Solvents & Samples
Polystyrenes for SEC calibration were from Agilent (EasiVial PS-M). All solvents were LC grade. Acetonitrile and tetrahydrofuran were purchased from Merck (Darmstadt, Germany) and VWR (Leuven, Belgium), respectively. A mixture of styrene-acrylonitrile copolymers (SAN) with varying percentages of acrylonitrile (0, 15.1, 25, 32, and 37.5% acrylonitrile) was prepared at a concentration of 2 mg/mL of each SAN in tetrahydrofuran.

Chromatographic conditions

### First Dimension
- **Column**: Agilent PLRP-S 100A, 2.1 × 150 mm, 3 μm
- **Solvent A**: Acetonitrile
- **Solvent B**: Tetrahydrofuran
- **Gradient**: 0% B at 0 min, 0% B at 5 min, 80% B at 60 min
- **Flow rate**: 0.664 mL/min
- **Temperature**: 25 °C

### Second Dimension
- **Column**: Agilent ResiPore, 10 × 50 mm, 3 μm
- **Solvent**: Tetrahydrofuran, isocratic
- **Flow rate**: 4.0 mL/min
- **Temperature**: 25 °C

#### Modulation
- 2-Position/4-port-duo valve equipped with two 80 μL loops, cocurrent setup
- **Modulation time**: 1 minute
- **Diode Array Detector**
  - **Wavelength**: 254 nm/4 nm, Ref. 360 nm/100 nm
  - **Data rate**: 40 Hz
- **Evaporative Light Scattering Detector**
  - **Evaporator temperature**: 79 °C
  - **Nebulizer temperature**: 55 °C
  - **Evaporator gas flow**: 1.0 SLM
  - **Smoothing**: 10
  - **Data output rate**: 40 Hz

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 (Figure 1A). 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 1B), but no information on the acrylonitrile content is obtained.

![Figure 1 – One-dimensional analysis of a SAN mixture using (A) 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) and (B) SEC (column: ResiPore, 10 × 50 mm, 3 μm; solvent: THF; flow: 1.0 mL/min) with evaporative light scattering detection.](image)

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, two-dimensional separation of the mixture of SAN is indispensable.

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.

For SEC calibration, individual second-dimension SEC chromatograms from the comprehensive 2D-LC analysis of polystyrenes (EasiVial PS-M) were exported from the LC×LC software and imported into the Cirrus GPC Offline GPC/SEC software. The resulting SEC calibration curve used a second-order polynomial curve fit and a coefficient of determination of 0.9997 was obtained. Individual second-dimension SEC chromatograms of the SAN with different acrylonitrile contents were used for evaluation of the molecular weight distributions, see Figure 3. 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.

Conclusions

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 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.

References: