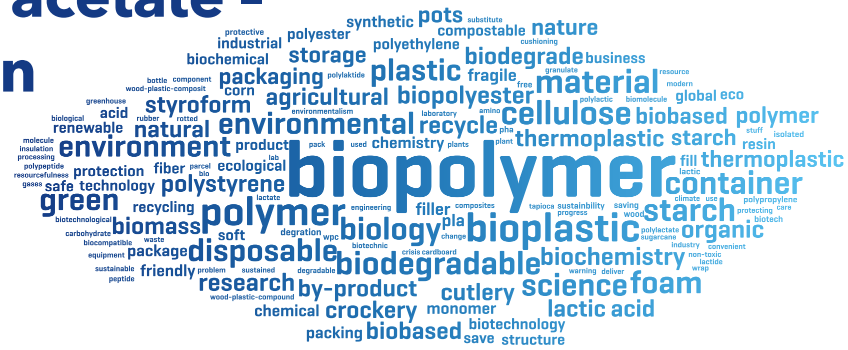
Science Together

Determining molecular weights for P(D,L)LA in ethyl acetate – an internal validation



Nils Lüttgert, Lisa Loxterkamp, Juliane Böttcher,
Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH,
Hegauer Weg 38, 14163 Berlin; www.knauer.net

SUMMARY

A method for the analysis of low molecular weight P(D,L)LA by GPC with respect to its number average molar mass (M_n) and weight average molar mass (M_w) was validated intralaboratory. To achieve this a KNAUER HPLC system was used in combination with a linear GPC column. The obtained molar masses were compared with the values from the absolute methods and deviations were discussed. The method was validated with respect to its repeatability and robustness to changes in flow rate, temperature, injection volume and sample concentration.

INTRODUCTION

Plastics are one of the most widely used most versatile materials in the 21st century. However, the majority of the world's annual production of approximately 370 million tons of plastics is neither biodegradable nor produced from renewable raw materials.(1) In contrast, poly lactic acid (PLA) is one of the most promising plastics with sustainable properties. The physical properties of plastics are mostly determined by their molar mass distribution, with the parameters M_n, M_w and the PD being the most important. GPC is the method of choice for the determination of these values. When no absolute method is available, the two most common calibrations are conventional and universal calibrations. For a conventional calibration, calibration standards of the polymer to be analyzed, or at the least a polymer of high chemical similarity, are required. For a universal calibration, first published by Benoit(2), a universal calibration curve is created via the Mark-Houwink relationship, so that a wide variety of polymers can be analyzed, for example with a narrow polystyrene (PS) calibration. In 1998, S. Mori published the results of a multistage round-robin test in which PS standards were analysed by GPC using conventional calibration (CC) with PS under various framework conditions.(3) The relative interlaboratory standard deviation (%RSD) for Mn of more than 20 % was reduced by setting strict framework conditions for the analysis, such as injection volume, sample concentration, and sample size.

Determining molecular weights for P(D,L)LA in ethyl acetate – an internal validation

INTRODUCTION

This shows that certain standards must be adhered to obtain reliable values when using GPC. Therefore, a method has been provided in this publication to analyse lower molecular weight P(D,L)LA using universal calibration. The method was developed according to ISO 13885-1 and its robustness and accuracy were investigated intralaboratory.(4) Ethyl acetate (EtOAc) was used as solvent, as it is significantly more environmentally friendly than the conventionally used solvents tetrahydrofuran (THF) and chloroform ($CHCl_3$).

SAMPLE PREPARATION

For calibration with PMMA, a ReadyCal-Kit in the molar mass range 800 Da - 2 200 kDa from PSS was used (12 standards). The calibration standards were dissolved in 1 ml EtOAc for one hour and then mixed with butylated hydroxytoluene (BHT) as a flow marker to a concentration of 1.5 mg/ml. The concentration of the calibration standard was 1.5 mg/ml. For the PLA samples, a 20 kDa PLA standard was used, for which M_n and M_w were specified in the certificate. For all samples, approximately 20 mg were weighed out and dissolved overnight (o.n.) in EtOAc to a concentration of 3 mg/ml. The following day, the samples were diluted to 1 ml with EtOAc and the flow marker BHT was added (1.5 mg/ml). All experiments were carried out by the same operator.

