Single Detector Gel Permeation Chromatography for Precise Molar Mass Determination of Polymers in Multiple Solvents

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Objective

- To demonstrate the advantages of using a dual flow differential refractive index (RI) detector for single detector GPC experiments, *e.g.* peak position calibration.
- To highlight the advantages of replacing a conventional RI detector with a dual flow RI detector for the determination of molar mass averages and distributions.
- To compare the baseline stability and precision of molar mass averages when a dual flow RI detector is coupled to both conventional and semi-micro GPC columns.

Introduction

- Since its inception the main utility of GPC has been to extract quantitative information in the form of molar mass averages and distributions of both synthetic and biopolymers with accuracy and precision.[1]
- Traditionally molar mass averages and distributions are obtained via a peak position calibration involving a series of linear narrow polydisperse standards of known molar mass and chemistry analyzed by GPC coupled to a differential refractive index detector (RI).

Introduction

- One major caveat of single detector GPC is the baseline stability of the RI detector. For peak position calibration, a drift in the RI baseline has been shown to drastically affect the accuracy and precision of molar mass averages and distributions.[2-4]
- Poor RI baseline stability results in uncertainty of baseline height and peak start and end points, as well as non-linear or unleveled baseline fitting, which in return results in errors ranging from 2%-25% in the determination of molar mass averages.[2-5]

Refractive Index (RI) Detectors

A conventional RI detector is constructed in such a way that there are two sides:

1. a reference side consisting of a stagnant pure solvent
2. the sample side, containing a flowing stream of analyte in the same solvent as in the reference side

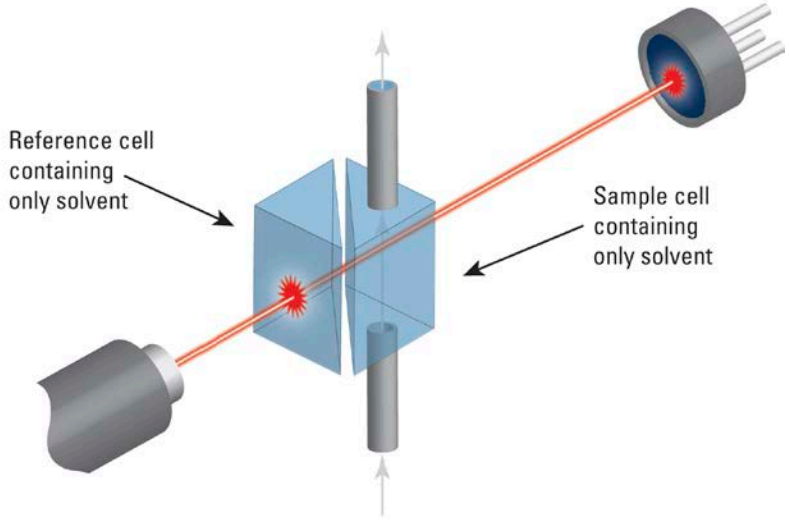


Figure 1: Depiction of a conventional RI detector flow cell when the contents of the reference and sample sides have the same refractive indices as each other, *i.e.*, both sides contain pure solvent only.

Refractive Index (RI) Detectors

Under ideal conditions:

1. When the contents of the reference and sample sides of the flow cell have the same refractive indices as each other, the photodiodes will produce equal signals.
2. When the contents of the reference and sample sides of the flow cell are different, *e.g.* have difference refractive indices, a voltage difference will result between the photodiodes.

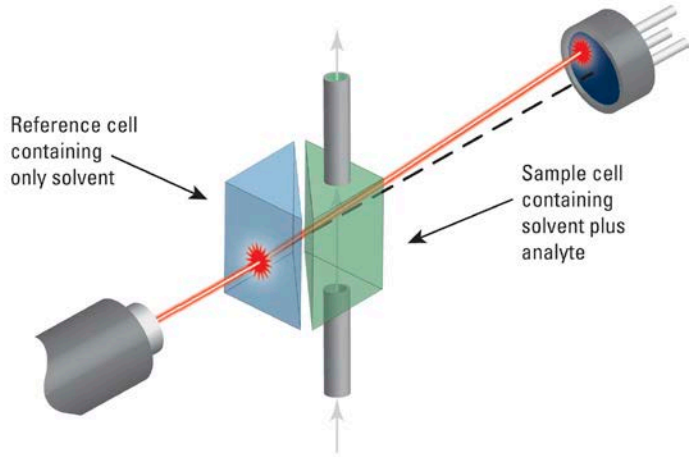


Figure 2: Depiction of a conventional RI detector flow cell when the contents of the reference and sample sides have different refractive indices as each other, *i.e.,* the reference cell contains pure solvent and the sample cell contains a dilute polymer solution.

Refractive Index (RI) Detectors

Typically:

The refractive index of an organic solvent slowly changes over time, resulting in a difference in the contents of the reference and sample sides of the flow cell thus causing a drifting RI baseline due to the slight difference in refractive indices and voltage between the photodiodes.

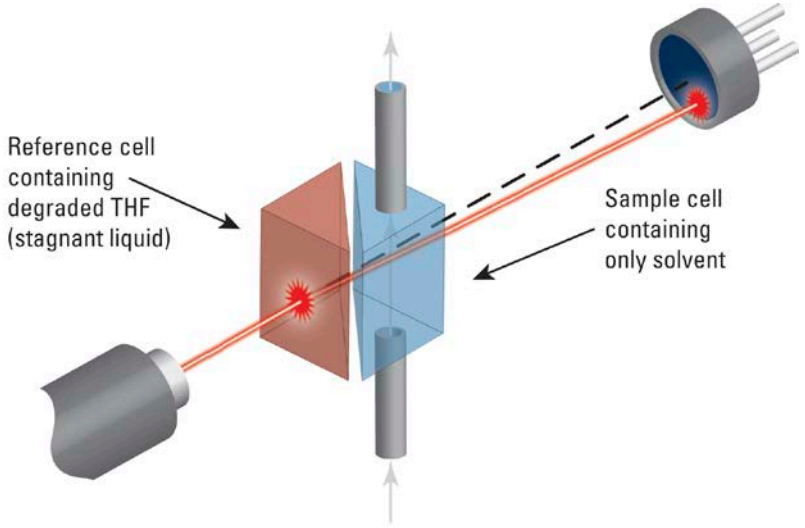


Figure 3: Depiction of a conventional RI detector flow cell showing the effects of THF degradation in the reference cell. Over time the reference side, consisting of stagnant pure solvent, will slowly change - resulting in baseline drift.

