Characterization of Styrene-Acrylonitrile Copolymers Using Comprehensive 2D-LC

Investigation of Two Mutually Dependent Distributions

Application Note

Energy and Chemicals

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Abstract

Synthetic polymers are complex mixtures with distributions in various molecular characteristics such as molecular weight and chemical composition. For a polymer with distributions in two molecular characteristics, full characterization can only be achieved using two-dimensional separation. This Application Note shows the analysis of styrene-acrylonitrile copolymers (SAN) using comprehensive two-dimensional liquid chromatography with interaction chromatography in the first dimension and fast size exclusion chromatography (SEC) in the second dimension. In the first dimension, separation according to the acrylonitrile content (chemical composition) is achieved, and the second-dimension SEC separation enables determination of the molecular weight distributions of the SAN with different acrylonitrile contents.
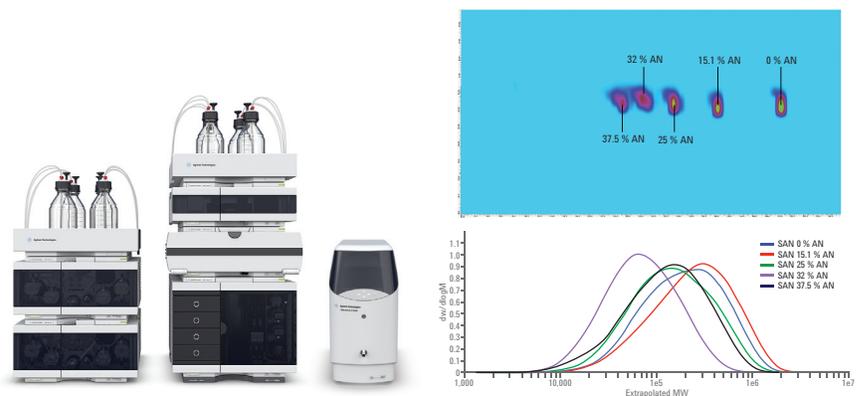
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Introduction

Synthetic polymers are not single molecular species but complex mixtures with distributions in various molecular characteristics such as molecular weight, composition, and chain architecture[1,2]. In the simplest case (homopolymers), the number of polymer repeat units vary, and a distribution of chain length or equivalently, molecular weight, exists. For polymers built up from different monomers (copolymers), a distribution of chemical composition is observed. Additional possible types of heterogeneity include topology, tacticity, and variations of end groups[1,3]. The different types of distributions present in a polymer are usually mutually dependent[4]. The properties of the polymer not only depend upon the average molecular structure, but are affected by the underlying distributions. To obtain characterization of the molecular distributions, separation of the different structures is indispensable[1,3,4].

The molecular weight distribution of a polymer can be analyzed using size exclusion chromatography (SEC), where molecules in solution are separated according to size (hydrodynamic volume). The separation of polymers based on chemical composition or functionality can be achieved by different forms of interaction chromatography[1,5]. For a polymer with distributions in two dimensions, for example, a copolymer with chemical composition and molecular weight distribution, full characterization can only be achieved using two-dimensional separations[1,6].

This Application Note shows the analysis of styrene-acrylonitrile copolymers (SAN) using comprehensive two-dimensional liquid chromatography (comprehensive 2D-LC). Interaction chromatography is applied in the first dimension to achieve separation according to the acrylonitrile content (chemical composition). In the second dimension, fast SEC enables determination of the molecular weight distributions of the SAN with different acrylonitrile contents. It is illustrated that the mutually dependent chemical composition and molecular weight distributions can only be

fully characterized using 2D-LC. For the analysis of polymers containing a chromophore, such as SAN, UV detection can be used. To enable more universal detection, the combination with evaporative light scattering detection (ELSD) is also shown.

