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응용 자료

Accurate Molecular Weight Determination of Polystyrene-Tetrahydrofuran Solutions using the Arc™ HPLC System with a Strong Solvent Compatibility Kit and Refractive Index (RI) Detector

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Abstract

Achieving accurate and reproducible molecular weight measurements is paramount to proper characterization of polymers. This application note illustrates accurate molecular weight determination of polystyrenes using an Arc HPLC System with a strong solvent compatibility kit and refractive index (RI) detector. Polystyrene standard solutions with narrowly distributed molecular weights and a sample were prepared in tetrahydrofuran (THF) solvent. Molecular weight calculations were performed using Empower™ Software with the gel permeation chromatography (GPC) option.

Benefits

- Accurate molecular weight determination for polystyrenes and excellent reproducibility using the Arc HPLC System with a strong solvent compatibility kit

- High resolution separation using Styragel HR columns for polymer analysis
- Quick and easy determination of molecular weight distribution of polymers using Empower Software with the GPC option

Introduction

Gel permeation chromatography (GPC) is a common technique for characterizing polymers, including molecular weight determination and molecular weight distribution. GPC is based on size exclusion chromatography (SEC), which separates molecules according to their hydrodynamic radius in solution.[1] Larger polymers elute earlier, while small molecular weight polymers elute later. In the polymer industry, molecular weight distribution is used to predict polymer properties such as mechanical strength and elasticity.[2] Polymer molecular weights are typically expressed as number average molecular weight (Mn), weight average molecular weight (Mw), size average molecular weight (Mz) and polydispersity index (PDI). The PDI is a ratio of Mw/Mn and is a measure of the range of the molecular weight distribution.

While GPC is a powerful tool for the analysis of polymers, accurate quantification of the molecular weight distributions is reported to be a main challenge[1]. Most polymers are poorly detectable by UV. Detectors such as refractive index (RI) and evaporative light scattering (ELSD) are often applied.

This application note illustrates the accurate molecular weight determination of polystyrene solutions using the Arc HPLC System installed with a strong solvent compatibility kit. The strong solvent compatibility kit allows the use of strong solvent, such as tetrahydrofuran (THF) and dimethylformamide (DMF), with the Arc HPLC System.[3] In this work, the molecular weight calibration was performed using a set of narrow polystyrene standards prepared in tetrahydrofuran solvent. The polystyrene sample was quantified against the calibration curve to determine molecular weight distribution. The GPC analysis demonstrated excellent calibration and accurate determination of molecular weight of the polystyrene sample.

Experimental

Tetrahydrofuran, HPLC grade, no preservatives, purchased from Fisher Chemicals, Catalog No.: T425–4.

Polystyrene Standards, Ready Cal PS Kit for APC, p/n: 186007223 < https://www.waters.com/nextgen/global/shop/standards--reagents/186007223-readycal-ps-kit-for-apc.html> : contains set of three narrow standards and unknown sample:

- Black vial cap: 2.25 mg each of polystyrene at MW 66K, 21.5K, 4.92K, 2.28K
- Blue vial cap: 2.25 mg each of polystyrene at MW 44.2K, 15.7K, 3.47K, 1.25K
- Green vial cap: 2.25 mg each of polystyrene at MW 28K, 9.13K
- White vial cap: 2.25 mg of polystyrene sample at MW 34.8K labeled as unknown for molecular weight confirmation

Sample Description

Standard Solutions

Polystyrene narrow standards and sample were prepared by adding 1.5 mL of tetrahydrofuran to each vial and allowing to dissolve for several hours. Concentration 1.5 mg/mL.

Method Conditions

System:	Arc HPLC System with quaternary solvent manager (QSM), flow through needle (FTN) and strong solvent compatibility kit (p/n: 715009279)
Mobile phase:	Tetrahydrofuran
Separation:	Isocratic
Flow rate:	1.0 mL/min
Columns:	All columns 7.8 x 300 mm with 5 µm, connected in series using joining tube (p/n: WAT084080) supplied with columns. 1. Styragel™ HR 4, 10,000 Å, molecular weight

	range: 5,000–600,000, p/n: WAT044225 2. Styragel HR 2, 500 Å, molecular weight range: 500–20,000, p/n: WAT044237 3. Styragel HR 1, 100 Å, molecular weight range: 100–5,000, p/n: WAT044234
Column temperature:	35 °C
Detection:	Refractive Index (RI) · Sampling rate: 10 pts/sec · Polarity: positive · Flow cell temperature: 35 °C
Injection volume:	50 µL
Vials:	LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV
Sample temperature:	15 °C
Wash solvents:	Sample/purge wash: tetrahydrofuran Needle wash: isopropyl alcohol

Data Management

Chromatography software:	Empower 3 Feature Release 5 Service Release 5 (FR5 SR5). GPC option used for data processing and reporting.

Results and Discussion

To ensure proper characterization of polymers, it is important to generate calibration curves using appropriate standards to establish separation for a set of columns tested. In this work, a bank of three columns was selected to cover the molecular range of the polystyrene narrow standards. Additionally, different porosity columns were chosen to produce adequate resolution for accurate determination of molecular weights. Columns were connected in series according to the pore size using the joining tubing, starting with the largest pore size to minimize back pressure. As the response of the refractive index detector can be influenced by changes in the eluent composition, gradient elution was not used.[1] The standard and sample solutions dissolved in THF solvent were run isocratically using THF as a mobile phase. The method successfully separated the polystyrene narrow standards and allowed the sample to be analyzed (Figure 1).

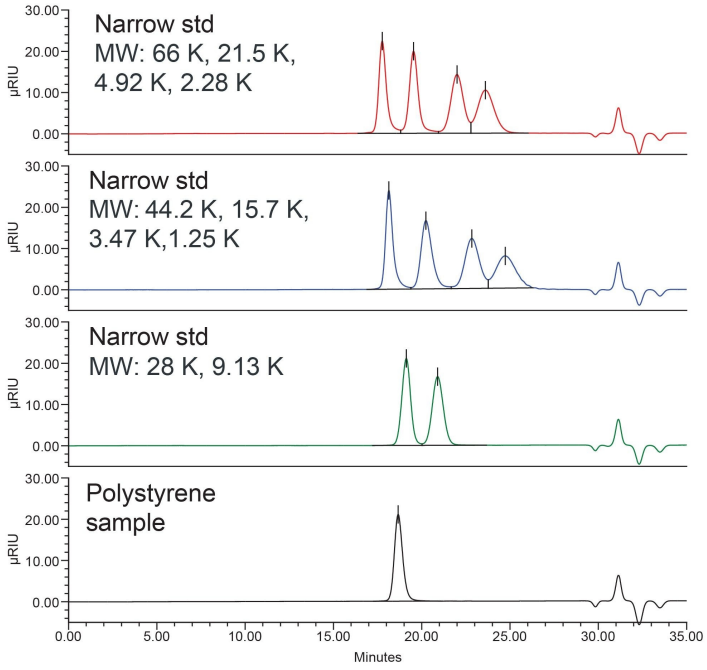

Figure 1. Chromatographic separation of polystyrene standards and sample solutions generated by an Arc HPLC System with a strong solvent compatibility kit and RI detector.

Polystyrene calibration and sample analysis

Data analysis and reporting was performed using Empower Software GPC option.[4] The GPC calibration was generated by analyzing a set of narrow polystyrene standards. The molecular weight at peak maximum (Mp) of each standard were utilized to generate a curve with molecular weights versus retention time. Applying a third order fit, method exhibited an acceptable relationship between the log molecular weight (Mp) vs retention times with correlation coefficient (R^2) of greater than 0.9997 (Figure 2).

