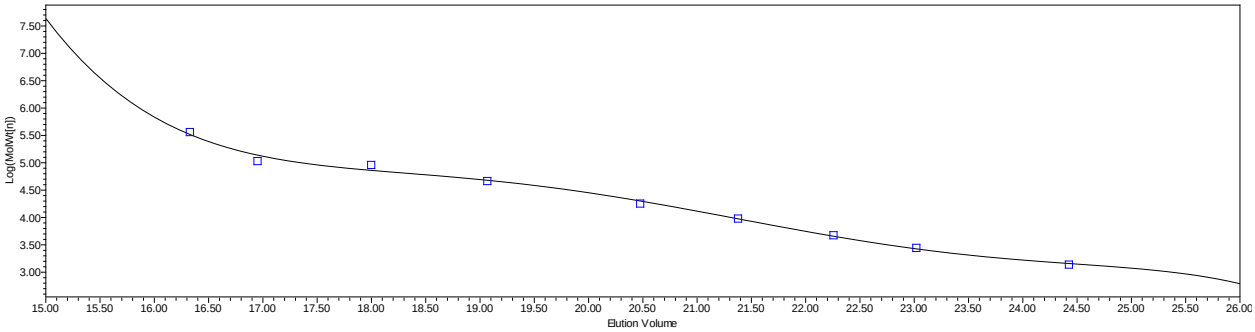


Figure 4. GPC calibration curve data (extracts run).

Table 3. GPC Calibration curve data (extracts run).

	Retention Time	Elution Volume	Mol Wt	Log Mol Wt	Hydrodynamic Volume	Log(MolWt[n])	Calculated Weight	% Residual
1	16.327	16.327	364000	5.561101	364000	5.561101	329157	10.586
2	16.950	16.950	107700	5.032216	107700	5.032216	137954	-21.931
3	17.997	17.997	91450	4.961184	91450	4.961184	72678	25.828
4	19.067	19.067	46380	4.666331	46380	4.666331	47941	-3.256
5	20.475	20.475	17970	4.254548	17970	4.254548	20053	-10.387
6	21.377	21.377	9570	3.980912	9570	3.980912	9469	1.062
7	22.256	22.256	4750	3.676694	4750	3.676694	4554	4.304
8	23.019	23.019	2790	3.445604	2790	3.445604	2673	4.374
9	24.425	24.425	1380	3.139879	1380	3.139879	1442	-4.323
10	26.064	26.064	580	2.763428	580	2.763428	575	0.869

Table 4. GPC Calibration curve Mathematical Parameters (extracts run).

Processing Method	Date Calibrated	R	R^2	Standard Error	v0	vt	Equation	Fit Type	RSD
190703SJH	7/3/2019 9:58:22 AM EDT	0.998174	0.996352	8.21E-02	15	26	Log(MolWt[n]) = 2.09e+003 - 4.99e+002 V^1 + 4.76e+001 V^2 - 2.26e+000 V^3 + 5.31e-002 V^4 - 4.96e-004 V^5	Fifth (5th Order)	21.854465

supplies:

Agilent Easical

Part Number:PL2010-0601
https://www.chem.agilent.com/store/productDetail.jsp?catalogId=PL2010-0601&catId=SubCat4ECS_32817

Phenogel Columns
https://www.phenomenex.com/Products/HPLCDetail/phenogel

Resipore:
Part Number:PL1113-6300
https://www.agilent.com/store/productDetail.jsp?catalogId=PL1113-6300

GPC-4D

GPC-4D was assayed as previously reported in literature [4 – 6]. Sample was dissolved in 0.2 um filtered chromatography grade Acetone (ACE) (Fisher Chemical cat#A949-4). Each sample was dissolved at the concentration indicated in results. After dissolution, sample was passed through a 0.2um (PTFE) filter (Tisch cat# SF14466) to remove particulates and placed directly into a septum capped 2 ml HPLC vial. The GPC-4D system at Akina consists of an Agilent 1260 Infinity II HPLC connected to Dawn Heleos II (MALLS) coupled to Dynapro Nanostar DLS via optical cable, Optilab T-rEX (RI detector) and Viscostar III viscometer operated by Astra 7 software. GPC analysis performed with separation by a linear gradient column (Tosoh Bioscience LLC, TSKgel GMHHR-L, 7.8 mm x 30 cm) at 0.6 ml/min flow of Acetone. Each sample was injected at 50.0 µL and eluted across the column with data collection from all detectors. The dndc was set as as experimentally determined for the particular LA:GA ratio [7]. The peak was selected for each sample using Astra Software and the relevant properties were determined using the software as well.

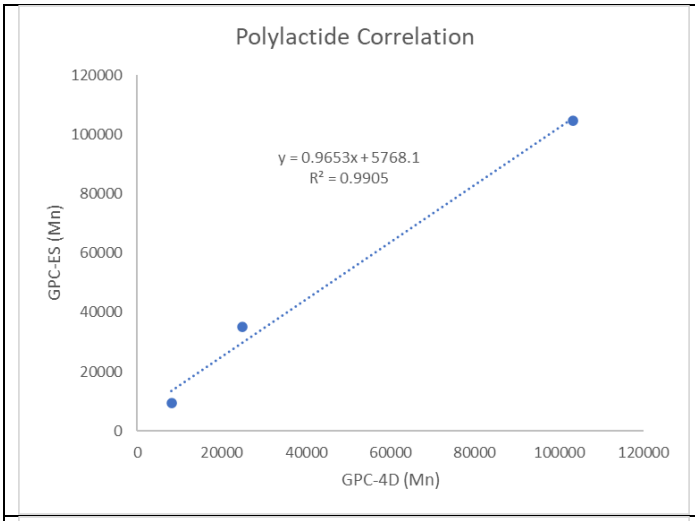
Results

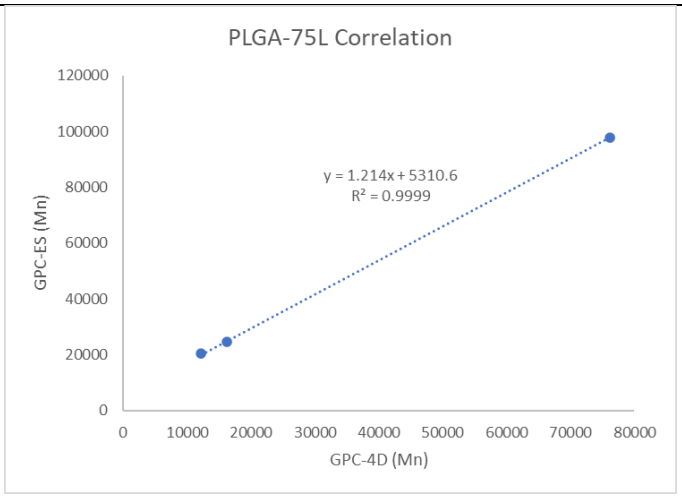
Table 5 below shows results from both methods for indicated standard sample.

Table 5. Number average molecular weight (Mn) achieved for PL(G)A standards using different techniques.

Polymer	Cat/lot#	GPC-4D (Mn)	GPC-ES (Mn)
P(DL)La	PLA 100L-H 180302RAI-A	103200	104474
	PLA 100L-M 180306FAJ-A	24900	34998
	PLA 100L-S 180312FAJ-A	8142	9350
PLGA-75L	PLGA 75L-H 180313RAI-B	76170	97812
	PLGA 75L-M 180323RAI-A	16290	24595
	PLGA 75L-S 18041RAI-A	12180	20557
PLGA-50L	PLGA-50L-H-E 201210RAI-A	49038	66100
	PLGA 50L-M 180406RAI-A	16280	24121
	PLGA 50L-S 180329RAI-A	5834	8415

The correlation between the achieved Mn results by method depended heavily on the LA:GA ratio of the polymer. This makes sense as the LA:GA ratio has a strong impact on solubility of the polymer and this, in turn, affects it's behavior in GPC columns [3].





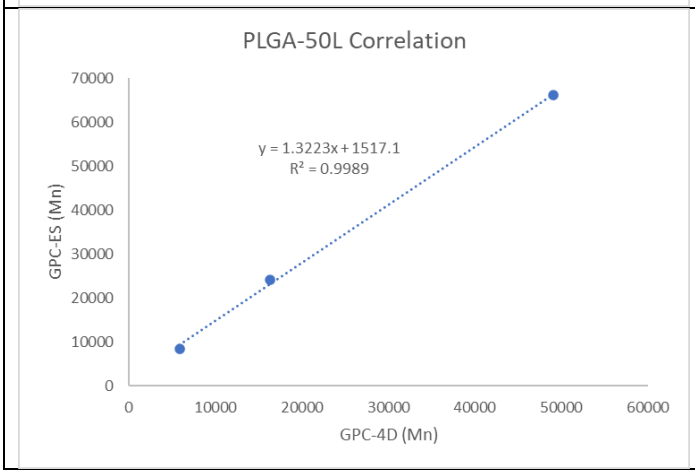


Figure 5. Correlation functions for comparison of GPC-ES results to GPC-4D results for number average molecular weight (Mn).

Based on slope and intercept the general trend is for GPC-ES results to yield slightly higher Mn than GPC-4D. However, this varies depending on LA:GA ratio and molecular weight of the material.

Conclusion

The method applied can have an impact on the resultant molecular weight. In addition to universal calibration compared to external standard there are a plethora of variances in column and instrumental configurations for GPC instruments which can affect the measured molecular weight. One method to evaluate and determine the correlation between two methods is to utilize PLGA standards with known properties and test these in parallel to evaluate the properties of a given GPC system.

References

1. Garner, John, Sarah Skidmore, Haesun Park, Kinam Park, Stephanie Choi, and Yan Wang. "A protocol for assay of poly (lactide-co-glycolide) in clinical products." International journal of pharmaceutics 495, no. 1 (2015): 87-92.
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3. Skidmore, Sarah, Justin Hadar, John Garner, Haesun Park, Kinam Park, Yan Wang, and Xiaohui Jiang. "Complex sameness: Separation of mixed poly (lactide-co-glycolide) s based on the lactide: glycolide ratio." Journal of Controlled Release (2019).
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5. Justin Hadar, Sarah Skidmore, John Garner, Haesun Park, Kinam Park, Yan Wang, Bin Qin, Xiaohui (Jeff) Jiang, Darby Kozak "Method matters: Development of characterization techniques for branched and glucose-poly(lactide-co-glycolide) polymers", Journal of Controlled Release Volume 320, 10 April 2020, Pages 484-494.
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7. 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