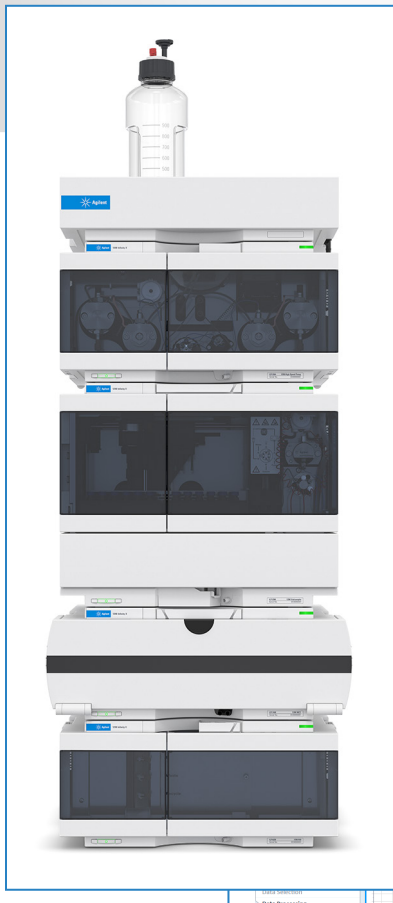
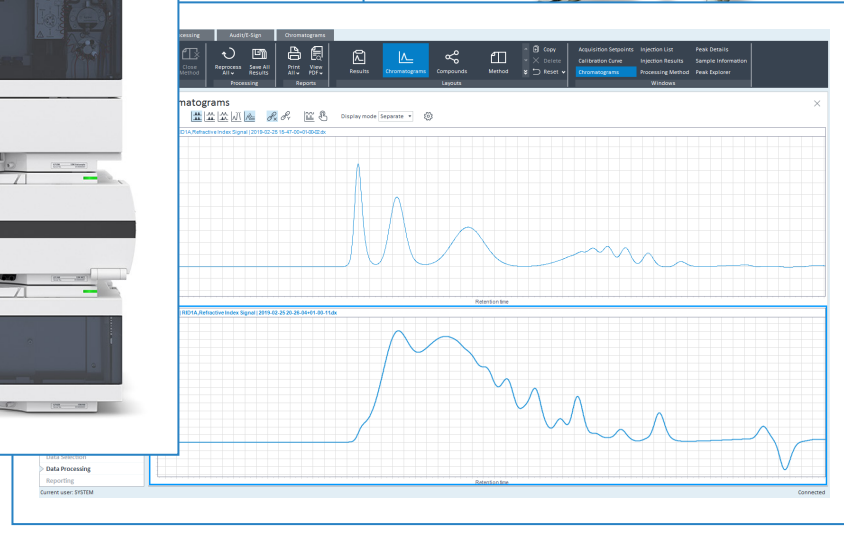
A UNIVERSAL REFRACTIVE INDEX DETECTOR FOR ANALYTICAL AND MICRO-SCALE GPC





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INTRODUCTION

Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) is an important liquid-chromatographic technique for determining a polymer's molecular-weight distribution and averages, and comparing batch-to-batch polymer quality.

Miniaturization (i.e. the use of smaller column dimensions and particle sizes) has been a popular approach in many liquid-chromatographic techniques. The benefits of miniaturization include reduced solvent costs, higher throughput, increased detector response, less chemical waste generation, and taking full advantage of the latest advances in liquid chromatography instrument design.

This white paper investigates how miniaturization can be applied to gel permeation chromatography, discusses critical considerations, and determines what benefits the approach brings for size-based separations.

It also describes the advantage of using the Agilent 1290 Infinity II GPC/SEC System, which includes the Agilent 1290 Infinity II Micro Refractive Index Detector (μRID). The Micro RID enables the use of GPC columns with narrow inner diameters (e.g., 4.6- and 2.1-mm columns). The use of such columns packed with smaller GPC particles leads to better resolution, faster runtime, and lower solvent consumption at the same, or better, polymer separation performance.

