Increased Precision in Polymer Analysis via Single-Detector Size Exclusion Chromatography

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Objective

- To demonstrate the advantages of using a dual-flow differential refractive index detector for single detector SEC experiments, *e.g.,* peak position calibration.
- To show that a dual-flow RI detector coupled to conventional and semi-micro SEC columns provides superb baseline stability and excellent precision in the determination of molar mass averages for polymers in neat, mixed, and complex solvent systems.
- To compare the repeatability, reproducibility, and baseline stability of a dual-flow differential refractive index detector with a conventional refractive index detector for calculations of molar mass averages via size exclusion chromatography (SEC).

Introduction

- Size exclusion chromatography (SEC) is the most widely accepted and used analytical method for obtaining molar mass averages and distributions of both synthetic and biopolymers.[1]
- Since its inception, the main utility of SEC has been to extract quantitative information from the elution curves with accuracy and precision.
- Traditionally, molar mass averages and distributions are obtained via a peak position (calibrant-relative) calibration involving a series of linear, narrow polydisperse standards of known molar mass and chemistry analyzed by SEC coupled to a differential refractive index (RI) detector.
- As new instrumentation evolves, there are many different configurations of additional detectors being coupled to SEC/RI, *e.g.*, static light scattering and differential viscometry.
- In the context of SEC, for simple polymers, single detector systems continue to be heavily employed as they provide excellent day-to-day reproducibility and are ideal for quality control procedures.

Introduction Continued

- One caveat to single detector SEC 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 baseline stability results in the uncertainty of baseline height and peak start and end points, as well as non-linear or unleveled baseline fitting, which in turn results in errors ranging from 2% to 25% in the determination of the number, weight, and *z*-average molar masses, M_n, M_w, and M_z, respectively.[2-5]

Refractive Index (RI) Detectors

- The most common type of differential refractive index (RI) detector is a deflection-type detector employing the principles of Snell's law of refraction. In this type of detector, light emitted from a source is transmitted through the flow cell of the RI detector and strikes a detector element.
- The flow cell is constructed in such a way that there are two chambers: (1) the reference chamber and (2) the sample chamber.
- As light passes through the reference side into the sample sides, the direction in which the light is travelling is changed *e.g.,* the path is bent. The amount of bending that takes place depends on the nature of the flow cell, the wavelength of light being used, the temperature, and the concentration of analytes in the cell. The light then strikes a mirror and reflects back through the cell and lens to the detector, which consists of either two photodiodes mounted on a single chip or of a photodiode array.

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Refractive Index (RI) Detectors Continued

- The photodiodes will produce equal signals, if the contents of the reference and sample chambers have the same refractive indices as each other.
- In contrast, if the reference and sample chambers have different refractive indices, a voltage difference will result between the photodiodes.
- The difference in refractive indices between the two chambers produces a voltage difference proportional to the concentration of the analyte in solution at the particular eluting slice.
- The difference between a conventional and dual-flow RI detector is in the construction of the RI flow cell.

Refractive Index (RI) Detectors Continued

- The flow cell in a conventional RI detector is constructed in such a way that there are two sides:

  (1) the reference side consisting of *stagnant* pure solvent

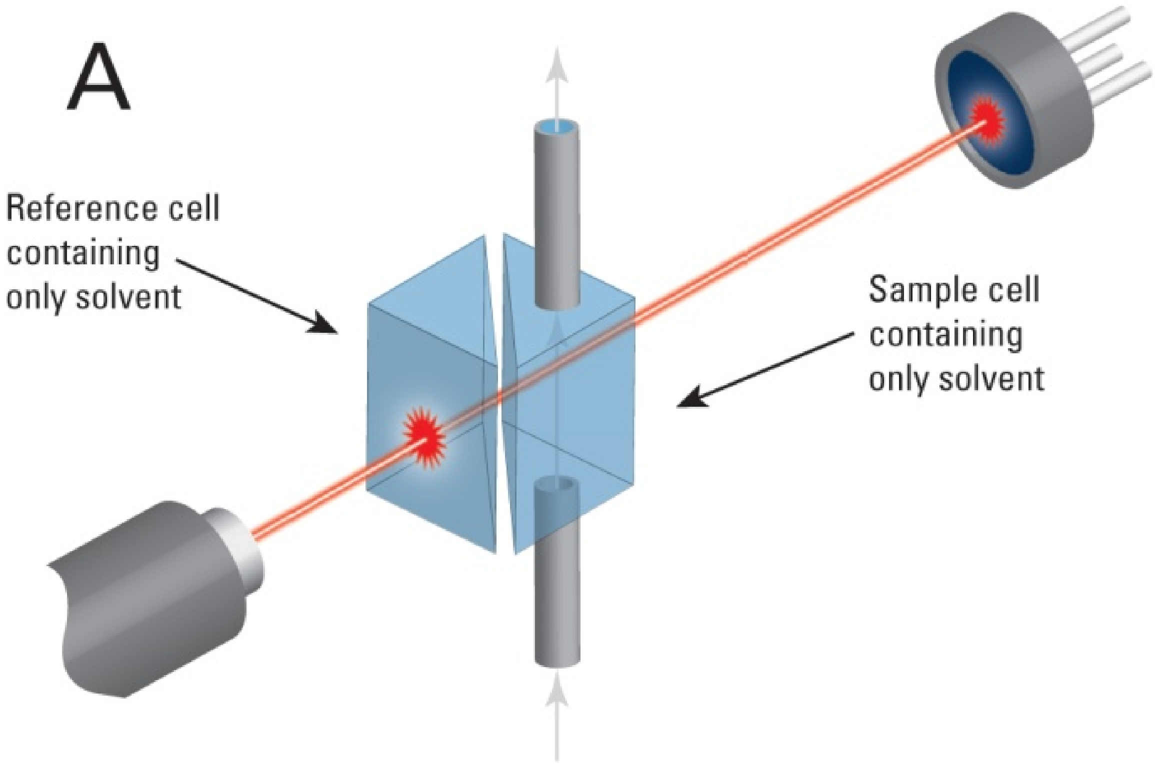
  (2) the sample side, containing a flowing stream of analyte in the same solvent as in the reference side

- The flow cell in 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) the reference side consisting of *a flowing stream* of pure solvent

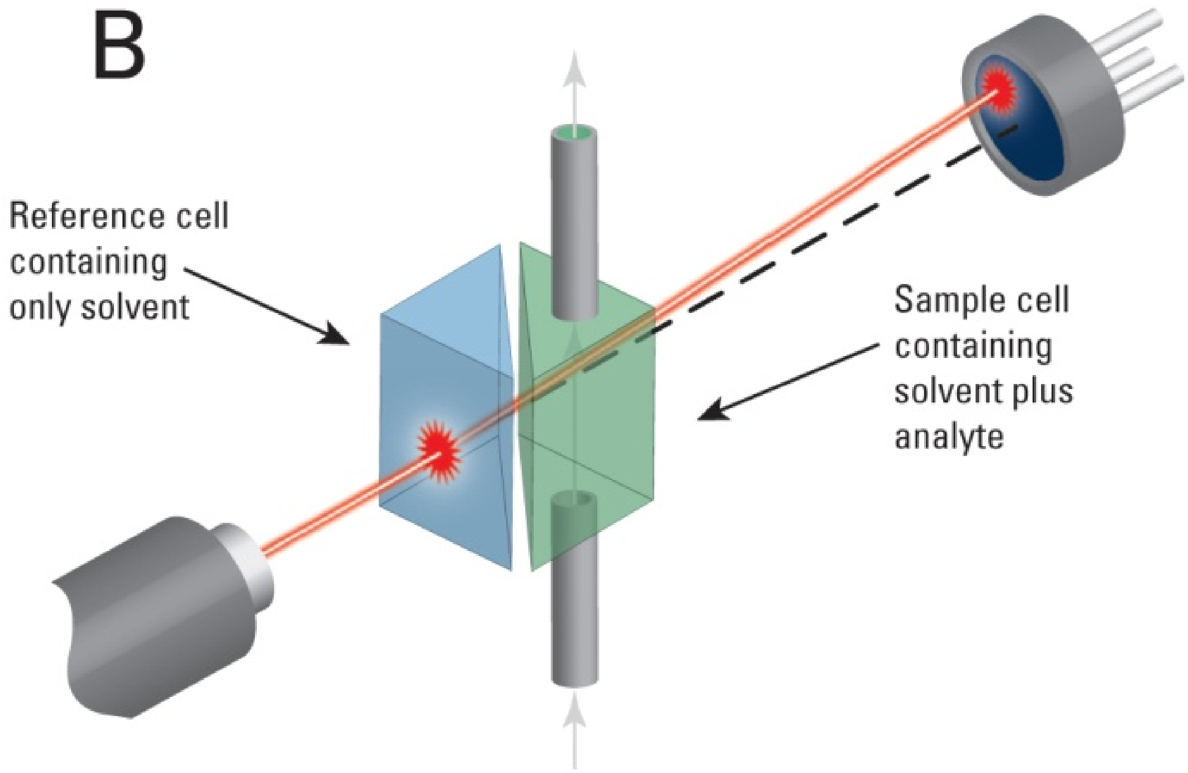
  (2) the sample side, containing a flowing stream of analyte in the same solvent as in the reference side.