RESULTS AND DISCUSSION

Initially, a calibration was made using PMMA, since polystyrene (PS) proved unsuitable in EtOAc due to interactions with the stationary phase **(VTN0021)**. The two largest molar masses were excluded due to the peak shape, resulting in an 10-point universal calibration with a 5th degree fit function. The overlay of calibration function and the chromatograms is shown in **Fig. 1**. For universal calibration with PMMA in EtOAc, the following Mark-Houwink parameters were used: $K = 21.1 \cdot 105$ dL/g, $\alpha = 0.64$.(5) For PLA $K = 15.8 \cdot 105$ dl/g and $\alpha = 0.78$ were used.(6)

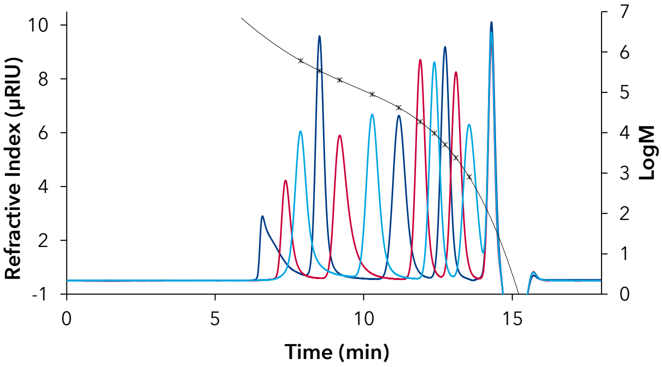


Fig. 1 10-point PMMA universal calibration in EtOAc by PSS (ReadyCal). Blue = green cup; Red = red cup; Light blue = white cup. Fit function of the 5th degree with deviations less than 5 %. $R^2 = 1$.

Accuracy

Tab. 1 shows the molar masses determined using CC and UC. The comparison with the values of the absolute method ^{1}H-NMR shows that the universal calibration provides much more accurate values. The values for M_n and M_w were taken from the certificate, with Mn determined by ^{1}H-NMR. The method used to determine the M_w value was not specified. The comparison of the molar masses determined using CC versus determination using absolute methods has shown that CC has large deviations of more than 100 %. In contrast, UC showed good results with a deviation of 8 % for M_n and 4 % for M_w. Round robin tests have shown that GPC methods, in the simplest case of polystyrene in THF using CC, have a high uncertainty in their reproducibility (interlaboratory) of 13–16 % for M_n and 6–10 % for M_w.(3) For more complex polymers, deviations can be larger.(7) There is a tendency for the M_n value to show greater uncertainty than the M_w value.

Tab. 1 Comparison of the determined molar masses using PMMA UC and CC with the molar masses determined by ^{1}H-NMR. *Average of 6-fold determination with (%)-deviation respective to the standard.

PLA standard (M_x) (Da)	UC average (Da)	UC values (Da)	CC average (Da)	CC values (Da)
M_n: 17 286 (by ^{1}HNMR)	18 618* (+8 %)	18 616	35 401* (+105 %)	35 295
		18 470		34 957
		18 607		35 281
		18 695		35 477
		18 623		35 919
		18 696		35 478
M_w: 20 700 (certificate)	21 519* (+4 %)	21 529	42 219* (+104 %)	42 214
		21 545		42 255
		21 508		42 167
		21 554		42 270
		21 591		42 192
		21 530		42 213

Repeatability and intermediate precision

To test repeatability, 6 injections of a 1 mg/ml concentrated PLA solution were injected consecutively. The relative standard deviation was determined to ± 83 Da (0.44 %RSD) for M_n and ± 28 Da (0.13 %RSD) for M_w. Furthermore, the intermediate precision was tested. For this purpose, the sample preparation was repeated on three consecutive days and a triple determination was performed in each case. The relative standard deviation was ± 85 Da (0.46 %RSD) for M_n and ± 48 Da (0.22 %RSD) for M_w. Both values meet the requirements of ISO 13885-1, which specifies values lower than 3 % for M_n and 2 % for M_w.

Robustness

Several method parameters were varied to investigate their influence on the result of the molar mass determination and to determine the respective standard deviation. First, the temperature was changed by ± 2 °C. **Fig. 2** shows that the method is robust for a change of ± 2 °C since M_n and M_w do not change significantly and do not show a trend. The standard deviations were between 48 and 116 Da (0.6 %RSD).