Refractive Index (RI) Detectors

A dual flow RI detector, such as that in the EcoSEC® GPC System, is constructed in such a way that there are two sides:

1. a reference side, consisting of a flowing stream of pure solvent
2. the sample side, containing a flowing stream of analyte in the same solvent as in the reference side

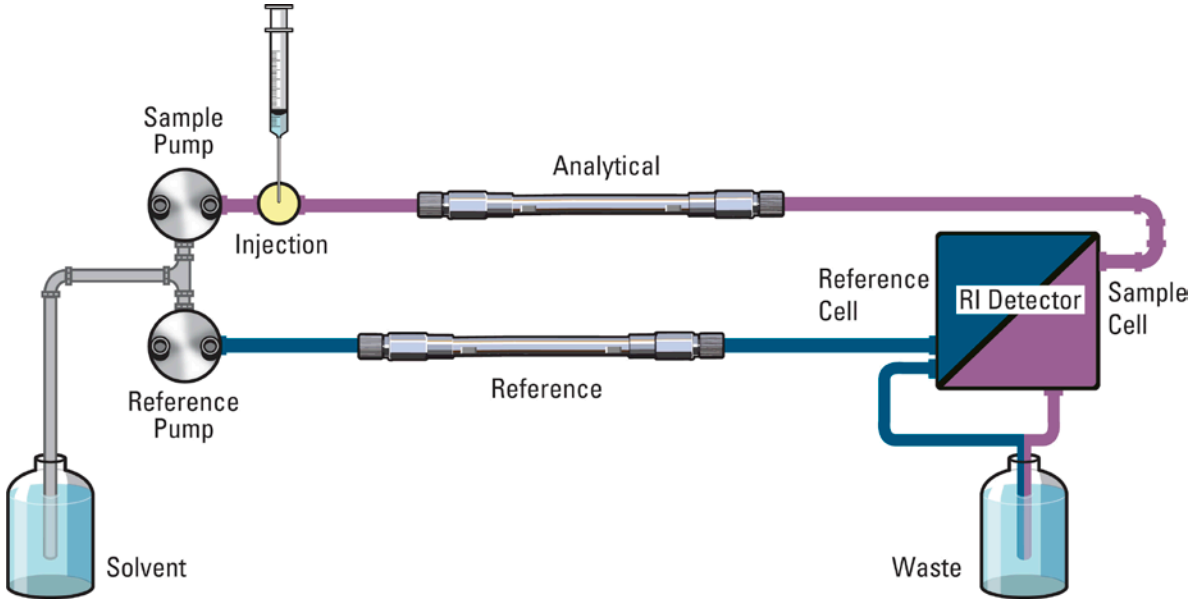


Figure 4A: Depiction of the flow paths in the EcoSEC GPC System, showing the dual flow RI detector flow cell when the contents of the reference and sample sides have different refractive indices as each other.

Refractive Index (RI) Detectors

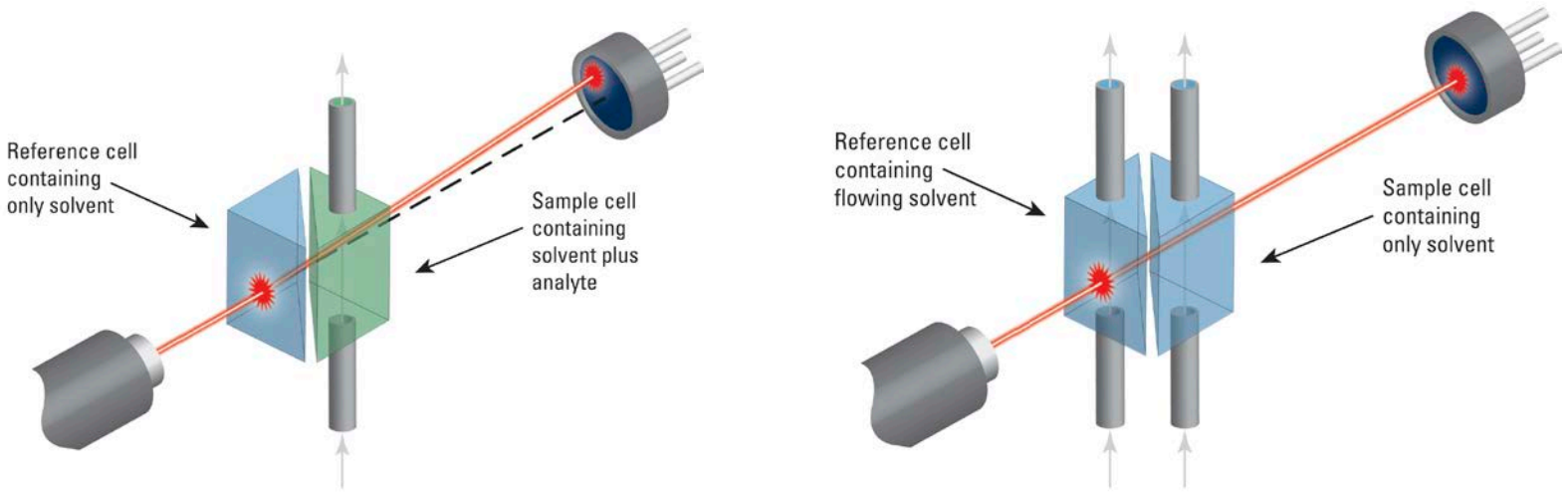


Figure 4B: Depiction of a dual flow RI detector flow cell showing the compensation of the changes in refractive index of the solvent over time.

Experimental

Instrumentation:

- EcoSEC GPC System (HLC-8320) equipped with a dual flow refractive index detector
- Modular HPLC or GPC system with an external conventional refractive index detector

Materials:

- Polystyrene standards, ranging in molar mass from 266 to 2.89×10^6 g/mol, with M_w/M_n = 1.01 (Tosoh Bioscience LLC)
- Dicyclohexyl phthalate, 99% pure (Aldrich Chemical)
- Uninhibited tetrahydrofuran (THF) (Fisher Chemical)
- Chloroform, dichloromethane, and hexafluoroisopropanol (HFIP) (Fisher Chemical, VWR, and Fluka Analytical, respectively)
- N,N,-Dimethylacetamide (DMAc) 99%, lithium bromide (LiBr) 99.9%, and tetraethylammonium bromide (Alfa Aesar)

Experimental

- For equal comparison between the dual flow and conventional RI detectors, all experiments were performed for both semi-micro and conventional GPC columns.
- The dual flow RI detector is housed within the EcoSEC GPC System, an all-in-one system engineered for low volume by reduced tubing lengths, low dead volume flow cells, and small stroke pumps, allowing the system to maintain the efficiency of semi-micro (4.6 mm ID × 15 cm) and conventional (7.8 mm ID × 30 cm) GPC columns.
- The conventional RI detector is coupled to a modular HPLC or GPC system optimized for the use of conventional GPC columns.

