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SUMMARY

Here, a KNAUER HPLC system was used in combination with a linear GPC column to perform calibrations of polystyrene (PS) and polymethylmethacrylate (PMMA) in tetrahydrofuran (THF), chloroform (CHCl_3) and ethyl acetate (EtOAc). The calibrations were tested using narrow PS standards. It was found that the methods for PS and PMMA in THF and chloroform provided excellent results. In addition, it was shown that calibration with PS in EtOAc is not suitable due to an apparent interaction of PS with the column. A linear column allowed all of the analytes to be analyzed in less than 15 minutes and with a solvent consumption of less than approximately 67 % when compared to conventional methods, in which 3 single-porosity columns are used.

INTRODUCTION

The demand for biobased plastics is increasing because, compared to petrochemical plastics, they can be produced sustainably and have a low environmental impact due to their degradability. An ever-increasing number of products are made using polymers derived from renewable raw materials. This also includes mixtures of polymers that have new properties. These properties are primarily determined by their polydispersity (PD), their number average molar mass (M_n) and their weight average molar mass (M_w). To determine these values, methodologies already exist that use a combination of three chromatography columns of

different individual porosities. Depending on the type of analysis needed the use of three columns is not always required (for example in product screening) and can result in longer analysis times. When considering green processes and sustainability the use of a three-column method should be avoided where possible as they consume more (often halogenated) solvents. The use of a linear column, where applicable, is a better solution as they provide both a shorter analysis time and a lower level of solvent consumption. In terms of sustainability, environmentally friendly solvents such as ethyl acetate are preferable.

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

INTRODUCTION

When developing new methods in GPC there are two challenges: on the one hand, the polymer must be soluble in the corresponding solvent, however, on the other hand, the solvent in combination with the column must be able to provide the correct results, i.e. an interaction-free chromatography must be guaranteed. Calibrations in CHCl_3 and THF were performed to compare them with the same calibrations in ethyl acetate, in order to confirm that no polymer-column interactions occur.

SAMPLE PREPARATION

For calibration with PS, an EasiCal PS-1/2-Kit in the molar mass range 3 670 Da–2 561 kDa from Agilent was used. The calibration standards were dissolved in 1 ml of the corresponding solvent for one hour. For calibration with polymethyl methacrylate, a PSS ReadyCal-Kit in the molar mass range 2 380 Da–2 200 kDa was used. The calibration standards were dissolved in 1 ml of the corresponding solvent for one hour. The PS standards were dissolved in 1 ml of the corresponding solvent at a concentration of 2.25 mg/ml. For all analyses, BHT was used as a flow marker at a concentration of 1.5 mg/ml.

RESULTS

Two universal calibrations were created in three solvents each: one with PS and one with PMMA. Subsequently, three narrow PS-standards were evaluated with these calibrations and the values were compared. For each measurement, butylhydroxytoluene (BHT) was used as a flow marker at a concentration of 1.5 mg/ml.

Fig. 1 shows a 16-point calibration with PS in chloroform in the molar mass range 580 Da to 2.7 million Da. The largest molar mass (6.57 million Da) is far outside the separation range of the column and was therefore not taken into account. The calibration points were fitted with a 5th degree polynomial for all six calibrations, with deviations of less than 5%. The 1st derivative of the function showed no local extrema in the calibration range.

The universal calibration of PMMA in chloroform is shown in **Fig. 2**. A ReadyCal-Kit from PSS in the molar mass range 800 Da–2.2 Mio. Da was used. The 12 calibration points were fitted with a 5th degree polynomial.

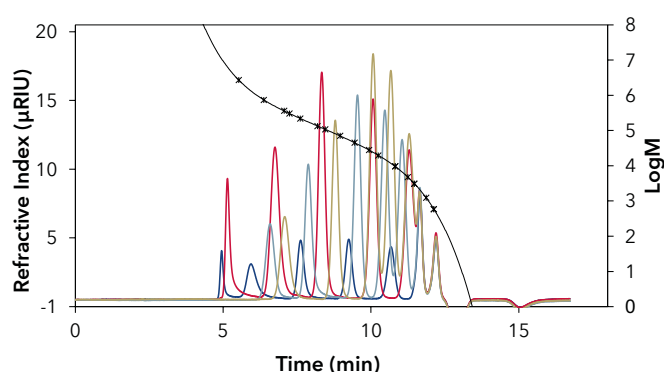


Fig. 1 16-point PS calibration in chloroform by Agilent (EasiCal) Blue=PS-1(A); Red=PS-1(B); Light blue=PS-2(A); gold=PS-2(B). Fit function of the 5th degree with deviations less than 5%. $R^2=1$

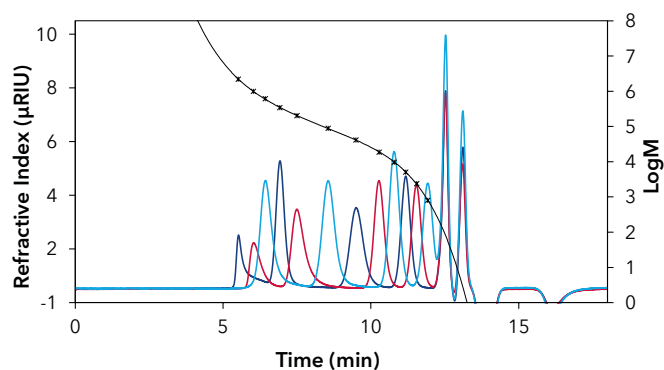


Fig. 2 12-point PMMA calibration in chloroform by PSS (ReadyCal). Blue=green cup; Red=red cup; Light blue=white cup. Fit function of the 5th degree with deviations less than 5%. $R^2=1$

The calibrations of PS and PMMA in ethyl acetate are shown in **Fig. 3** and **Fig. 4**. As with the calibrations in chloroform, the calibration standards were fitted with a polynomial of the 5th degree. In the case of PS calibration, the highest value for the calibration due to the peak shape was not considered. The two lowest were overlapped with the flow marker signal, to resolve this a 15-point calibration in the range 1 230 Da–2.7 million Da was created. For the PMMA calibration, all 12 points of the calibration set were used. **Fig. 5** and **Fig. 6** show the chromatograms for PS and PMMA in THF. For the

calibrations in THF, the same methods were used, whereas for the PMMA calibration the lowest value overlapped with the flow marker and was not taken into account.

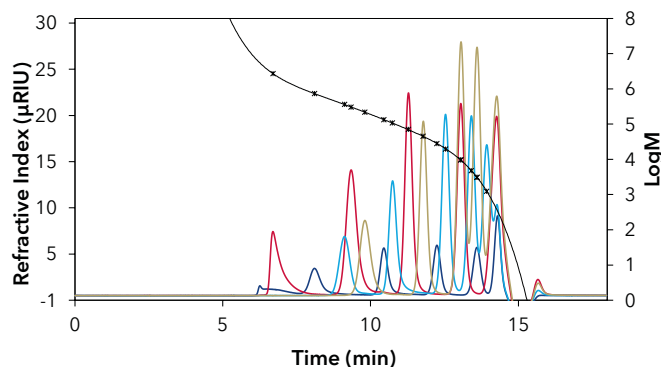


Fig. 3 15-point PS calibration in EtOAc by Agilent (EasiCal). Blue=PS-1(A); Red=PS-1(B); Light blue=PS-2(A); Gold=PS-2(B). Fit function of the 5th degree with deviations less than 5%. $R^2=1$