Conventional RI Detector



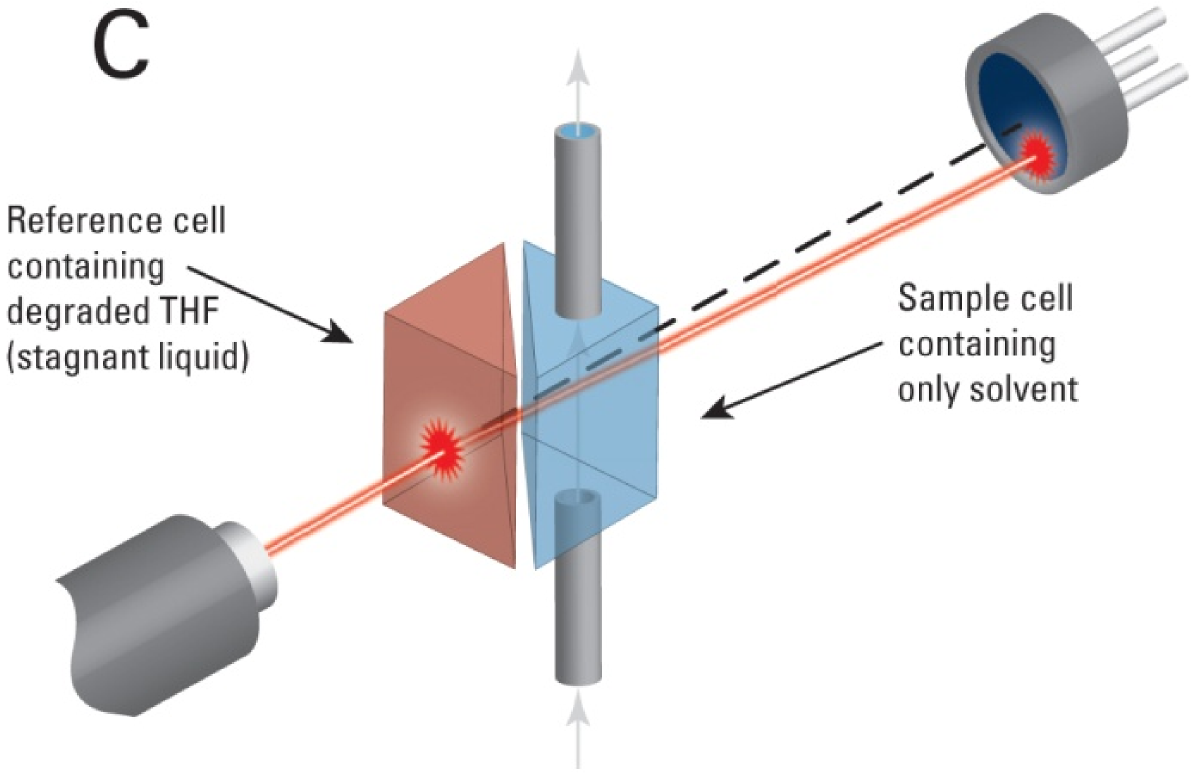
Depiction of a conventional RI detector flow cell when the contents of the reference and sample sides have the same refractive indices as each other, *i.e.*, both sides contain pure solvent only.