Experimental

Instrumentation

The Agilent 1290 Infinity II 2D-LC Solution was composed of the following modules:

- Agilent 1290 Infinity II High-Speed Pumps (2x G7120A) equipped with ultraclean tubing kit (G4220-68070) for compatibility with tetrahydrofuran:

    - First dimension pump: Channel B pump head equipped with black PTFE piston seals (p/n 5063-6589) for compatibility with tetrahydrofuran; maximum pressure 600 bar

    - Second dimension pump: Channel A and channel B pump heads equipped with black PTFE piston seals (p/n 5063-6589) for compatibility with tetrahydrofuran; maximum pressure 600 bar

        Easy seal exchange towards the black PTFE piston seals is possible using the easy maintenance pump heads (easy maintenance upgrade for a 1290 Infinity II High-Speed Pump, G4223A)

- Agilent 1290 Infinity II Multisampler (G7167B) with cooler (option #100)

- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with 2-position/4-port-duo valve (2D-LC valve head 1,300 bar: p/n 5067-4244) equipped with two 80 μL loops

- Agilent 1290 Infinity II Diode Array Detector (G7117B) with a 10-mm Max-Light cartridge cell (G4212-60008)

- Agilent 1290 Infinity II Evaporative Light Scattering Detector (G7102A)

Use of tetrahydrofuran

Be aware that the use of tetrahydrofuran can shorten instrument lifetime, and may reduce maintenance intervals.

Software

- Agilent OpenLAB CDS ChemStation Edition rev. C.01.07 [27] with Agilent 1290 Infinity 2D-LC acquisition software product version A.01.02 SP1

- GC Image LCxLC-HRMS Edition software version 2.5b0 for 2D-LC data analysis from GC Image LLC., Lincoln, NE, USA

- Agilent Cirrus GPC Offline GPC/SEC Software version 3.4.1

Columns

First dimension

Agilent PLRP-S 100A, 2.1 × 150 mm, 3 μm (p/n PL1912-3300)

Second dimension

Agilent ResiPore, 10 × 50 mm, 3 μm (custom)

Chemicals

Polystyrenes for SEC calibration were from Agilent (EasiVial PS-M, p/n PL2010-0301). All solvents were LC grade. Acetonitrile and tetrahydrofuran were purchased from Merck (Darmstadt, Germany) and VWR (Leuven, Belgium), respectively.

Standards

Individual styrene-acrylonitrile copolymers with varying percentages of acrylonitrile (0, 15.1, 25, 32, and 37.5 % acrylonitrile) were dissolved in tetrahydrofuran at a concentration of 10 mg/mL. Aliquots of the individual solutions were combined to a total concentration of 2 mg/mL of each styrene-acrylonitrile copolymer in the mixture.

For SEC calibration, 0.5 mL of tetrahydrofuran was added to the preweighed EasiVial PS-M vials containing the polystyrene standards. Three mixtures, each containing polystyrenes of four different narrow molecular weight distributions (Mp in the range of 162–465,600 g/mol), were analyzed.

2D-LC Method

Parameter	Value		
First-dimension pump			
Solvent A	Acetonitrile		
Solvent B	Tetrahydrofuran		
Flow rate	0.064 mL/min		
Gradient and flow rate	Time (min)	%B	Flow (mL/min)
	0	0	0.064
	5	0	0.064
	60	80	0.064
	61	100	0.500
	64	100	0.500
	65	0	0.500
	70	0	0.500
	71	0	0.064
Stop time	75 minutes		
Second-dimension pump			
Solvent	Tetrahydrofuran isocratic		
Flow rate	4.0 mL/min		
Modulation time	1.00 minute		
Modulation on	0 to 60 minutes		
Agilent 1290 Infinity II Multisampler			
Injection volume	3 µL		
Sample temperature	15 °C		
Needle wash	10 seconds in acetonitrile/tetrahydrofuran (30/70, v/v)		
Agilent 1290 Infinity II Multicolumn Thermostat			
First and second-dimension column	25 °C		
2-Position/4-port-duo valve	The 2-position/4-port-duo valve was switched automatically after each second-dimension modulation cycle of 60 seconds. The loops were used in a cocurrent manner (filling and elution of the loops in the same flow direction).		
Agilent 1290 Infinity II Diode Array Detector			
Wavelength	254 nm/4 nm, Ref.: 360 nm/100 nm		
Data rate	40 Hz		
Stop time	60 minutes		
Agilent 1290 Infinity II Evaporative Light Scattering Detector			
Evaporator temperature	70 °C		
Nebulizer temperature	55 °C		
Evaporator gas flow	1.0 SLM		
Smoothing	10		
Data output rate	40 Hz		
Stop time	60 minutes		

Results and Discussion

To enable characterization of a SAN mixture, information on the acrylonitrile content (chemical composition) and on the molecular weight distribution has to be obtained. One-dimensional interaction chromatography enables the separation of SAN according to the acrylonitrile content, as shown in Figure 1. However, no information on the molecular weight distribution can be obtained.

In contrast, one-dimensional SEC enables the separation of SAN according to size (Figure 2), but no information on the acrylonitrile content is obtained. Even using both interaction chromatography and SEC individually, it is not possible to obtain information on the molecular weight distribution of SAN with a specific acrylonitrile content, or to compare the molecular weight distributions of SAN with different acrylonitrile contents in a mixture. To obtain such information, two-dimensional separation of the mixture of SAN is indispensable.
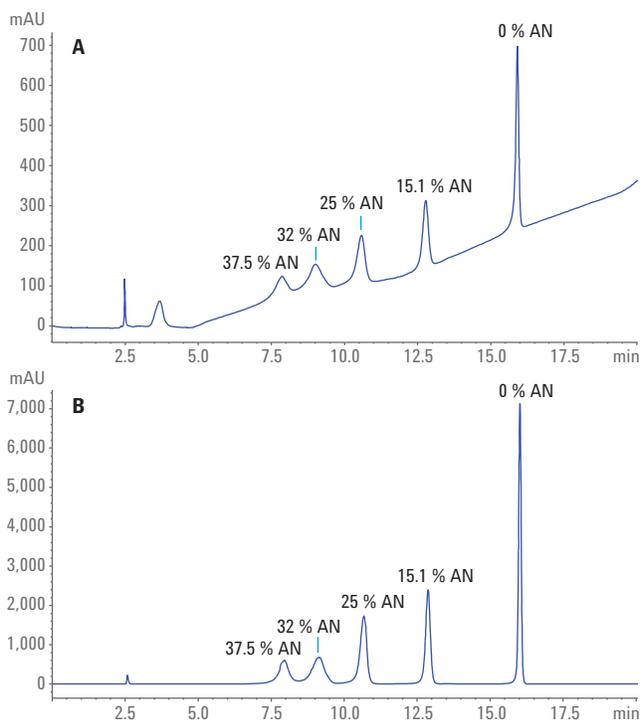

Figure 1. One-dimensional analysis of a mixture of SAN using interaction chromatography; column: PLRP-S 100A, 2.1 × 150 mm, 3 µm; solvent: 0 to 80 % THF in ACN in 19 minutes; flow: 0.2 mL/min; (A) Agilent 1290 Infinity II Diode Array Detector at 254 nm; (B) Agilent 1290 Infinity II Evaporative Light Scattering Detector.
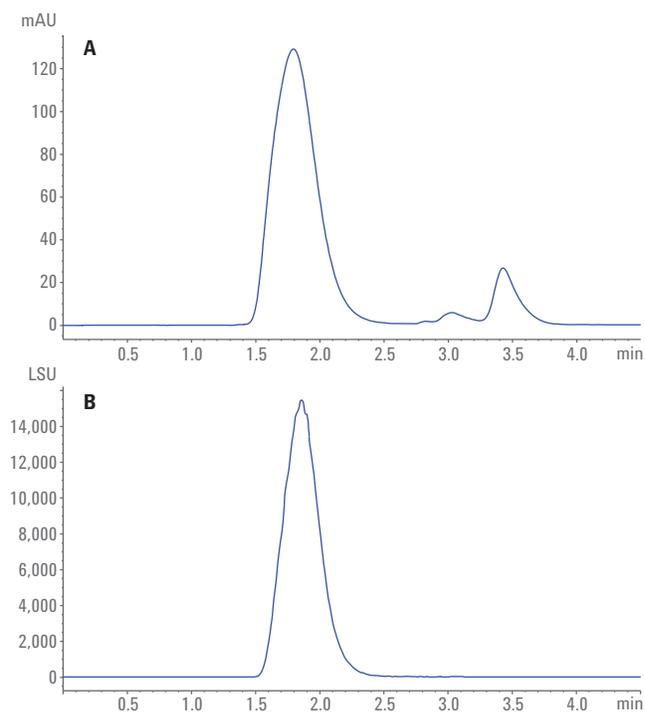

