

Size exclusion chromatography of polylactic acid in three different solvents

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



SUMMARY

Several different polylactic acid (PLA) samples have been successfully examined with respect to their molar mass and polydispersity (PD) using a KNAUER HPLC system in combination with a gel permeation chromatography (GPC) column. Calibrations of polystyrene (PS) and polymethyl methacrylate (PMMA) in three different solvents, tetrahydrofuran (THF), chloroform and ethyl acetate (EtOAc) have been recorded. In this study a 20 kDa PLA standard and two real samples were evaluated using these calibrations and the results compared with one another. It could be shown that EtOAc is suitable as an environmentally friendly alternative for the analysis of low molecular weight P(D,L)LA samples.

INTRODUCTION

PLA is one of the most promising sustainable plastics. It can be produced on an industrial scale using simple, bacteria-driven, bio-based synthesis whilst also being biodegradable, meaning it could help solve problems such as long-term pollution of the environment by non-degradable plastics.⁽¹⁾ In addition, PLA can be used in a wide range of applications such as medical applications, as packaging material, for disposable tableware or in horticulture. Work is currently underway to make PLA more widely applicable and more recyclable through fibre reinforcement.⁽²⁾ The macroscopic properties of a

polymer, such as melting point, hardness and elasticity, are mainly determined by the number average molar mass (M_n), the weight average molar mass (M_w) and the polydispersity. The method of choice to determine these parameters is GPC. In this application, three methods are presented to determine the molecular weight of three different PLA standards. For this purpose, two calibrations in three different solvents, THF, CHCl_3 and EtOAc, were investigated/recorded. In addition, two real samples were analysed: PLA filaments for 3D-printing and a PLA disposable drinking bottle.



Additional
Information

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SAMPLE PREPARATION

For calibration with polystyrene, an EasiCal PS-1/2-Kit in the molar mass range 580 Da - 6 570 kDa from Agilent was used (17 points). For calibration with PMMA, a ReadyCal-Kit in the molar mass range 800 Da - 2 200 kDa from PSS was used (12 points). The calibration standards were dissolved in 1 ml of the corresponding solvent for one hour and then mixed with butylated hydroxytoluene (BHT) as a flow marker to a concentration of 1.5 mg/ml. The number of calibration points for each solvent was adjusted to cover the optimal separation range of the column. The PLA standards were dissolved in the corresponding solvent over night at room temperature without stirring to a concentration of 1 mg/ml and then mixed with BHT as a flow marker to a concentration of 1.5 mg/ml. The PLA samples to be investigated were dissolved overnight at RT without stirring in the corresponding solvent to a target concentration of 3 mg/ml and then filtered over a 0.45 μm PTFE filter. BHT was added as a flow marker to a concentration of 1.5 mg/ml.

RESULTS

A linear column was used for the analysis of the PLA samples. To calibrate a PS and a PMMA calibration were recorded in all three solvents. BHT was used as the flow marker. **Fig. 1** shows the calibration with PS in chloroform while **Fig. 2** displays the calibration with polymethyl methacrylate (PMMA). The calibration points were fit with a 5th degree function and showed deviations of less than 5%. The following Mark-Houwink constants from the literature were used for the evaluation by means of universal calibration: $K = 22.1 \text{ dl/g} \cdot 10^5$, $\alpha = 0.77$ for PLA in CHCl_3 and the respective M_n value were determined. ⁽³⁾ **Tab. 1** shows the results compared to the M_n value of the certificate determined by $^1\text{H-NMR}$. There are significant deviations of over 20% for the lower molecular weight polymers. It is striking that both PMMA and PS calibration show deviations in the same range. The calibrations have been checked against each other and provide good results.