An additional advantage of using the 1290 Infinity II GPC/SEC System is the ability to run both analytical and microscale separations on one system. The 1300 bar pressure range makes it possible to run any type of GPC/SEC column.

EXPERIMENTAL DESIGN

High-performance GPC/SEC columns deliver significantly increased resolution compared with a conventional GPC/SEC column set. The use of high-performance GPC/SEC columns allows the use of fewer columns in series and higher linear velocities, without sacrificing separation quality.

To demonstrate the performance of miniaturization, polystyrene and epoxy resin samples were separated with columns that would resolve numerous oligomer peaks. The parameters used in this experiment are listed in **Table I**.

RESULTS AND DISCUSSION

Miniaturization. Conventional analytical-scale GPC/SEC separations are performed using columns with a length of 300 mm, an internal diameter of 7.5 mm and a particle size of dp = 5 um. **Table II** shows how various parameters affect the results and their potential benefits. Optimal linear velocities in GPC separations are relatively low and, as a result, flow rates of 1.0 mL/min are used. To achieve acceptable resolution, multiple columns

Table I: Parameters used in the experiment.

Parameter	Value
Solvent	THF, isocratic, channel B
Flow Rates	0.06, 0.3, 0.6, 1.0 mL/min
Slop Time	22, 11, or 7 minutes
Column Temperature	35 °C, 2 columns in series connected with 75 µm i.d. capillaries
Injection Volume	20 µL, 4 µL
RID	Optical unit temperature: 35 °C Data rate: 18 Hz Signal polarity: positive

Table II: Effect of various parameters on the separation.

Parameter	Change	Benefit	Consideration
Flow rate	Lower	Decreased solvent consumption	Pump flow rate precision
Peak volume	Lower	Increased sensitivity	System dispersion volume
Peak width	Narrower	Higher resolution	Detector data (sampling) rate

are often connected in series, leading to typical runtimes of 30–45 minutes per analysis, with solvent consumption of as much as 45 mL. The effect of GPC/SEC miniaturization is summarized later in this paper.

Peak dislocations. A choice of columns can also prove important when moving down to micro scale. Using columns of different pore sizes in series has disadvantages compared to multiporous stationary phases.

Figure 1 illustrates the calibration curves using polystyrene standards for three different individual columns with various pore sizes: 50Å (blue), 100Å (red), and 500Å (green). The point at which the calibration curves overlap can cause problems with peak shapes. The Agilent PlusPore columns feature mixed, multiporous stationary phases (gray). These columns enable extremely linear calibrations.

The example shown in **Figure 2** illustrates when individual pore-size columns overlap, it can lead to peak "dislocations," which can lead to misinterpretation of the sample distribution and the calculated molecular weight. Hence, such experimental artifacts can be eliminated by using multiporous columns.

Silica-based packing materials of less than 3 µm could yield increased performance. However, problems with shear degradation and deformation could arise and also interaction between the polymer and stationary phase could take place. Therefore, it is important that the sample is not adsorbed on to the packing material, which will likely cause peak tailing and inaccurate results —varying as much as 10–20% from the true value.

Identical molecular weights at different flow rates. To prove that identical molecular weight results are obtained at different flow rates, the molecular weight averages (Mp, Mw, Mn, and *Đ*) of an epoxy resin was determined (**Figure 3**). The molecular-weight distributions all show good resolution, with some degradation of the larger oligomers at the highest flow rate. However, the

Figure 1: A comparison of calibration curves generated using individual pore-size columns with multiporous GPC columns.