Baseline Stability and Molar Mass Precision for Polymers in THF

Experimental

Instrumentation:	EcoSEC GPC System (HLC-8320) equipped with a dual flow refractive index detector Modular HPLC or SEC system with an external conventional refractive index detector
Columns:	TSKgel® SuperMultiporeHZ-M, 4 µm, 4.6 mm ID × 15 cm × 2 + guard column TSKgel GMHXL-L, 6 µm, 7.8 mm ID × 30 cm + guard column
Solvent/ mobile phase:	THF
Flow rate:	0.35 and 1.0 mL/min
Temperature:	40 °C (pump and column ovens and RI detector in the EcoSEC GPC System) 40 °C (column oven and RI detector for modular system)

Figure 5A: Comparison of Baseline Drift of a Dual Flow Refractive Index Detector and Conventional Refractive Index Detector using Semi-micro GPC Columns

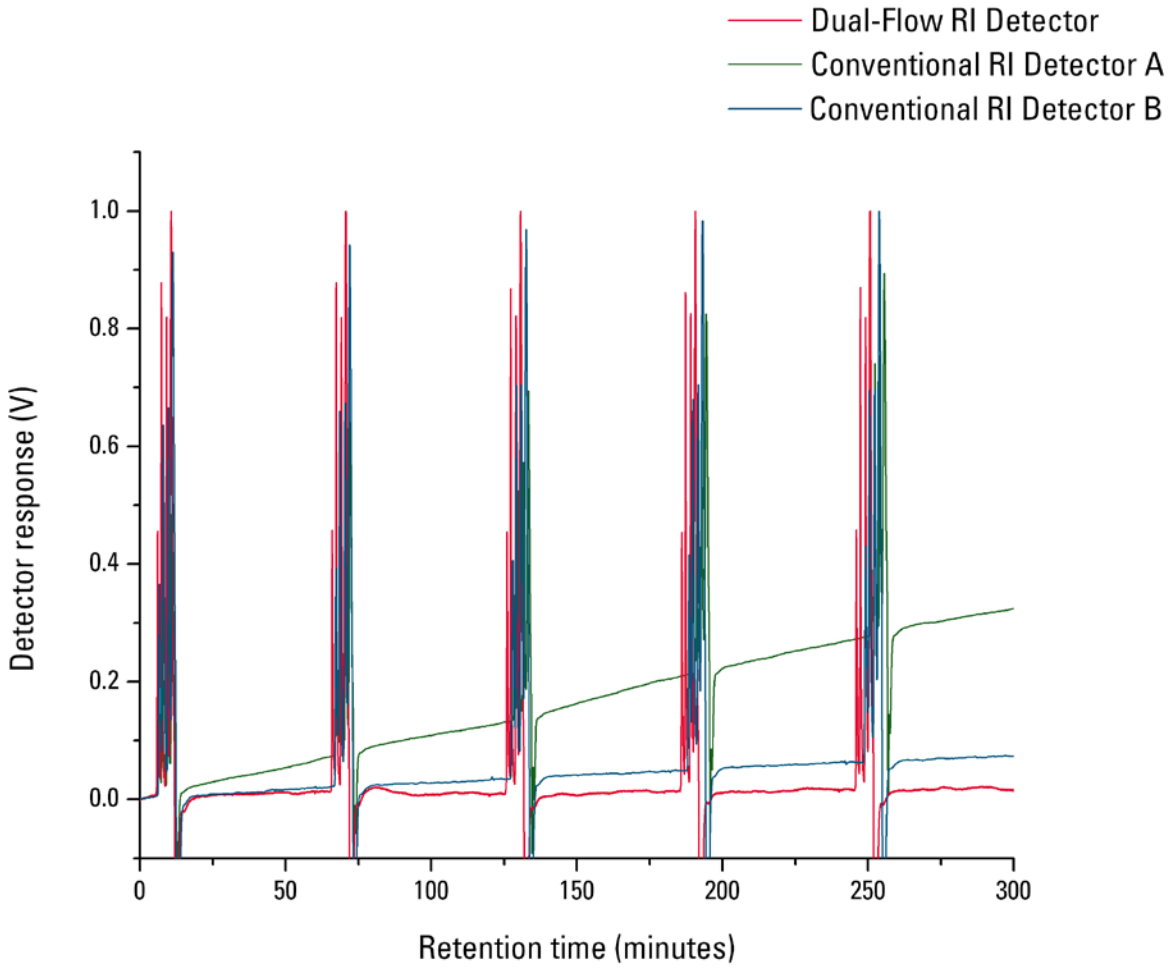
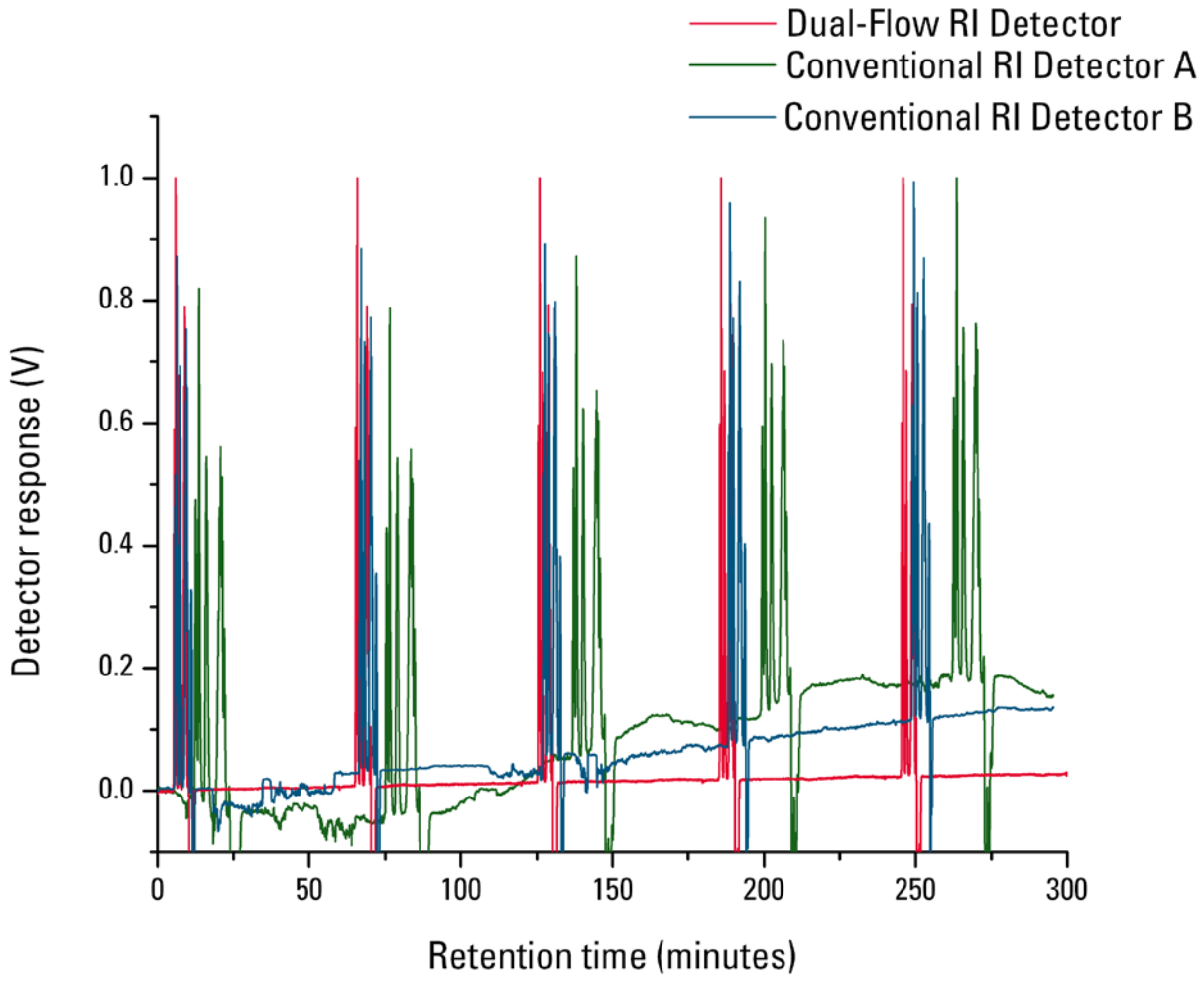


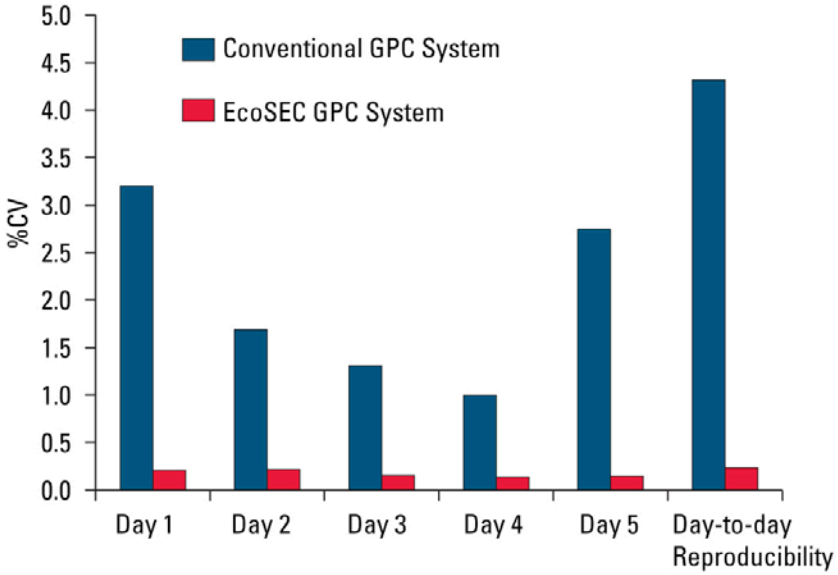
Figure 5B: Comparison of Baseline Drift of a Dual Flow Refractive Index Detector and Conventional Refractive Index Detector using Conventional GPC Columns



Figures 5A & 5B Conclusions

- As shown in Figures 5A & 5B, five consecutive injections of polystyrene standards, on semi-micro GPC columns at 0.35 mL/min and conventional GPC columns at 1.0 mL/min, with run times deliberately extended to one hour without auto zeroing the detector between injections for a total of five hours, resulted in an extremely stable baseline with low baseline drift on the dual flow RI detector and a significantly drifting baseline on the two conventional RI detectors for both column lengths.
- In comparison to the conventional GPC systems, the EcoSEC GPC System has both lower baseline drift and a better signal to noise ratio.

Figure 6: Comparing M_w Reproducibility of a Dual Flow Refractive Index Detector to that of a Conventional Refractive Index Detector



TSKgel SuperMultiporeHZ-M, 4.6 mm ID × 15 cm, × 2

Mobile phase:	THF
Flow rate:	0.35 mL/min
Temperature:	40° C
Injection vol.:	10 µL
Samples:	poly(vinyl chloride-co-vinyl acetate)

Figure 6 Conclusions

- The repeatability and reproducibility of the molar mass averages as obtained via dual flow and conventional RI detectors were compared.
- The reproducibility of the weight-average molar mass, M_w, of the dual flow RI detector was determined to be superior by a factor of 3 to that of a conventional RI detector.
- Additionally, the day-to-day reproducibility and repeatability for the determination of molar mass averages was shown to vary by less than 0.5% for the dual flow RI detector, while the conventional RI detector produced day-to-day variations in molar mass averages between 1% and 3%.

Baseline Stability and Molar Mass Precision for Polymers in Neat Solvents

Experimental

Instrumentation:	EcoSEC GPC System (HLC-8320) equipped with a dual flow refractive index detector
Columns:	TSKgel SuperHZM-M, 3 & 5 µm, 4.6 mm ID × 15 cm × 2 + guard column
Solvent/ mobile phase:	chloroform
Flow rate:	0.35 mL/min
Temperature:	40 ^{o}C (pump and column ovens and RI detector in the EcoSEC GPC System)

Figure 7: Baseline Drift of a Dual Flow Refractive Index Detector using Semi-micro GPC Columns in Chloroform

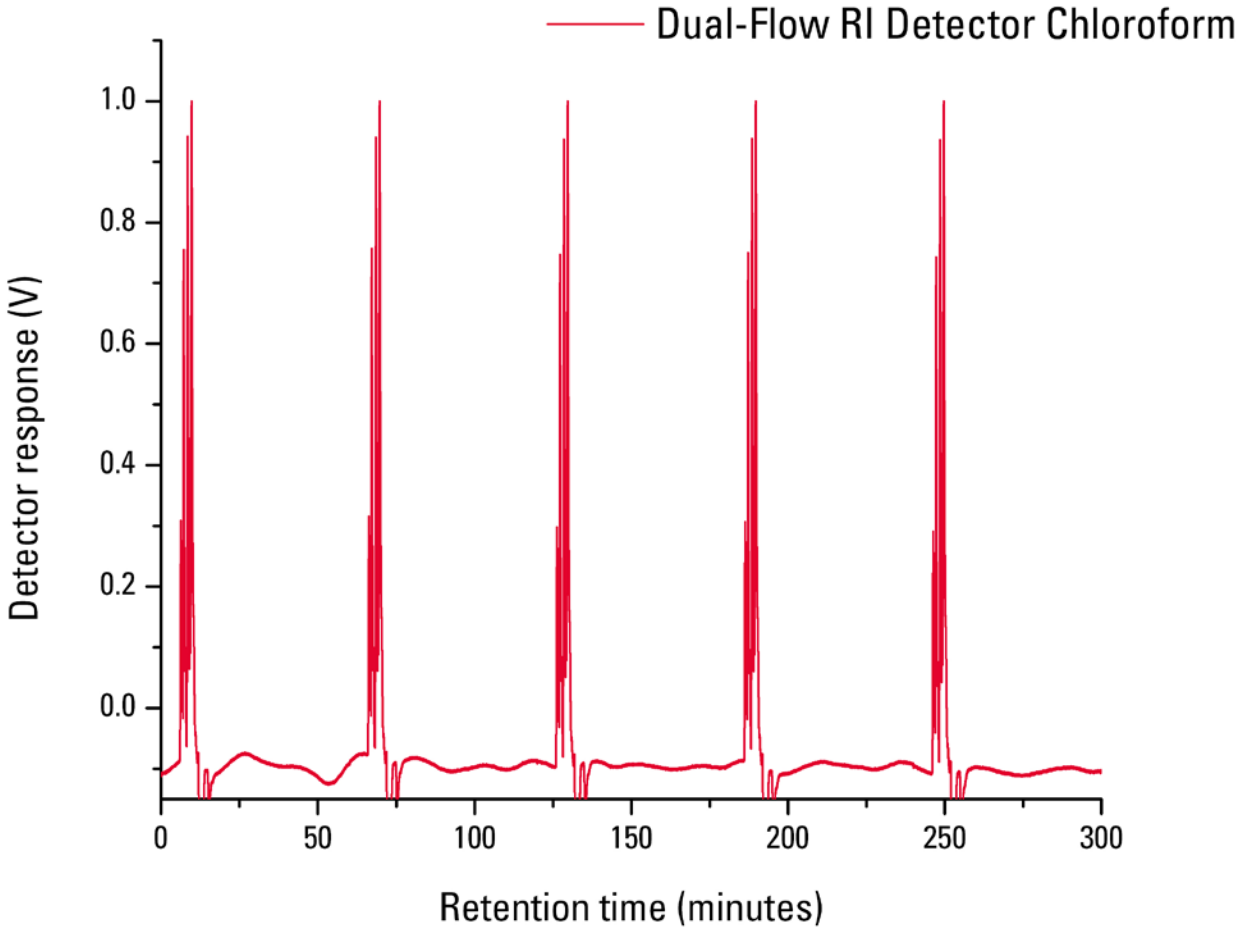
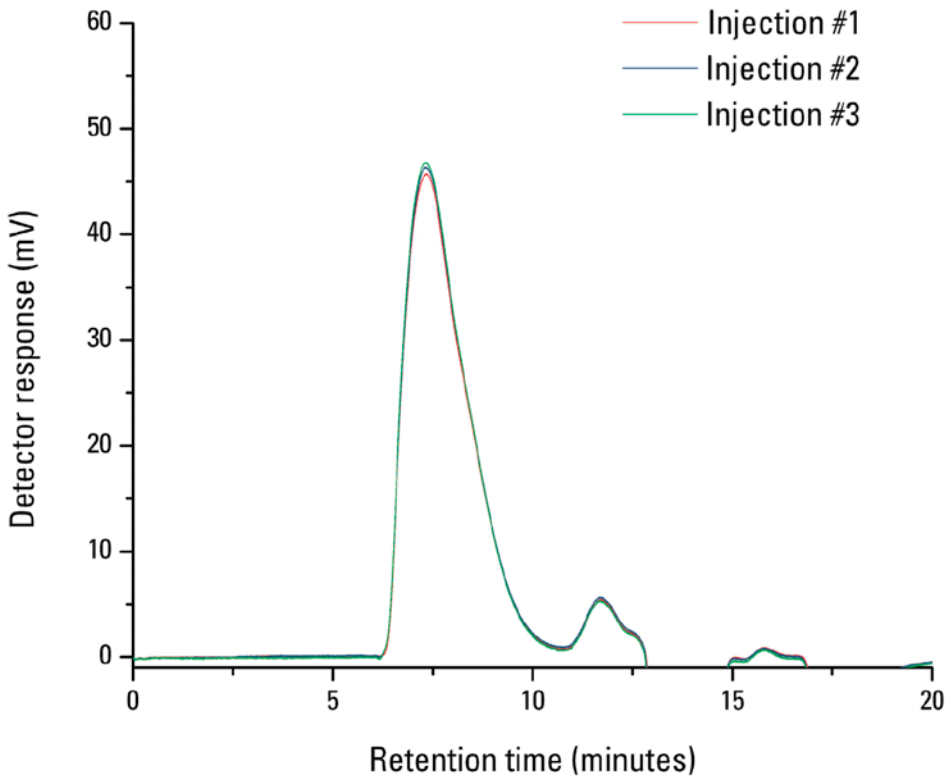


Figure 7: Conclusions

As shown in Figure 7, five consecutive injections of polystyrene standards in chloroform, on semi-micro GPC columns at 0.35 mL/min, with run times deliberately extended to one hour without auto zeroing the detector between injections for a total of five hours, resulted in an extremely stable baseline with low baseline drift on the dual flow RI detector.

Figure 8: Baseline Stability of a Dual Flow Refractive Index Detector in Chloroform



TSKgel SuperHZM-M, 4.6 mm ID × 15 cm × 2

Mobile phase:	chloroform
Flow rate:	0.30 mL/min
Temperature:	35° C
Injection vol.:	25 µL
Sample:	poly-hydroxybutyrate

Table 1: Molar Mass Reproducibility of a Dual Flow Refractive Index Detector in Chloroform

Injection Number	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)
1	2.10×10^5	1.04×10^6	1.99×10^6
2	2.00×10^5	1.04×10^6	2.03×10^6
3	2.08×10^5	1.05×10^6	2.08×10^6
Average	2.06×10^5	1.04×10^6	2.03×10^6
Standard Deviation	$\pm 0.05 \times 10^5$	$\pm 0.05 \times 10^6$	$\pm 0.04 \times 10^6$
% CV	2.56	0.55	2.22

Figure 8 and Table 1 Conclusions

- The reproducibility and reliability of a dual flow refractive index detector in chloroform is shown in Figure 8 through the overlay of three consecutive injections of a poly-hydroxybutyrate sample.
- The preciseness of the molar mass averages for the poly-hydroxybutyrate sample in chloroform is shown in Table 1. The coefficients of variations for all molar mass determinations were less than 2.6%. Most importantly the coefficients of variations for the weight average molar mass, $M_{w,}$ (the most highly characterized average) was well below 1%.

Baseline Stability and Molar Mass Precision for Polymers in Mixed Solvents

Experimental

Instrumentation:	EcoSEC GPC System (HLC-8320) equipped with a dual flow refractive index detector
Columns:	TSKgel SuperHM-H, 3 µm, 6 mm ID × 15 cm × 2 + guard column
Solvent/ mobile phase:	95:5 Dichloromethane:HFIP with 5 mmol/L tetraethylammonium bromide
Flow rate:	0.35 mL/min
Temperature:	40 °C (pump and column ovens and RI detector in the EcoSEC GPC System)

Figure 9: Baseline Drift of a Dual Flow Refractive Index Detector using Semi-micro GPC Columns in 95:5 Dichloromethane:HFIP

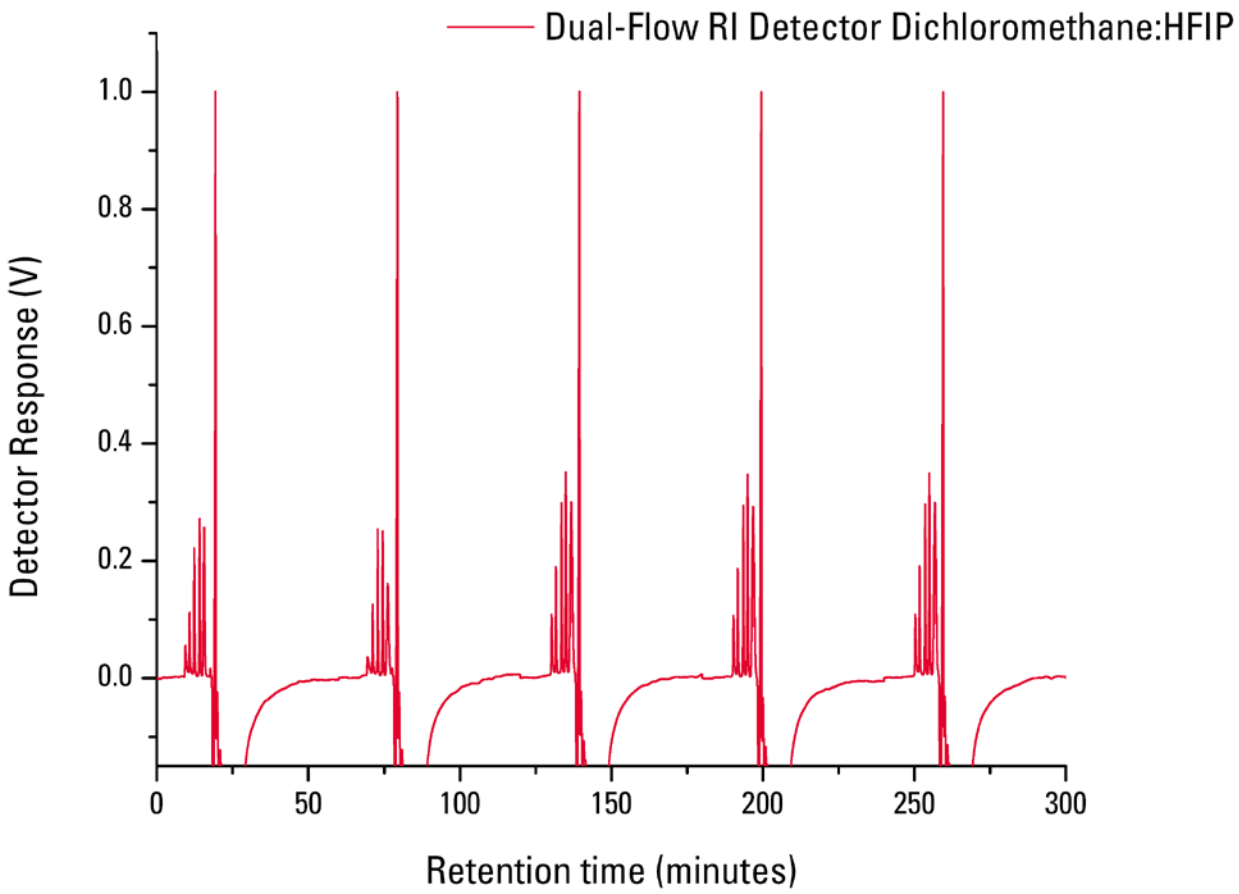
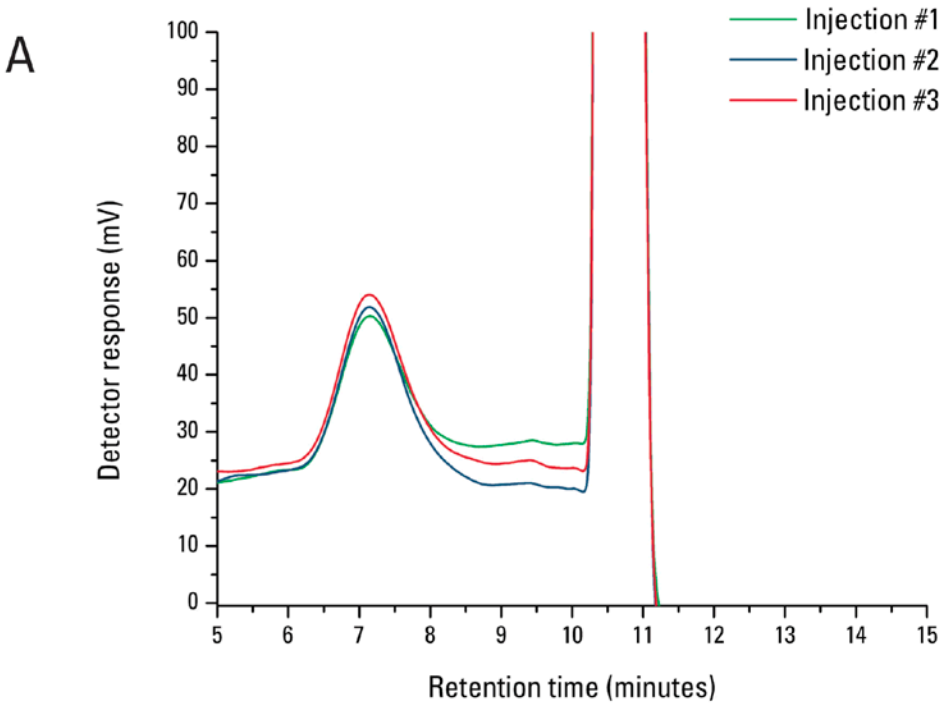


Figure 9: Conclusions

As shown in Figure 9, five consecutive injections of polystyrene standards in 95:5 Dichloromethane:HFIP with 5 mmol/L tetraethylammonium bromide, on semi-micro GPC columns at 0.35 mL/min, with run times deliberately extended to one hour without auto zeroing the detector between injections for a total of five hours, resulted in a stable baseline with low baseline drift on the dual flow RI detector.