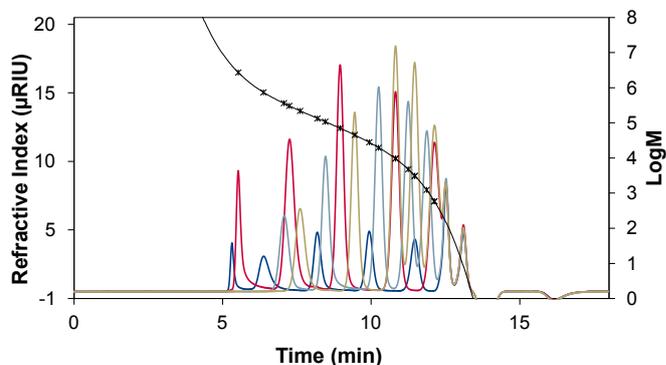


Fig. 1 16-point PS calibration in chloroform by Agilent (EasiCal) in the molar mass range of 1 230 Da - 2 703 kDa. Blue = PS-1(A); red = PS-1(B); light blue = PS-2(A); gold = PS-2(B). Fit function of the 5th degree with deviations less than 5%. $R^2 = 1$.

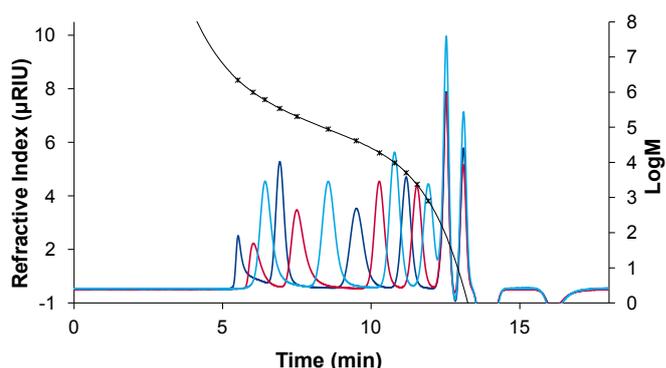


Fig. 2 12-point PMMA calibration in chloroform by PSS (ReadyCal) in the molar mass range of 800 Da - 2 200 kDa. Blue = green cup; red = red cup; light blue = white cup. Fit function of the 5th degree with deviations less than 5%. $R^2 = 1$.

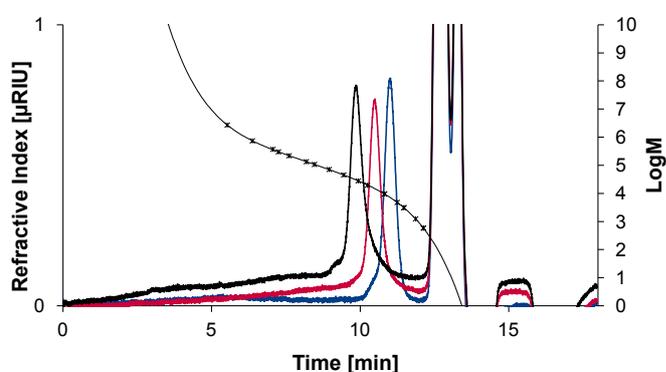


Fig. 3 PLA standards in chloroform. Blue \approx 5 kDa, red \approx 10 kDa, black \approx 20 kDa. $T = 25^\circ\text{C}$. 1 mg/ml, injection volume = 20 μl . PS calibration shown.

The following Mark-Houwink constants were used for the analysis of PLA in EtOAc: $K = 15.8 \text{ dl/g} \cdot 10^5$, $\alpha = 0.78$.⁽⁴⁾ **Tab. 2** shows the results of the analysis of the 20 kDa PLA standard in EtOAc with a PMMA and PS calibration. The PS calibration shows strong negative deviations of up to -150%. The PMMA calibration, on the other hand, delivers good results. The following Mark-Houwink constants were used for the analysis of PLA in THF: $K = 17.4 \text{ dl/g} \cdot 10^5$, $\alpha = 0.74$.⁽⁵⁾ **Tab. 3** shows the results of the PLA standard in THF. In this instance both the PS and PMMA calibration show good results.

