Time saving, high resolution GPC/SEC using micro columns and optimized RI detection

Application Note Chemical Manufacturing

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Efficient and fast separation for low molar mass polymers and proteins can be achieved using a combination of micro columns packed with smaller particles and optimized detectors. Although traditional analytical RI detectors, such as the SECcurity RI, profit from the higher resolution of micro columns, less peak broadening and higher resolution are the benefits that dedicated micro cell detection units offer.

Introduction
As in HPLC before there is now a trend in GPC/SEC to separate low molar mass polymers and oligomers on columns filled with particles of small particle size. Smaller particles generally have the advantage that higher resolution is achieved.

Unlike in UHPLC in µGPC/SEC it is not the extremely high pressures that are a challenge but the cell volumes of the typical GPC/SEC detectors and the dead volumes especially when working with multi detection. If the cell volume is too large, the previously separated oligomers will be back- mixed in the detector cell and the advantage of small particle sizes and dedicated micro columns is lost.

Cells with small volumes are already available for many UV/DAD detectors and can be replaced easily. However for the most common GPC/SEC detector, the refractive index RI, only very few models are suited and there is no easy exchange of cells. With the PSS SECcurity µ-RI an RI with smallest cell volume (1.7 µL), specifically designed for the use with micro columns is now available. The detector can be seamlessly retrofitted at any time in PSS SECcurity GPC systems as well as in Agilent 1260/1290-Systems.

System Requirements

	Conditions
Pump	PSS SECcurity GPC1260 isocratic pump • flow rate [mL/min]: 0.33 • mobile phase: THF
Injection system	PSS SECcurity GPC1260 Autosampler
Columns	• PSS SDV precolumn (4.6*30 mm) • PSS SDV, 3 µm, 100 Å (4.6 x 250 mm) Temperature: Ambient Optimum flow-range: 0.33 -0.7 mL/min
Calibration	PSS PS ReadyCals low
Loading	Samples dissolved in THF • 1 mg/mL, 5 µL injection volume

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Detector(s)	• PSS SECcurity µRI Applicable flow-range: 0.1 - 1.0 mL/min • PSS SECcurity RI Applicable flow-range: up to 3.0 - 5.0 L/min depending on solvent viscosity and back pressure (limit: 5 bar)
Software	PSS WinGPC UniChrom with ChromPilot

Procedure, Results & Discussion

Smaller particles are used to obtain higher resolution. If these particles are packed in GPC/SEC columns with smaller inner diameters (compared to traditional analytical columns) an efficient separation can be achieved that needs less mobile phase. Traditionally micro columns are run at a reduced flow-rate between 0.35 ml/min, but modern materials can be also operated in the flow-rate range of 0.5 - 0.7 mL/min to save not only mobile phase, but also time.

Typical column dimensions for micro columns are e.g. 150 to 250 mm length and 4.6 mm inner diameter. The limitations of this approach with respect to molar mass and polymer type are currently being evaluated. Potential problems for higher molar masses might be shear forces from column frits or the material itself and chromatographic artifacts.

For this application note PSS SDV columns, a polymeric crosslinked styrene-divinyl benzene material, have been used to investigate the resolution and the performance of the PSS µRI for oligomeric polystyrene. An advantage of polymeric stationary phases over silica based materials is that less interactions are observed. In addition it is easier to construct a column set that is mismatch free to cover a wider molar mass range.

Figure 1 shows a comparison of the separation of an oligomeric polystyrene on 2 polymer based PSS SDV micro columns with small particles using a analytical SECcurity RI with 8µL cell and the PSSµRI with a 1.7 µL cell.