Figure 2. One-dimensional SEC analysis of a mixture of SAN; column: ResiPore, 10 × 50 mm, 3 µm; solvent: THF; flow: 1.0 mL/min; (A) Agilent 1290 Infinity II Diode Array Detector at 254 nm; (B) Agilent 1290 Infinity II Evaporative Light Scattering Detector.

Figure 3 shows the comprehensive two-dimensional analysis of a SAN mixture using interaction chromatography in the first dimension and SEC in the second dimension. The use of a short (50 mm) SEC column at a high flow rate (4 mL/min) enabled an SEC run time of only one minute and, therefore, the application in a comprehensive 2D-LC setup with a modulation time of one minute. As can be seen in Figure 3, UV detection and ELS detection provide comparable results. In contrast to ELS detection, using UV detection, background signals resulting from the recurring injections of first-dimension effluent to the second-dimension separation are detected. In addition, a low molecular weight impurity present in the SAN mixture is only detected using UV detection, as can be observed from the one-dimensional analyses.

From the comprehensive 2D-LC analysis, differences in the molecular weight distributions of the SAN with different acrylonitrile contents can be observed. For example, SAN with 32 % acrylonitrile shows a higher retention time in the second-dimension SEC analysis and consequently a lower molecular weight compared to the other SAN contained in the mixture.
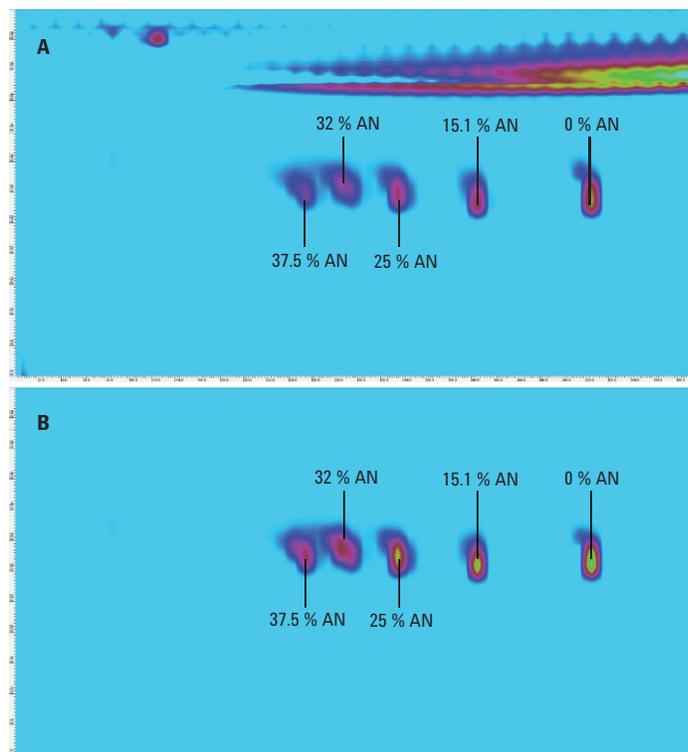

Figure 3. Comprehensive 2D-LC analysis of a mixture of SAN with interaction chromatography in the first dimension and SEC in the second dimension; (A) Agilent 1290 Infinity II Diode Array Detector at 254 nm; (B) Agilent 1290 Infinity II Evaporative Light Scattering Detector.

To enable calibration of the SEC column, polystyrenes (EasiVial PS-M) were analyzed using comprehensive 2D-LC. Figure 4 shows the resulting separations with ELS detection. In addition to separation according to chemical composition in the first dimension, some nonlinear dependency of the first-dimension retention time from molecular weight was observed. For each of the analyzed mixtures of polystyrenes, an unexpected low molecular weight impurity was detected. Following the ELSD settings (with an evaporator temperature of 70 °C), the polystyrene with the lowest molecular weight (Mp 162 g/mol, contained in PS-M green) could not be detected. Lower evaporator temperatures have not been applied.