Tab. 1 By means of PS- and PMMA calibration, determined M_n values of three PLA standards in CHCl_3

PLA standard (M_n)	With PS calibration	With PMMA calibration
5 883 (by $^1\text{HNMR}$)	4 757 (-19.1 %)	4 396 (-25.3 %)
11 883 (by $^1\text{HNMR}$)	8 843 (-25.6 %)	8 472 (-28.7 %)
17 286 (by $^1\text{HNMR}$)	15 991 (-7.5 %)	15 266 (-11.7 %)

Tab. 2 By means of PS- and PMMA calibration, determined M_n values of three PLA standards in EtOAc

PLA standard (M_n)	With PS calibration	With PMMA calibration
5 883 (by $^1\text{HNMR}$)	14 688 (+150 %)	6 532 (+11.0 %)
11 883 (by $^1\text{HNMR}$)	21 577 (+81,6 %)	11 300 (-5,2 %)
17 286 (by $^1\text{HNMR}$)	31 820 (+84.1 %)	18 958 (+9.7 %)

Tab. 3 By means of PS- and PMMA calibration, determined M_n values of three PLA standards in THF

PLA standard (M_n)	With PS calibration	With PMMA calibration
5 883 (by $^1\text{HNMR}$)	5 600 (-4.8 %)	6 410 (+9.0 %)
11 883 (by $^1\text{HNMR}$)	10 131(-14.7 %)	11 779 (-0.9 %)
17 286 (by $^1\text{HNMR}$)	17 504 (+1.3 %)	20 358 (+17.7 %)

To test the performance of the method in chloroform and THF, two PLA filaments for use in 3D printing as well as one PLA-drinking bottle were analyzed. The chromatograms of the filaments differ greatly. **Fig. 4** shows the overlay of filament A with a 10 kDa PLA standard. In addition to the RID signal, two UV signals with a wavelength of 245 and 280 nm are shown. The size ratios of the signals to one another match with the PLA standard, so that it can be assumed that it is pure PLA. The same pattern is observable in the chromatogram of the PLA-drinking bottle, depicted in **Fig. 5**. In contrast, **Fig. 6** shows the chromatogram of filament B in THF.

While the PLA standard shows no absorption at 280 nm in THF, a strong absorption signal at 280 nm of filament B can be seen. Furthermore, the ratio between the RID and 245 nm signal is too large in relation to the signal of the corresponding standard. In addition, the UV signal is strongly shifted in its retention time in comparison to the RID signal. As a result, it can be inferred that filament B has been exposed to another, possibly polymer-specific, additional substance like another polymer (blend) or macromolecule that absorbs UV in this region. **Tab. 4** shows the determined molar masses of a PLA-drinking bottle as well as the filaments A and B. All of them have relatively similar molar masses and PD's.

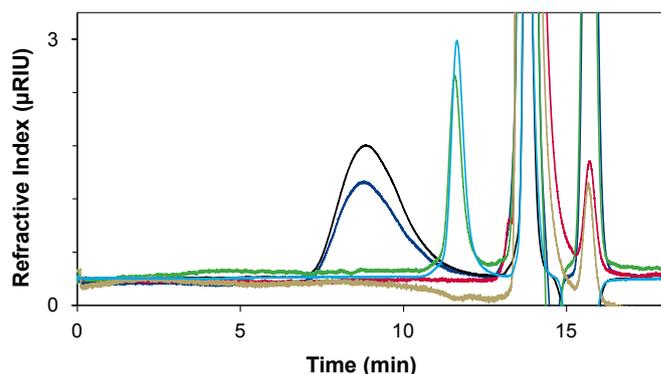


Fig. 4 Overlay of PLA 10 kDa standard with a PLA Filament A for 3-D Printing. Black & light blue = RID, dark blue & green = 245 nm, red & gold = 280 nm. 3 mg/ml in THF, 20 µl injection volume.