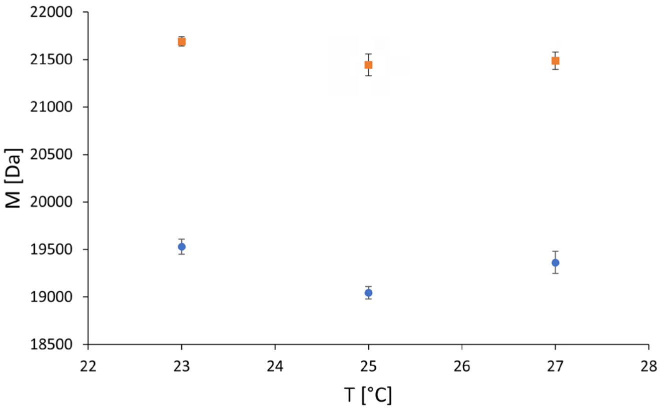


Fig. 2 M_n (dots) and M_w (squares) values at 23-, 25-, and 27 °C. Triple determination with relative standard deviation. 1 mg/ml, 20 µl injection volume.

The flow rate was changed by ±0.2 ml/min. **Fig. 3** shows that the method is robust even when the flow rate is changed by ±0.2 ml/min, since the %RSD is low, and no trend can be detected. The flow marker shows its strength here, as M_n and M_w are also very stable in this experiment when applying flow correction.

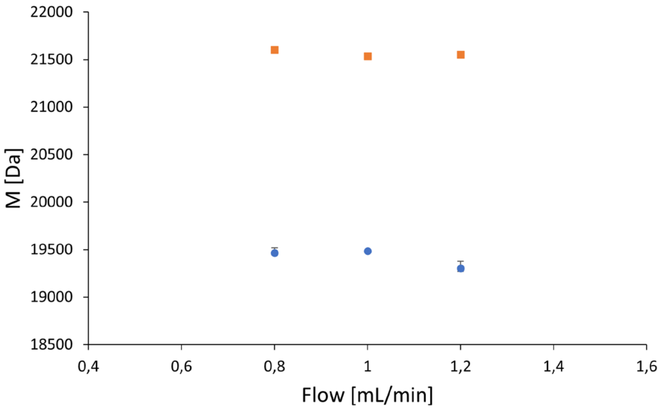


Fig. 3 M_n (dots) and M_w (squares) values at 0.8-, 1.0-, and 1.2 ml/min. Triple determination with relative standard deviation. 1 mg/ml, 20 µl injection volume.

For all experiments, 20 µl of PLA sample were injected. The aim was to investigate the influence of the injection volume on the molar mass determination. **Fig. 4** shows the result. There is no significant effect on the molar mass determination in the range 10–30 µl injection volume. The %RSD is low and no trend can be identified.

Determining molecular weights for P(D,L)LA in ethyl acetate – an internal validation

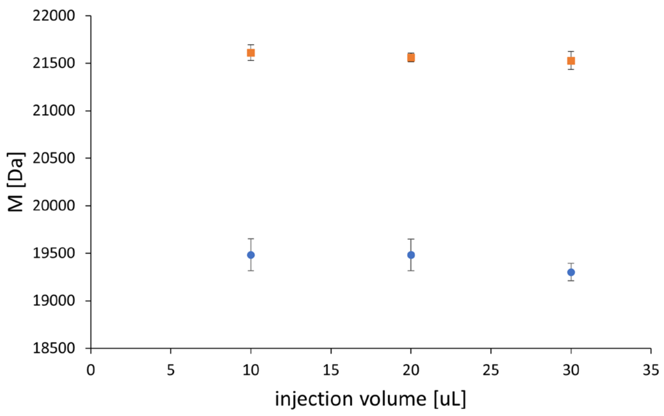


Fig. 4 M_n (dots) and M_w (squares) values at 10-, 20-, and 30 µL injection volume. Triple determination with relative standard deviation. 1 mg/ml injection volume.

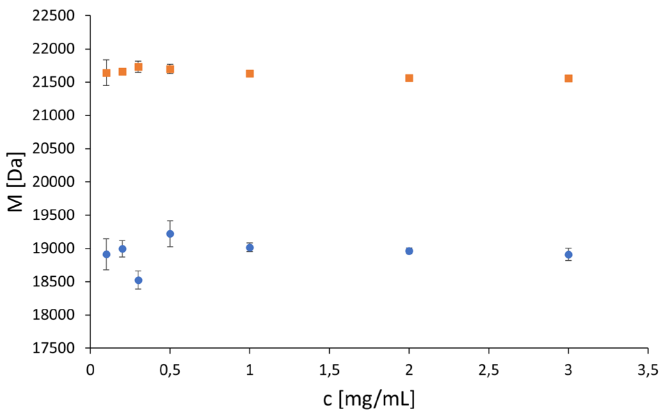


Fig. 5 M_n (dots) and M_w (squares) values at 0.1-, 0.2-, 0.3-, 0.5-, 1.0-, 2.0-, and 3.0 mg/ml sample concentration. Triple determination with relative standard deviation. 20 µl injection volume.

When determining high molar masses, concentration effects can reduce the hydrodynamic volume so that the molar mass is underestimated. This is also the reason why ISO 16014-3 specifies a sample concentration of 0.5 mg/ml for polymers with a molar mass with an order of magnitude of or greater than 106 Da, whereas sample concentrations of up to 5 mg/ml are not a problem when the M_w is less than 105 Da. Since the PLA standard for validation is in the magnitude of 104 Da no concentration effects should occur at an injection concentration of 1 mg/ml. If this were the case, the molar mass would have to increase with decreasing sample concentration. To test this, M_w and M_n were determined for different concentrations in the range 0.1–3 mg/ml. The results in **Fig. 5** show that there is no trend towards higher molar masses at lower concentrations, this means that the method can be considered robust in the concentration range 0.1 to 3 mg/ml.

CONCLUSION

These experiments have shown that the determination of the M_n and M_w of low molecular weight P(D,L)LA is easily possible using a KNAUER HPLC system combined with a gel permeation chromatography (GPC)-column. The intralaboratory validation proves that the developed method is robust within the limits shown here with respect to changes in temperature, concentration, flow rate and injection volume. Furthermore, the repeatability of the molar mass determination as well as the intermediate precision of the sample preparation was verified. It was shown that the CC fails for this column/solvent/polymer combination, whereas the UC provides good results. The conventional calibration did not fulfil the specification because the different hydrodynamic radii of PLA and PMMA in EtOAc were not considered. The flow rate has a very strong influence on the molar mass determination and must be as constant as possible. Slight fluctuations affect the retention volume. In order to take fluctuations into account, a flow marker, in this case BHT, was used and is recommend as mandatory for all GPC/SEC applications.

MATERIALS AND METHODS

Standards, Eluents and Samples

Chemical	CAS	Purity	Manufacturer
P(D,L)LA	26780-50-7 (Batch: MKCL3830)	not specified	Sigma-Aldrich
BHT	128-37-0	≥ 95 %	Supelco
EtOAc	141-78-6	≥ 99.8 %	VWR
PS-CAL-Kit	(Batch: 6374186/6381821)	not specified	Agilent
PMMA-CAL-Kit	(Batch: mmkitr1-07)	not specified	PSS

Method parameters	
Column temperature	25 °C
Injection volume	20 µl
Injection mode	Partial Loop
Detection	RID
Data rate	10 Hz

Pump parameters	
Eluent (A)	EtOAc
Flow rate	1 ml/min
Pump program	isocratic

System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L HPG	APH38ED
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	AZURA RID 2.1L	ADD31
Thermostat	AZURA CT 2.1	ATC00
Pre-Column	AppliChrom ABOA StyDiViBe 10E5A-BPT,500–1.5 MioDa, 50x8 mm, 100.000 A	05GW470ABJ
Column	AppliChrom ABOA StyDiViBe 10E5A-BPT,500–1.5 MioDa, 300x8 mm, 100.000 A	30GW470ABJ
Software	ClarityChrom 8.2.3 – Workstation, autosampler control included	A1670
Software	ClarityChrom 8.2.3 – SEC/GPC extension	A1678

REFERENCES

[1] https://de.statista.com/statistik/daten/studie/167099/umfrage/weltproduktion-von-kunststoff-seit-1950/. Stand: 2019. 25.08.2021, 14:08 Uhr.

[2] Benoit, F., Grubisic, Z., Rempp, P.: Journal of Polymer Science 1967, B, 5, 753.

[3] S. Mori, International Journal of Polymer Analysis and Characterization: 1998, 4, 6, 531-546.

[4] ISO 13885-1: Gel permeation chromatography (GPC) – Part 1: Tetrahydrofuran as eluent, 2020.

[5] A. Eriksson, Acta Chem. Scand: 1953, 7, 623-642.

[6] X. Kaitian et al., Journal of Applied Polymer Science: 1996, 59, 561-563

[7] R. Bruessau, Macromol. Symp.: 1996, 110, 15-32.

RELATED KNAUER APPLICATIONS

VCH0017 – Molecular weight distribution of a broad polystyrene standard

VEV0084 – Analysis of Poly [(R)-3-hydroxybutyric acid] in chloroform using GPC and universal calibration

VEV0085 – Size exclusion chromatography of polylactide acid in three different solvents

VEV0087 – Determining molecular weights for P(D,L) LA in THF – an internal validation

VTN0021 – Green SEC/GPC – comparison of two calibrations in three different solvents