For SEC calibration, individual second-dimension SEC chromatograms of the polystyrenes were exported from the LCxLC software, as is exemplarily shown for the polystyrene with an Mp of 465,600 g/mol of PS-M red in Figure 5. The individual second-dimension SEC chromatograms were imported into the Cirrus GPC Offline GPC/SEC software, and a SEC calibration curve was generated (Figure 6). For SEC calibration, a second-order polynomial curve fit was used, and a coefficient of determination of 0.9997 was obtained.
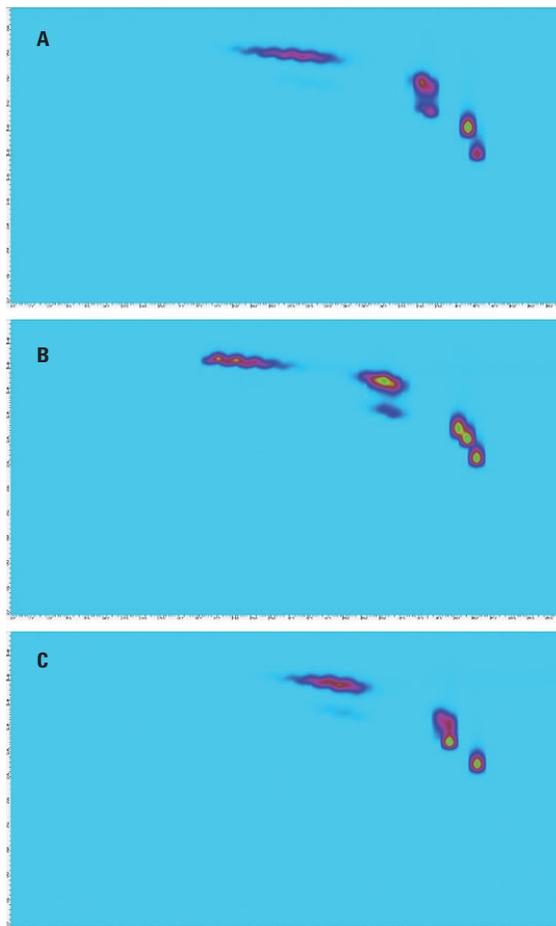

Figure 4. Comprehensive 2D-LC analysis of polystyrenes (EasiVial PS-M) with interaction chromatography in the first dimension and SEC in the second dimension; detection: Agilent 1290 Infinity II Evaporative Light Scattering Detector; (A) PS-M red; (B) PS-M yellow; (C) PS-M green.
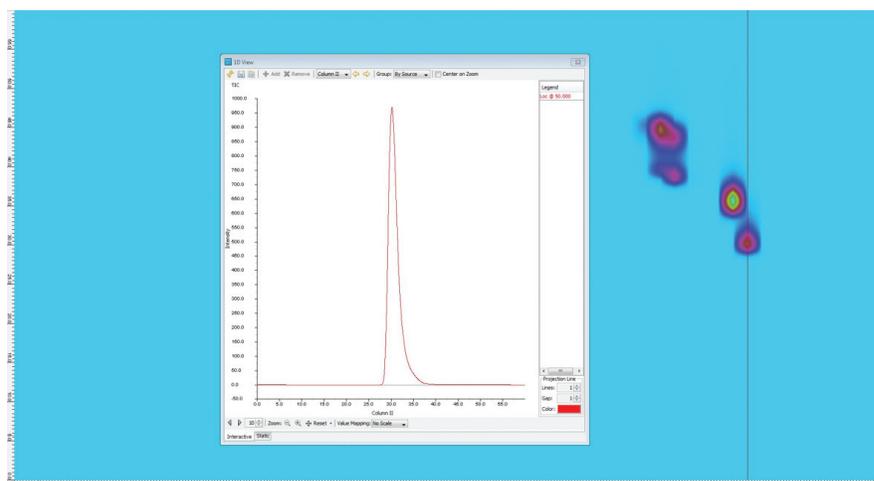

Figure 5. Comprehensive 2D-LC analysis of polystyrenes PS-M red (EasiVial PS-M); detection: Agilent 1290 Infinity II Evaporative Light Scattering Detector; display of the individual second-dimension SEC chromatogram for the polystyrene with an Mp of 465,600 g/mol for exportation.