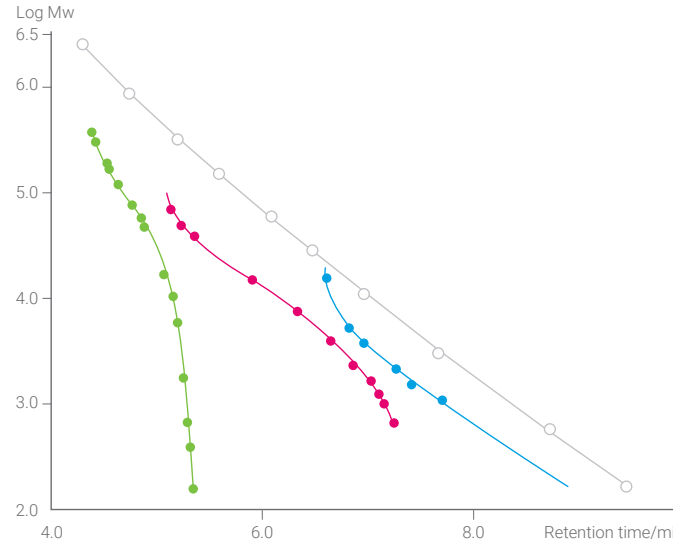
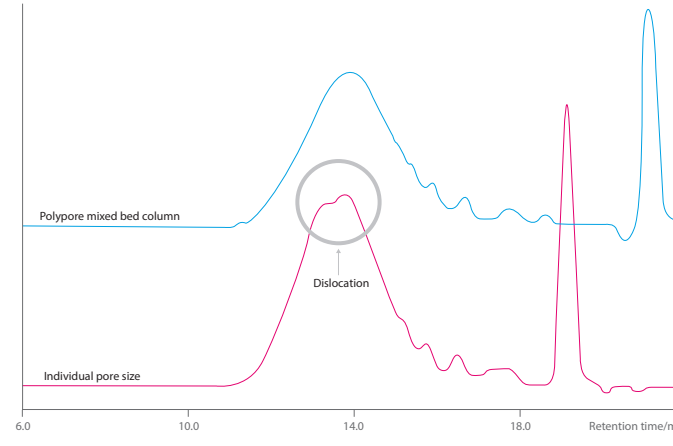


Figure 2: The effect of individual pore-size column combinations on peak shape.



> "Multiporous columns help to eliminate peak dislocation effects."

results of the calculated molecular weights are nearly identical (table in **Figure 3**).

The fast runtimes of 2 x PLgel columns (two 250 x 4.6 mm, 3 µm) gave excellent oligomeric resolution for the PS 580 part of the calibration mixture. Resolution was largely maintained at the 1.0 mL/min flow rate, where runtime was less than six minutes. An example is shown in **Figure 4**.

Figure 3: Comparison of the molecular weight of an epoxy resin on an Agilent PLgel column (two 250 x 4.6 mm, 3 µm) at different flow rates (A) 0.3; B) 0.6; C) 1.0 mL/min tetrahydrofuran) and detection with an Agilent 1290 Infinity II Micro RID.

Flow Rate (mL/min)	RT (min)	Mp (g/mol)	Mn (g/mol)	Mw (g/mol)	Đ
0.3	11.678	7261	2614	6415	2.45
0.6	5.876	7278	2638	6340	2.40
1.0	3.544	7297	2606	6238	2.39

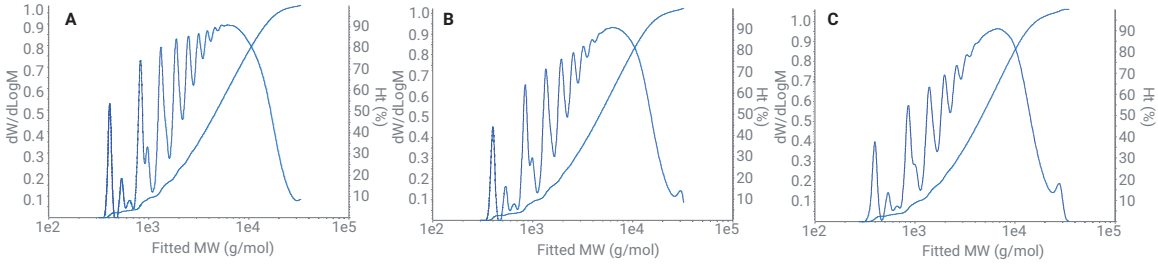


Figure 4: Comparison of the separation of a low molecular weight polystyrene mixture on PLgel columns (two 250 x 4.6 mm, 3 µm) at different flow rates (A) 0.3; B) 0.6; C) 1.0 mL/min tetrahydrofuran) and detection with an Agilent 1290 micro RID.