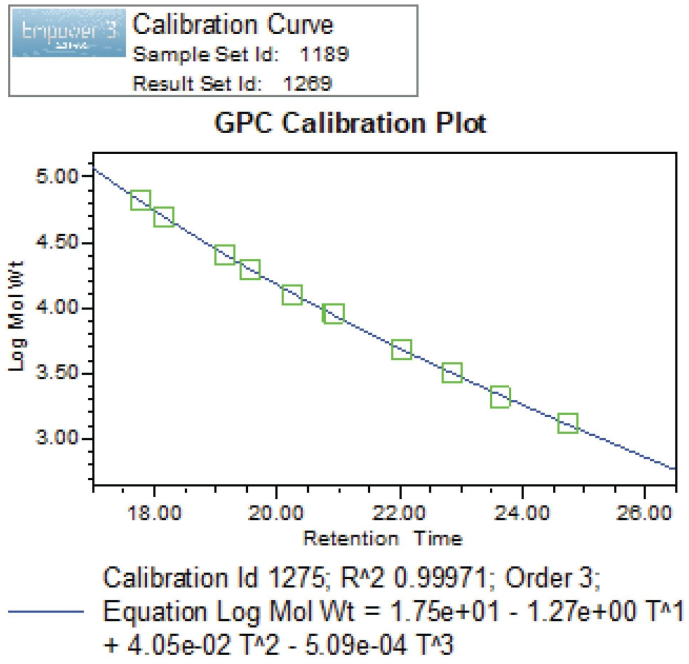

Figure 2. GPC calibration curve generated using polystyrene narrow standard standards.

The polystyrene sample was quantified against a calibration curve to calculate the molecular weight information. The GPC measurements included peak average molecular weight (Mp), weight-average molar mass (M_w), number-average molar mass (M_n), and polydispersity of molar mass distribution (Figure 3). Six replicate injections of the sample solution produced relative standard deviation (RSD) ranging from 0.20 to 0.67%. Additionally, the calculated molecular weights were compared to the target values for Mp, Mw, and Mn of 34,500, 34,000, and 33,200 (Dalton), respectively. This resulted in 100.6%, 100.0%, and 98.9% accuracy for the molecular

weight measurements. Additionally, the distribution plots in the Empower GPC option were used to display molecular weight information for the polymer sample. The plots showed molecular weight distribution (Figure 4), allowing for correct and accurate characterization of polymers.

GPC_Mw_peak data
Result Set Id: 1269 Calibration Id: 1275
Processed Channel Descr.: W2414 RI

Name: Green 1

	Sample Name	Name	RT	MP (Daltons)	Mw (Daltons)	Mn (Daltons)	Mz (Daltons)	Polydispersity
1	White cap: 1.5mg/mL	Green 1	18.678	34809	34050	32862	35046	1.04
2	White cap: 1.5mg/mL	Green 1	18.681	34738	34060	32970	35009	1.03
3	White cap: 1.5mg/mL	Green 1	18.681	34722	33990	32722	34959	1.04
4	White cap: 1.5mg/mL	Green 1	18.683	34676	34100	33162	34985	1.03
5	White cap: 1.5mg/mL	Green 1	18.685	34645	33966	32826	34916	1.03
6	White cap: 1.5mg/mL	Green 1	18.686	34620	33813	32510	34845	1.04
Mean			18.682	34701	33997	32842	34960	1.0
Std. Dev.			0.003	68.89	102.37	220.74	71.43	0.00
% RSD			0.02	0.20	0.30	0.67	0.20	0.41

Figure 3. Polystyrene broad sample analysis for molecular weight distribution. Six replicate injections.