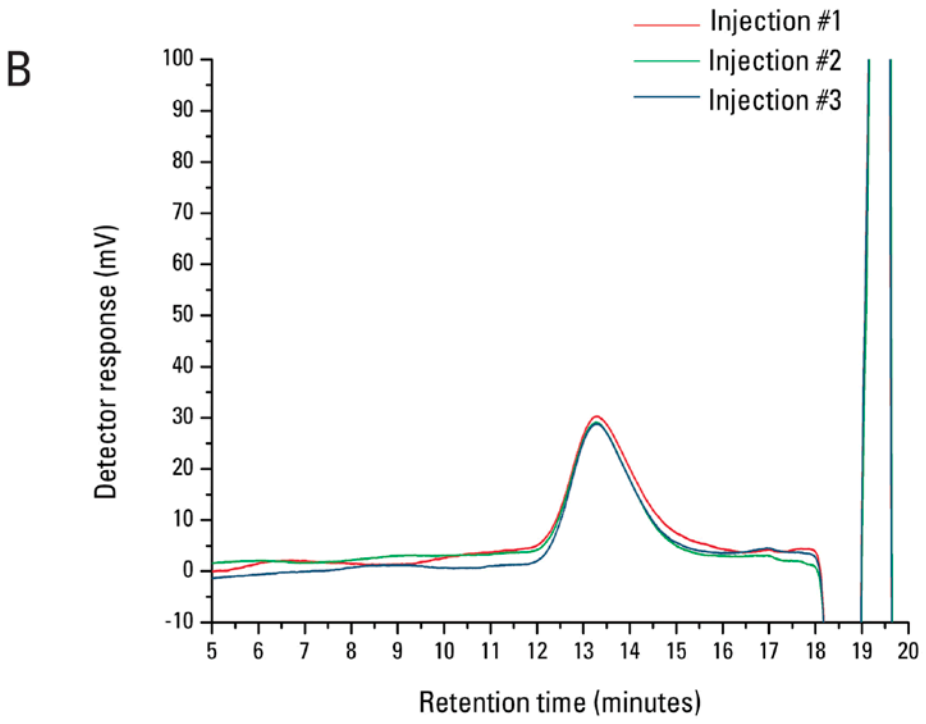
Figure 10A: Baseline Stability of a Dual Flow Refractive Index Detector using Conventional GPC Columns in 95:5 Dichloromethane:HFIP



TSKgel GMH$_{HR}$-M, 7.8 mm ID × 30 cm

Mobile phase:	95:5 Dichloromethane:HFIP
Flow rate:	1.0 mL/min
Temperature:	35° C
Injection vol.:	150 µL
Sample:	copolyester

Figure 10B: Baseline Stability of a Dual Flow Refractive Index Detector using Semi-micro GPC Columns in 95:5 Dichloromethane:HFIP



TSKgel SuperHM-H, 6.0 mm ID × 15 cm × 2

Mobile phase:	95:5 Dichloromethane:HFIP
Flow rate:	0.35 mL/min
Temperature:	35° C
Injection vol.:	10 µL
Sample:	copolyester

Table 2: Molar Mass Reproducibility of a Dual Flow Refractive Index Detector in 95:5 Dichloromethane:HFIP

Injection Number	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)
1	3.29×10^4	5.49×10^4	8.22×10^4
2	3.32×10^4	5.54×10^4	8.28×10^4
3	3.28×10^4	5.48×10^4	8.18×10^4
Average	3.29×10^4	5.51×10^4	8.23×10^4
Standard Deviation	$\pm 0.02 \times 10^4$	$\pm 0.03 \times 10^4$	$\pm 0.05 \times 10^4$
% CV	0.55	0.68	0.63

Figures 10A, 10B and Table 2 Conclusions

- Typically, mixed solvent systems such as 95:5 Dichloromethane:HFIP wreak havoc on single detector GPC systems as they decrease the reproducibility of the molar mass averages and stability of the RI baseline. Figures 10A and 10B show the baseline stability of a dual flow RI detector for both conventional and semi-micro GPC columns.
- Table 2 confirms the stability of the dual flow RI detector in Dichloromethane:HFIP, as the molar mass averages obtained for a polyester sample in Dichloromethane:HFIP have coefficients of variation well below 1%.
- A dual flow RI detector increases the reproducibility of the molar mass averages obtained for polymers dissolved in mixed solvent systems by increasing the stability of the RI baseline.

Baseline Stability and Molar Mass Precision for Polymers in Complex Solvents

Experimental

Instrumentation:	EcoSEC GPC System (HLC-8320) equipped with a dual flow refractive index detector
Columns:	TSKgel SuperHZM-H, 3 & 5 µm, 6 mm ID × 15 cm × 2 + guard column
Solvent/ mobile phase:	DMAc or DMF with 0.02 mol/L LiBr
Flow rate:	0.35 mL/min
Temperature:	40 °C (pump and column ovens and RI detector in the EcoSEC GPC System)

Figure 11: Baseline Drift of a Dual Flow Refractive Index Detector using Semi-micro GPC Columns in DMAc with 0.02 mol/L LiBr