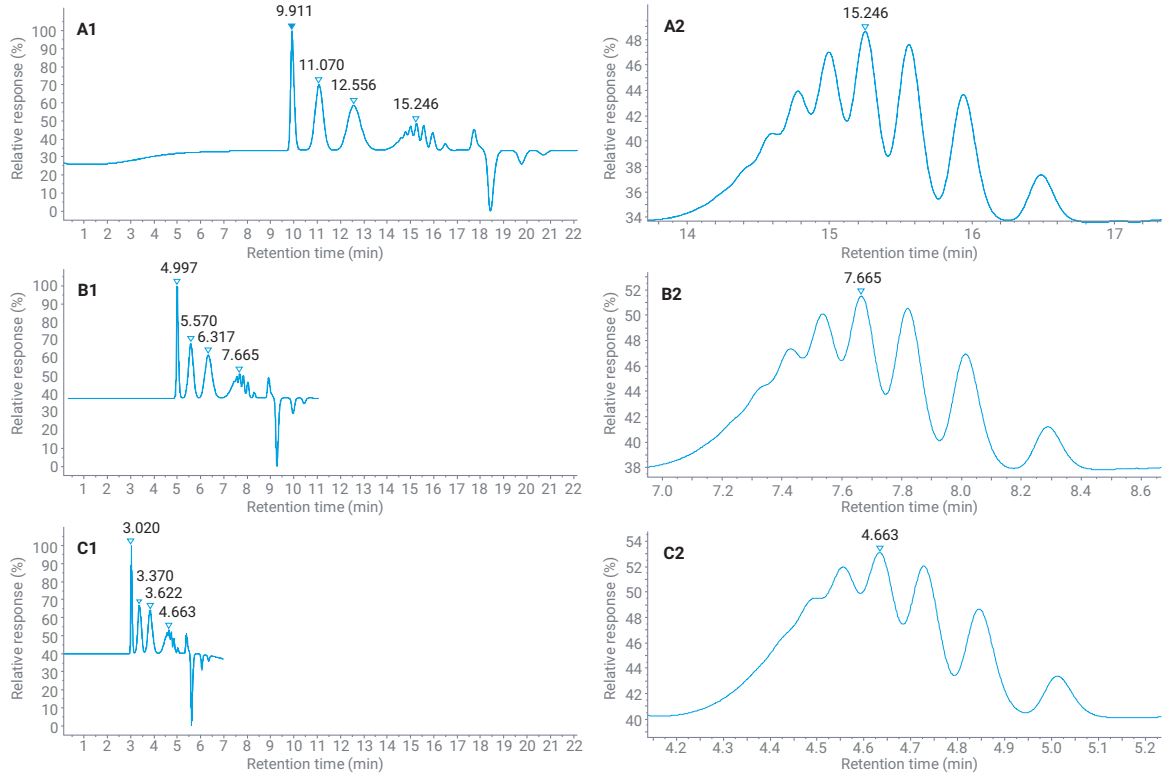


Figure 5: Polyol mixture. Data courtesy of H. Eghbali et al., *Analytical Science,* The Dow Chemical Company.