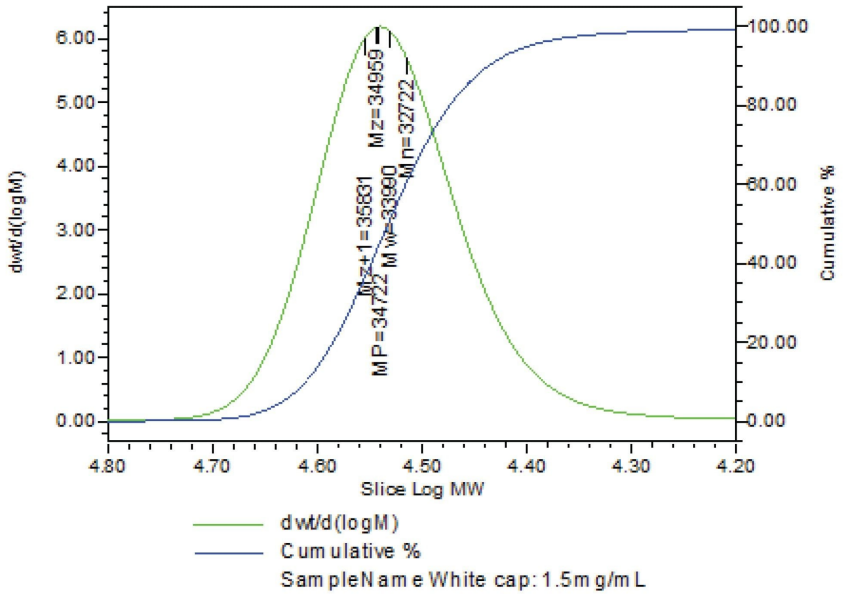

Figure 4. Molecular weight distribution plot for polystyrene sample.

Inter-Day Performance

Inter-day performance of the method for the same analysis ensures that the method generates consistent results, which is paramount for accurate characterization of polymers. For performance verification, polystyrene standards and sample were analyzed on different days to assess the calibration curve and accuracy of the molecular weight determination. Over the days studied, method generated excellent calibration and accuracy for the molecular weight measurements.

Solutions	Parameter	Day 1 (initial)	Day 3	Day 5
Polystyrene standards	Calibration, R^2	0.99970	0.99971	0.99970
Polystyrene sample ▪ Calculated average molecular weight ▪ Accuracy of measurements	Mp (Dalton)	▪ 34646 ▪ 100.4%	▪ 34701 ▪ 100.6%	▪ 34684 ▪ 100.5%
	Mw (Dalton)	▪ 34039 ▪ 100.1%	▪ 33997 ▪ 100.0%	▪ 33991 ▪ 100.0%
	Mn (Dalton)	▪ 32888 ▪ 99.1%	▪ 32842 ▪ 98.9%	▪ 32796 ▪ 98.8%

Table 1. Inter-day performance for polystyrene analysis including calibration curve and sample analysis. Target molecular weights for sample: Mp = 34,000 Dalton, Mw = 34,000 Daltons, and Mn = 33,200 Daltons.

Conclusion

A set of narrow polystyrene standards and polystyrene sample were analyzed using the Arc HPLC System with strong solvent compatibility kit and processed using Empower Software with the GPC option. The method demonstrated excellent repeatability and accurate molecular weight determination for analysis of polystyrene solutions. The Empower Software GPC option enabled quick data analysis to effectively characterize polymer molecular weight determination and molecular weight distribution.

References

1. Knol WC, Pirok BW, Peters RAH. Detection Challenges in Quantitative Polymer Analysis by Liquid Chromatography. *Journal of Separation Science*, July 2020.
2. Walsh D, Schinski DA, Schneider RA, Guironnet D. General route to design polymer molecular weight distributions through flow chemistry. *Nature Communications*, 2020.

3. Arc HPLC QSM-FTN Strong Solvent Compatibility Kit Product Solutions, 715009279 <https://www.waters.com/webassets/cms/support/docs/715009279v00.pdf> .

4. Empower GPC Software Getting Started Guide, Waters Corporation User Guide, 71500031303. <https://www.waters.com/webassets/cms/support/docs/71500031303ra.pdf>

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Arc HPLC System <https://www.waters.com/nextgen/global/products/chromatography/chromatography-systems/arc-hplc-system.html>

ACQUITY UPLC Refractive Index Detector <https://www.waters.com/134726507>

Empower Chromatography Data System <https://www.waters.com/10190669>

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