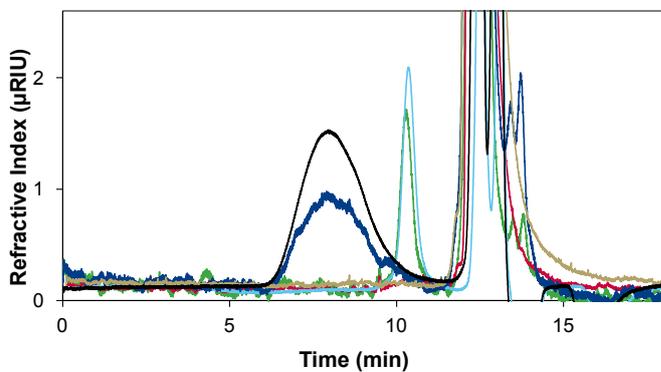


Fig. 5 Overlay of PLA 10 kDa standard with a PLA drinking bottle. Black & light blue = RID, dark blue & green = 245 nm, red & gold = 280 nm. 3 mg/ml in CHCl_3 , 20 µl injection volume.

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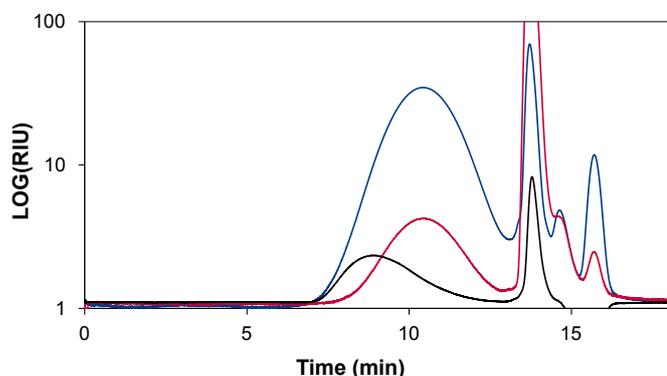


Fig. 6 PLA Filament B for 3-D Printing. Black = RID, dark blue = 245 nm, red = 280 nm. 3 mg/ml in THF, 20 μ l injection volume.

Tab. 4 Determined molar masses of PLA samples in THF using PS calibration

PLA Sample	M_n	M_w	M_p	PD
Filament A	79 405	131 994	131 134	1.7
Filament B	70 918	125 582	124 363	1.8
Bottle	64 848	108 015	101 498	1.7

DISCUSSION

In 1998, S. Mori published the results of a multistage round-robin test in which PS standards were analyzed using GPC and a conventional calibration with PS under various framework conditions.⁽⁶⁾ In compliance with strict specifications regarding injection volume, sample concentration, calibration kit and baseline determination, the relative standard deviation for M_n was 13–16%. In this work a universal calibration was applied, for which Mark-Houwink constants from the literature were used. Taking this additional uncertainty into account, deviations in the range of 20% compared to the absolute method can be regarded as acceptable. The extreme deviations of polystyrene in ethyl acetate were investigated by analyzing further narrow PS standards. There was a clear systematic deviation as shown in **Tab. 4**. The smaller the molar mass of the PS standard, the greater the underestimation. This result clearly indicates an interaction of PS with the stationary phase (StyDiViBe).

Tab. 5 Four different PS Narrow Standards evaluated with universal PMMA Calibration in EtOAc

PS standard (M_n)	With PMMA calibration
2 557 000	1 813 831 (-24%)
292 000	185 233 (-34%)
69 350	37 978 (-44%)
9 580	2 336 (-75%)

CONCLUSION

Considering the relatively high uncertainty of the universal calibration, it could be shown that a low molecular weight PLA standard can be analyzed in three different solvents using two different calibrations. Due to interactions with the stationary phase, polystyrene in EtOAc is not suitable when using a polystyrene GPC column. This could be shown due to systematic deviations of several PS standards. The analysis of two PLA filaments with a KNAUER HPLC system in combination with a GPC column has shown that real samples can be analyzed quickly and easily using this suggested method. When using a Diode-Array Detector (DAD), additional information about the sample composition can be obtained. Particularly noteworthy is the possibility of analyzing PLA in EtOAc, as EtOAc is significantly more environmentally friendly when compared to CHCl_3 and THF.

MATERIALS AND METHODS

Standards, Eluents and Samples

Chemical	CAS	Purity	Manufacturer
P(D,L)LA	26780-50-7 (Batch: MKCL3830)	not specified	Sigma-Aldrich
CHCl_3	67-66-3	$\geq 99\%$	Merck KGaA
THF	109-99-9	HPLC grade	Supelco
EtOAc	141-78-6	$\geq 99.8\%$	VWR
PS-CAL-Kit	(Batch: 6374186/6381821)	not specified	Agilent
PMMA-CAL-Kit	(Batch: mmkitr1-07)	not specified	PSS
Real Samples	-	-	unknown

Method parameters

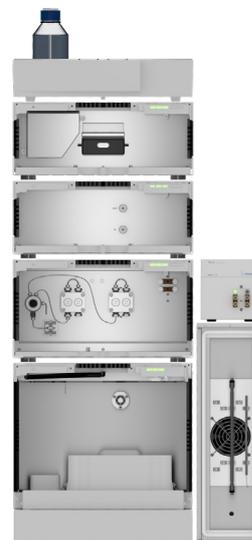
Column temperature	25 °C
Injection volume	20 µl
Injection mode	Partial Loop
Detection 1	RID
Detection 2	DAD 245/280 nm
Data rate	10 Hz
Time constant	0.1 s

Pump parameters

Flow rate	1 ml/min
Pump program	isocratic
Eluent (A1)	CHCl ₃
Eluent (A2)	THF
Eluent (A3)	EtOAc

System configuration

Instrument	Description	Article No.
Pump	AZURA P 6.1L HPG	APH38ED
Autosampler	AZURA AS 6.1L	AAA00AA
Detector 1	AZURA RID 2.1L	ADD31
Detector 2	AZURA DAD 2.1L	ADC01
Thermostat	AZURA CT 2.1	ATC00
Pre-Column 1 (THF)	AppliChrom ABOA StyDiViBe 10E5A-BPT,500 - 1.5 MioDa, 50x8 mm, THF, 100.000 A	05GW470ABJ
Column 1 (THF)	AppliChrom ABOA StyDiViBe 10E5A-BPT,500 - 1.5 MioDa, 300x8 mm, THF, 100.000 A	30GW470ABJ
Pre-Column 2 (CHCl ₃)	AppliChrom ABOA StyDiViBe 10E5A-BPT,500 - 1.5 MioDa, 50x8 mm, CHCl ₃ , 100.000 A	05GW470ABJ
Column 2 (CHCl ₃)	AppliChrom ABOA StyDiViBe 10E5A-BPT,500 - 1.5 MioDa, 300x8 mm, CHCl ₃ , 100.000 A	30GW470ABJ
Pre-Column 3 (EtOAc)	AppliChrom ABOA StyDiViBe 10E5A-BPT,500 - 1.5 MioDa, 50x8 mm, EtOAc, 100.000 A	05GW470ABJ
Column 3 (EtOAc)	AppliChrom ABOA StyDiViBe 10E5A-BPT,500 - 1.5 MioDa, 300x8 mm, EtOAc, 100.000 A	30GW470ABJ
Software	ClarityChrom 8.2.3 - Workstation, autosampler control included	A1670
Software	ClarityChrom 8.2.3 - SEC/GPC extension	A1678

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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

[VTN0021](#) - Green SEC/GPC - comparison of two calibrations in three different solvents