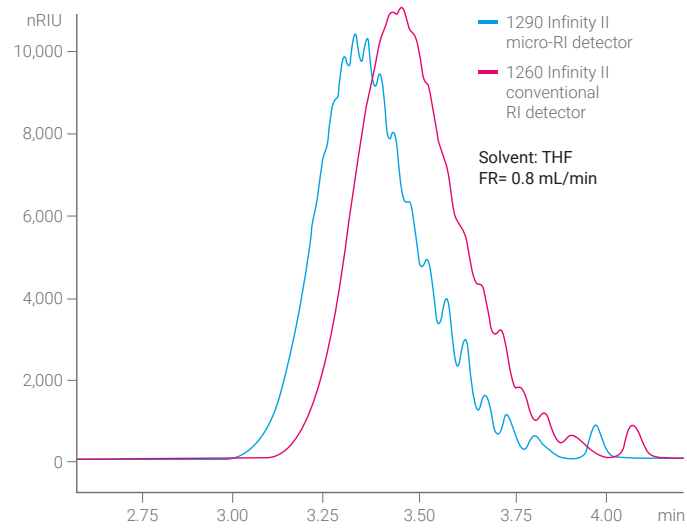
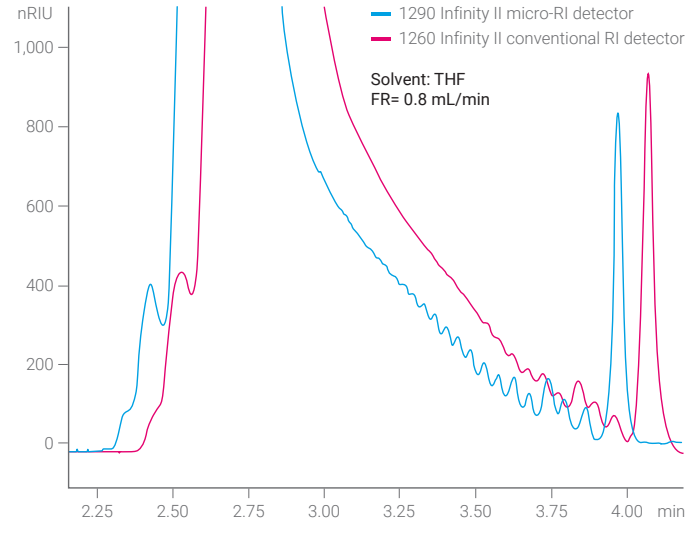


Figure 6: Polyol mixture. Data courtesy of H. Eghbali et al., *Analytical Science,* The Dow Chemical Company.



Micro separations. Since it is possible to run any type of GPC/SEC column, it is also possible to achieve very high resolution at very short retention times. However, this technique is particularly useful for oligomeric fingerprinting.

Figure 5 shows a comparison between a standard 8 µl flow cell (as in the Agilent 1260 Infinity II RID) vs. the 2 µl flow cell (as in the Agilent 1290 Infinity II RID). The improvements in resolution are clearly observed at the lower end of the molecular weight scale.

> "The high-precision solvent delivery is available at low flow rates, enabling highly reproducible results."