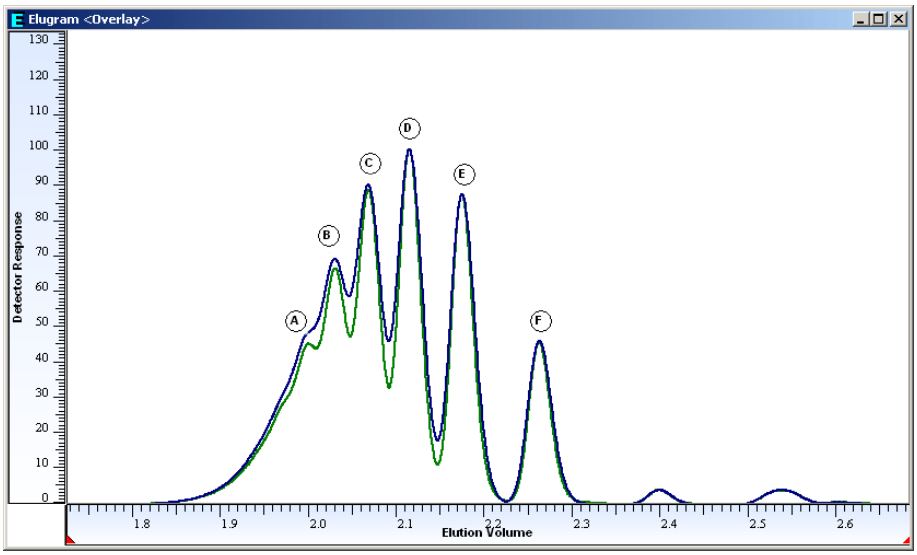

Figure 1: Oligomeric polystyrene separated in < 3 mL on one PSS SDV micro column with 3 µm particle size using an analytical SECcurity RI (blue trace) and a SECcurity µRI (green trace).

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Although the analytical RI has already a very good performance it is possible to detect more oligomers with the µRI Detector. Table 1 shows the results for the area analysis of the peaks as well as the molar masses for each oligomer.

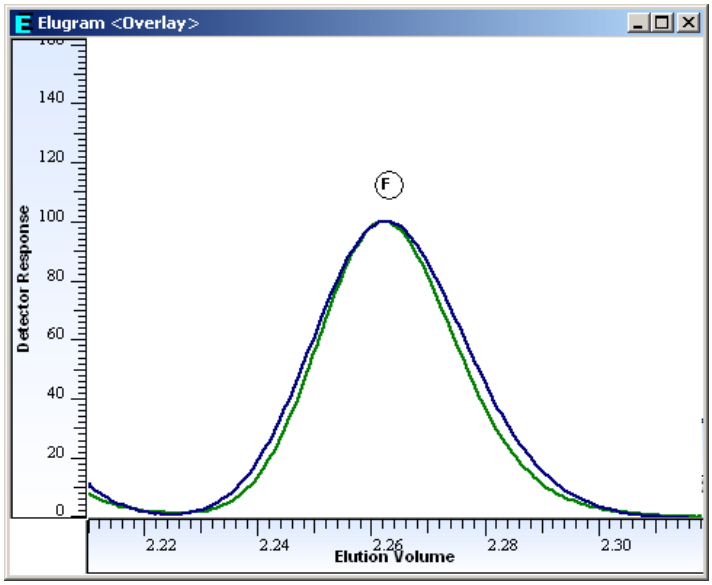
Table 1: Molar masses and peak area results for the analytical SECcurity RI (blue) and the SECcurity µRI (green) shown in figure 1

Peak	Molar mass [Da]	Area%
A	786	- / 16.46
B	682	30.40* / 14.41
C	578	19.41 / 18.66
D	474	21.08 / 20.75
E	370	17.89 / 17.92
F	266	9.24 / 9.40

* not resolved

Besides the higher resolution the µRI detector shows also less signal broadening. Figure 2 shows a magnified comparison of the 2 traces for Peak F. Here it is clearly visible that the peak for the µRI is narrower. This also explains the small differences for the peak area obtained with the analytical RI and the more accurate µRI.

Figure 2: Comparison of dimeric styrene measured on a micro column with an analytical SECcurity RI (blue trace) and a µRI (green trace). The signal obtained with the µRI is narrower due to less broadening effects in the smaller detector cell.



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