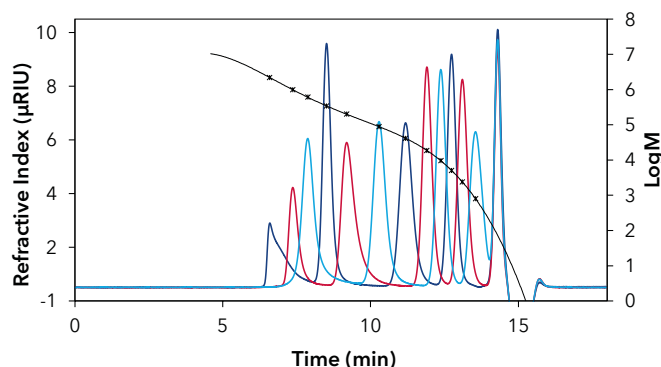


Fig. 4 12-point PMMA calibration in EtOAc by PSS (ReadyCal). Blue=green cup; Red=red cup; Light blue=white cup. Fit function of the 5th degree with deviations less than 5%. $R^2=1$

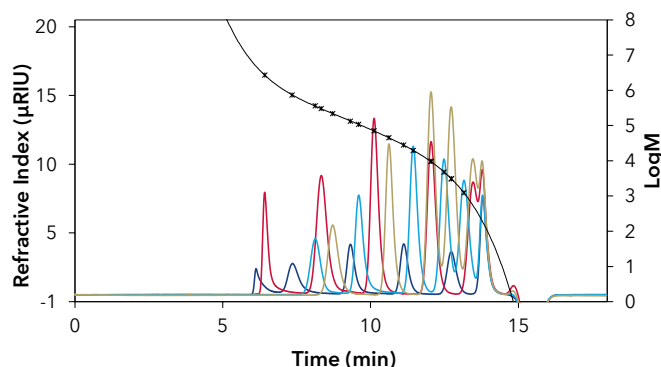


Fig. 5 15-point PS calibration in THF by Agilent (EasiCal). Blue=PS-1(A); Red=PS-1(B); Light blue=PS-2(A); Gold=PS-2(B). Fit function of the 5th degree with deviations less than 5%. $R^2=1$

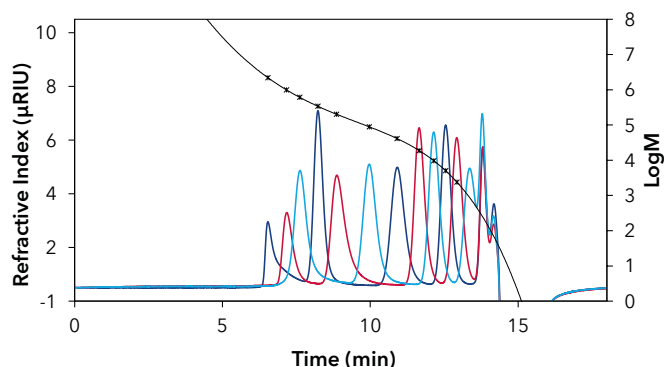


Fig. 6 11-point PMMA calibration in EtOAc by PSS (ReadyCal). Blue=green cup; Red=red cup; Light blue=white cup. Fit function of the 5th degree with deviations less than 5%. $R^2=1$

The PS standards used for the review were part of a PSS ReadyKal kit (black cup). Universal calibration was applied to the standards, considering the Mark-Houwink parameters K and α , which were taken from the literature as seen in **Tab. 7**. **Tab. 1** shows the results with the PS calibration in THF. The calibration covers the molecular range for PS very well with only a small deviation of less than 10%.

Tab. 1 Results of the evaluation of PS narrow standards with a PS calibration in THF

Standard Mn/Mw (Da)	Mn (Da)	Mw (Da)
59 300/62 500	63 167 (6.5%)	65 527 (4.8%)
14 700/15 500	14 451 (1.7%)	15 092 (2.6%)
4 380/4 710	4 305 (1.7%)	4 622 (1.9%)

Tab. 2 shows the results of the PMMA calibration in THF. The deviations are slightly over 10% but show no trend and remain within an acceptable range. Comparable results were obtained for the calibrations of PS/PMMA in chloroform (**Tab. 3** and **Tab. 4**).