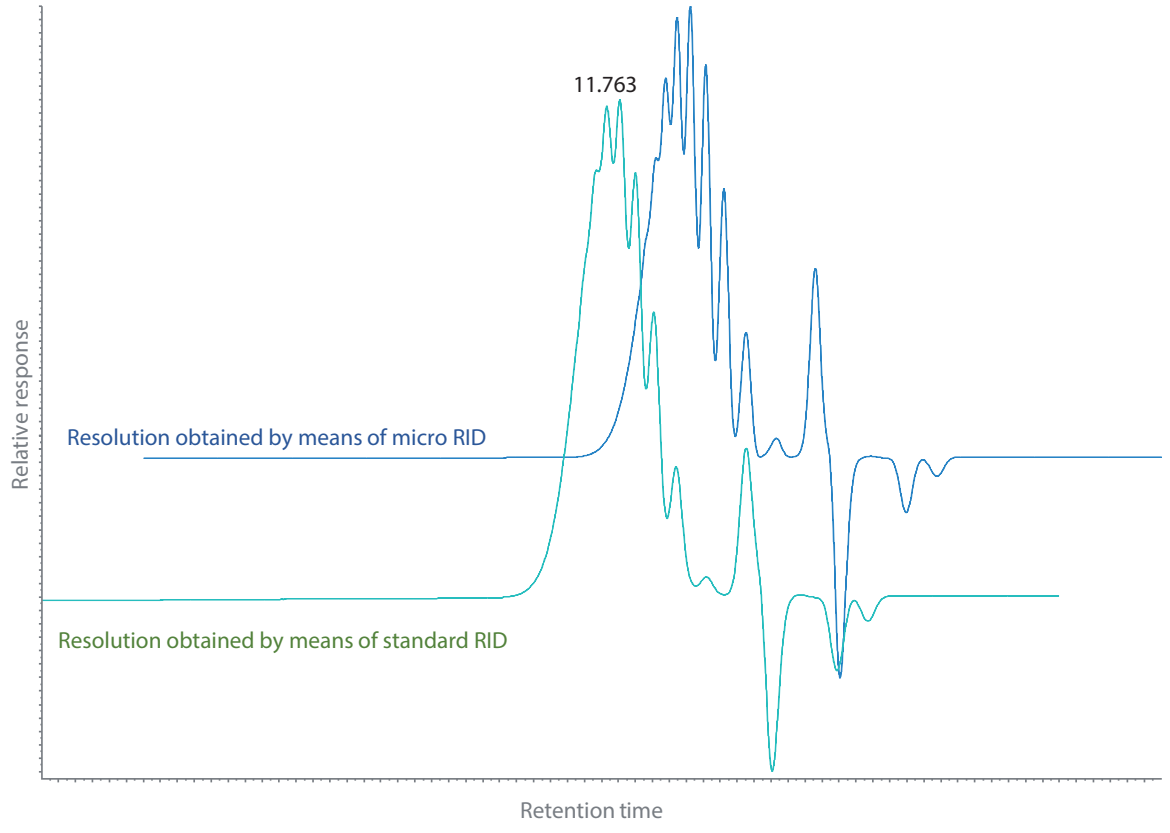
Figure 6 highlights the enhancements in performance that can be achieved by using a Micro RID. In this specific case, increased resolution can be assessed both at the higher and lower end of the molecular weight scale.

SUMMARY

The use of the Micro RID in the 1290 Infinity II GPC/SEC System enables the use of narrow-bore GPC/SEC columns with 2.1 mm ID for microscale analysis. The calculation of the applied flow rate from the previously shown 4.6 mm ID column to the 2.1 mm ID column resulted in a corresponding flow rate of 60 µL/min. The 1290 Infinity II High-Speed Pump provided the required flow accuracy to maintain retention times compared to the larger ID columns. Another advantage is the lower dispersion volume of the Micro RID cell (2 µL) compared to the standard RID (8 µL), which provides better resolution of lower molecular weight oligomers (**Figure 7**).

The PS/DVB particles packed in polypore high-performance columns are a proven technology for GPC/SEC separations, meaning that methods can be transferred with confidence without the risk of adsorption effects or other interactions between the analytes and stationary phase. Linear dynamic range is comparable with conventional RI detectors.

Figure 7: Comparison of the resolution of standard RID versus micro RID for a low molecular weight PS 580 (two Agilent OligoPore columns, 250 × 2.1 mm, 60 µL/min flow rate). Due to the stacked-display mode, there is no retention time scale shown.



> "Miniaturization to high-performance GPC/SEC columns with a 2.1 mm internal diameter allows solvent consumption to be reduced by 92% compared with 7.5-mm columns."

GPC/SEC miniaturization does require the careful control of system dispersion volume that is a feature of UHPLC instruments. In combination with a low-volume detector flow cell and high-detector data rate, miniaturized high-performance GPC/SEC columns can generate excellent resolution and faster runtimes.

Miniaturization to high-performance GPC/SEC columns with a 2.1 mm internal diameter allows solvent consumption to be reduced by 92% compared with 7.5-mm columns.

The high-precision solvent delivery is available at low flow rates, enabling highly reproducible results.

In order to get the optimal performance from the Agilent micro RI detector it is important to include ultra-low dispersion tubing 0.075 mm ID between the columns and the detector. Taking this into account the micro RI detector yields reduced interdetector delay and dispersion and better peak shape with respect to conventional RID; and rapid baseline stability and low noise and drift.