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In the same manner, individual second-dimension SEC chromatograms of the SAN with different acrylonitrile contents were used for evaluation of the molecular weight distributions. Figure 7 and Table 1 show the molecular weight distributions of the SAN with different acrylonitrile contents, and summarize the molecular weight information obtained. Differences in the molecular weight distributions of the SAN with different acrylonitrile contents that were already visible from the comprehensive 2D-LC chromatogram can now be more closely investigated.

Conclusion

Comprehensive 2D-LC with interaction chromatography in the first dimension and fast SEC in the second dimension enables the separation of SAN according to the acrylonitrile content (chemical composition) and the determination of the molecular weight distributions of the SAN with different acrylonitrile contents. Such information on the mutually dependent chemical composition and molecular weight distributions can only be obtained using two-dimensional separation.
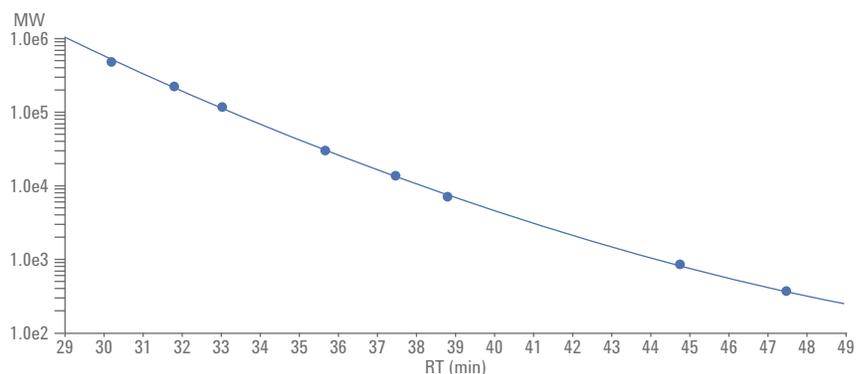

Figure 6. SEC calibration curve from the comprehensive 2D-LC analysis of polystyrenes (EasiVial PS-M).
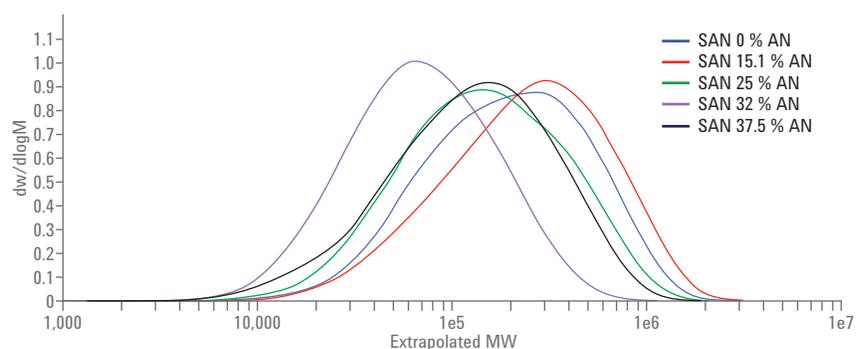

Figure 7. Molecular weight distributions of the SAN with different acrylonitrile contents obtained from the comprehensive 2D-LC analysis of the mixture of SAN.

Table 1. Molecular weight information of the SAN with different acrylonitrile contents obtained from the comprehensive 2D-LC analysis of the mixture of SAN.

SAN	Mp	Mn	Mw	PD
SAN 0 % AN	282631	110158	273515	2.4829
SAN 15.1 % AN	319674	130723	340225	2.6026
SAN 25 % AN	151636	83193	207518	2.4944
SAN 32 % AN	71568	44027	94477	2.1459
SAN 37.5 % AN	164110	65939	178206	2.7026

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