Tab. 5 shows the results of the PMMA calibration in ethyl acetate. The values deviate very strongly and show an increasing variance with decreasing molar mass. This is an indication of interactions with the stationary phase. Most likely, PS interacts with the styrene-divinylbenzene copolymer (StyDiViBe) of the stationary phase. These interactions are considered in the calibration with PS, which is why the deviations in **Tab. 6** are below 10%.

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

Tab. 2 Results of the evaluation of PS narrow standards with a PMMA calibration in THF

Standard Mn/Mw (Da)	Mn (Da)	Mw (Da)
59 300/62 500	67 903 (14.5 %)	69 831 (11.7 %)
14 700/15 500	16 856 (8.8 %)	17 616 (13.7 %)
4 380/4 710	4 887 (3.8 %)	5 257 (11.6 %)

Tab. 3 Results of the evaluation of PS narrow standards with a PS calibration in chloroform

Standard Mn/Mw (Da)	Mn (Da)	Mw (Da)
59 300/62 500	64 987 (9.6 %)	67 136 (7.4 %)
14 700/15 500	15 085 (2.6 %)	15 687 (1.2 %)
4 380/4 710	4 509 (2.9 %)	4 819 (2.3 %)

Tab. 4 Results of the evaluation of PS narrow standards with a PMMA calibration in chloroform

Standard Mn/Mw (Da)	Mn (Da)	Mw (Da)
59 300/62 500	58 662 (1.1 %)	60 296 (3.5 %)
14 700/15 500	14 382 (2.2 %)	14 993 (3.3 %)
4 380/4 710	3 825 (12.7 %)	4 170 (11.5 %)

Tab. 5 Results of the evaluation of PS narrow standards with a PMMA calibration in ethyl acetate

Standard Mn/Mw (Da)	Mn (Da)	Mw (Da)
59 300/62 500	36 150 (-39.0 %)	37 874 (-39.4 %)
14 700/15 500	4 847 (-67.0 %)	5 270 (-66.0 %)
4 380/4 710	1 009 (-77.0 %)	1 130 (-76.0 %)

Tab. 6 Results of the evaluation of PS narrow standards with a PS calibration in ethyl acetate

Standard Mn/Mw (Da)	Mn (Da)	Mw (Da)
59 300/62 500	65 113 (9.8 %)	67 016 (7.2 %)
14 700/15 500	15 624 (6.3 %)	16 294 (5.1 %)
4 380/4 710	4 804 (9.7 %)	5 164 (5.0 %)

CONCLUSION

Using the KNAUER GPC/SEC system it is possible to create universal calibrations with PMMA and PS in THF and chloroform that can be used for the analysis of polymers using a linear column. It was also demonstrated that when using a StyDiViBe column with ethyl acetate as mobile phase, a universal calibration with PMMA standards is not possible. Interactions of PS with the stationary phase (e.g. π -interactions) are most likely responsible for this.

MATERIALS AND METHODS

Tab. 7 Mark-Houwink constant from the literature for PS/PMMA in the solvents used

Polymer	K[dl/g*10 ⁵], α in THF	K[dl/g*10 ⁵], α in CHCl ₃	K[dl/g*10 ⁵], α in EtOAc
PS	14, 0.7, (25°C) ⁽¹⁾	7.86, 0.76, (25°C) ⁽³⁾	42.96, 0.57, (25°C) ⁽³⁾
PMMA	19.9, 0.66, (T=n.a.) ⁽²⁾	4.8, 0.8, (25 °C) ⁽⁴⁾	21.1, 0.64, (20°C) ⁽⁵⁾

Method

Column temperature	25 °C
Injection volume	20 μ l
Injection mode	Partial Loop
Detection	RID
Data rate	10 Hz

Pump parameters

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

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System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L HPG	APH38ED
Autosampler	AZURA AS 6.1L	AAA00AA
Detector 1	AZURA RID 2.1L	ADD31
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