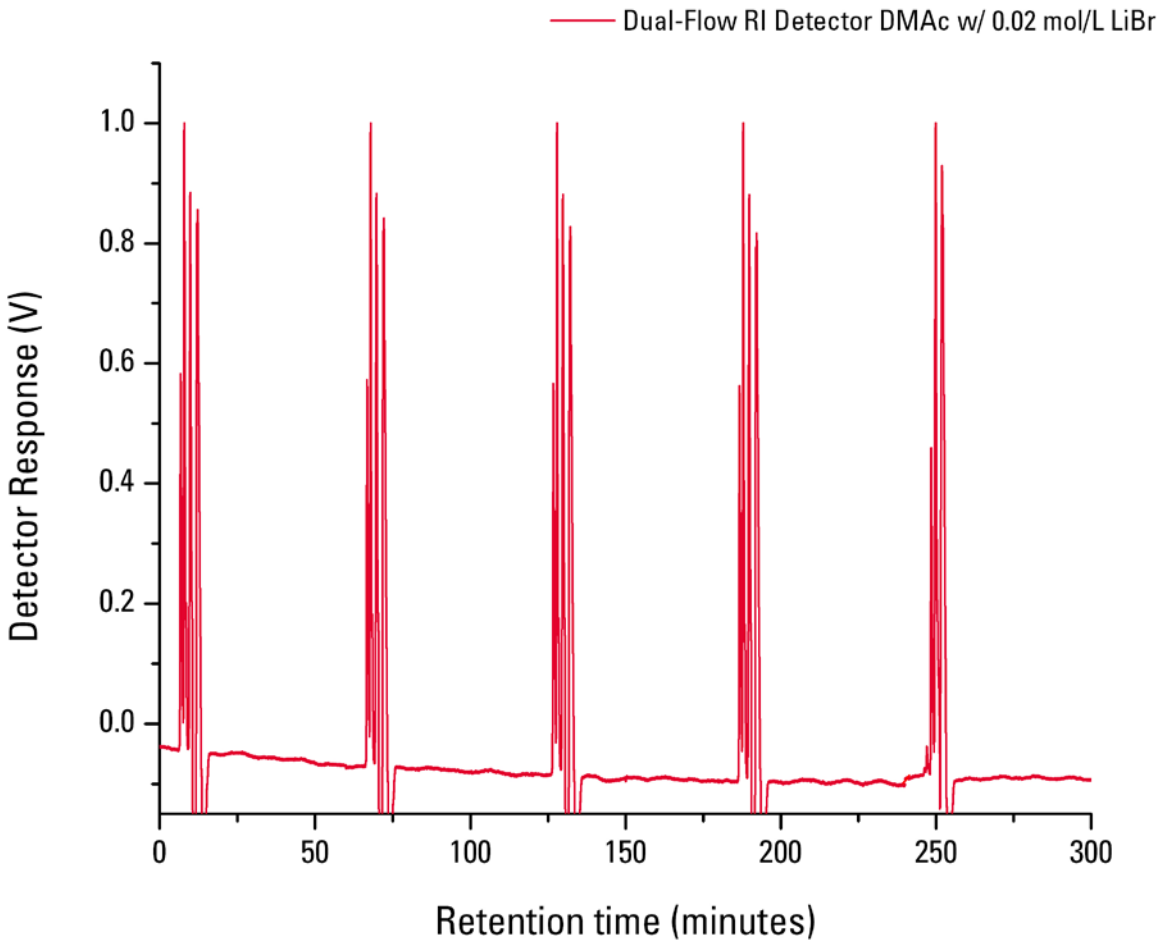
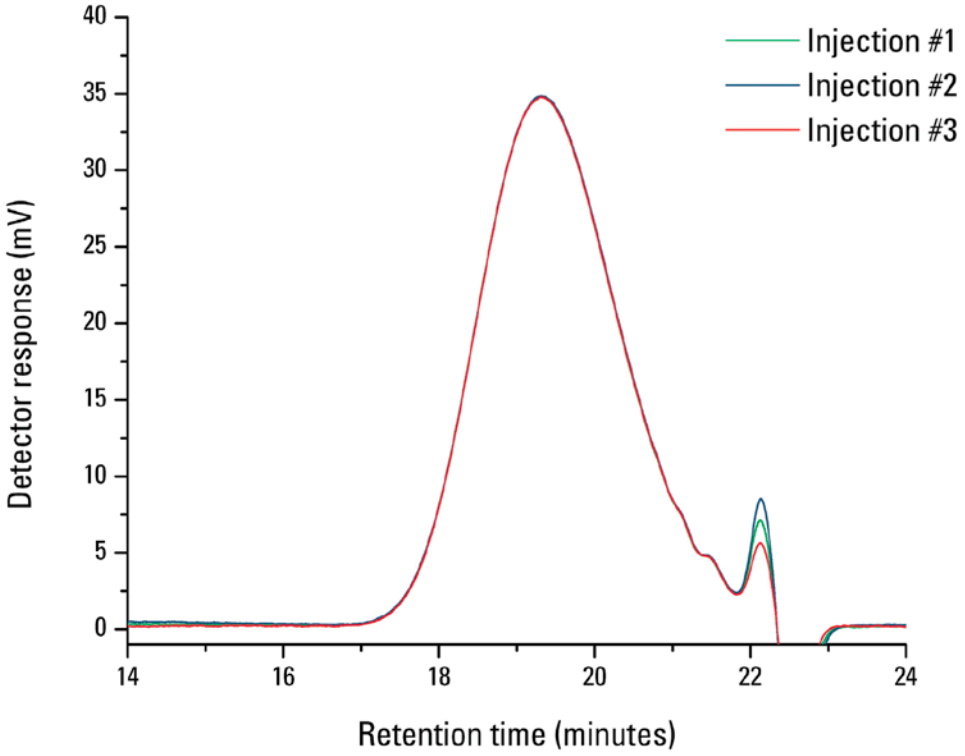


Figure 11 Conclusions

As shown in Figure 11, five consecutive injections of polystyrene standards in DMAc with 0.02 mol/L LiBr, on semi-micro GPC columns at 0.35 mL/min, with run times deliberately extended to one hour without auto zeroing the detector between injections for a total of five hours, resulted in a stable baseline with low baseline drift on the dual flow RI detector.

Figure 12: Baseline Stability of a Dual Flow Refractive Index Detector in DMF with 5 mmol/L LiBr



TSKgel GMHXL, 7.8 mm ID × 30 cm × 2

Mobile phase:	DMF with 5 mmol/L LiBr
Flow rate:	1.0 mL/min
Temperature:	40° C
Injection vol.:	100 µL
Sample:	polyimide

Table 3: Molar Mass Reproducibility of a Dual Flow Refractive Index Detector in DMF with 5 mmol/L LiBr

Injection Number	M_n (g/mol)	M_w (g/mol)	M_z (g/mol)
1	1.86×10^4	2.86×10^4	3.95×10^4
2	1.86×10^4	2.87×10^4	3.96×10^4
3	1.86×10^4	2.86×10^4	3.94×10^4
Average	1.86×10^4	2.87×10^4	3.95×10^4
Standard Deviation	$\pm 0.04 \times 10^4$	$\pm 0.05 \times 10^4$	$\pm 0.08 \times 10^4$
% CV	0.21	0.19	0.20

Figure 12 and Table 3 Conclusions

- Highly reproducible data is needed to observe subtle molar mass distribution trends from various synthetic routes for polymers.
- The high precision of the molar mass averages obtained from a dual flow RI detector in complex solvent systems is shown in Figure 12 and Table 3 from three consecutive injections of a polyimide sample in DMF with 5 mmol/L LiBr using conventional GPC columns. The molar mass averages have a coefficient of variation around 0.20%.

Conclusions

- A stable RI detector baseline is required for successful experiments and, more importantly, repeatable and reproducible molar mass averages.
- Extreme care must be taken when molar mass averages and distributions are determined via peak position calibration by GPC coupled to a RI detector, as uncertainties and instabilities in the RI baseline can result in relatively large errors, inconsistencies, and deviations in molar mass averages and distributions.

Conclusions continued

- The repeatability and reproducibility of the molar mass averages were shown to increase greatly when a conventional RI detector was replaced with a dual flow RI detector.
- The dual flow RI detector has unmatched baseline stability, excellent retention time reproducibility, and day-to-day consistency compared to conventional RI detectors for polymers in neat, mixed, and complex solvent systems.
- A dual flow RI detector is ideal for single detector GPC experiments which rely on accurate and precise instrumentation and multi-detector GPC experiments which require excellent baseline stability and consistent instrumentation.

References

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