Conventional RI Detector Continued



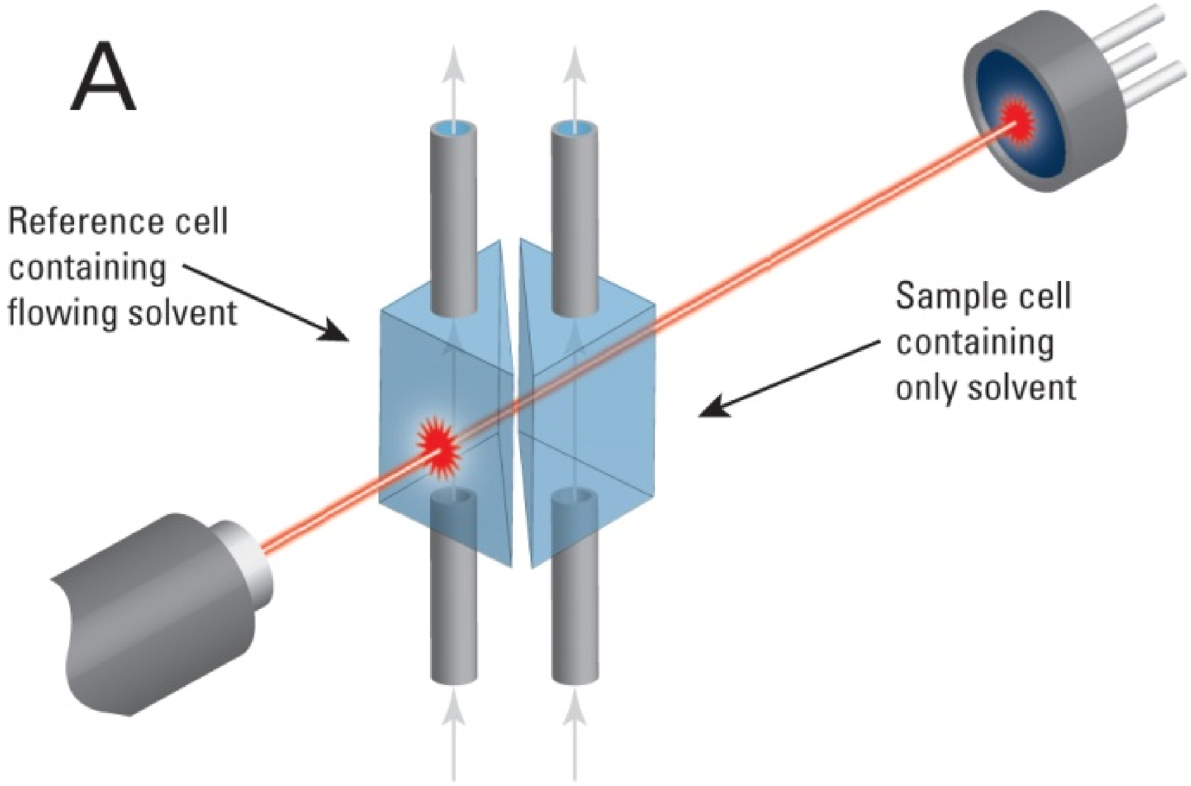
Depiction of a conventional RI detector flow cell when the contents of the reference and sample sides have different refractive indices as each other, *i.e.*, the reference cell contains pure solvent and the sample cell contains a dilute polymer solution.

Conventional RI Detector Continued



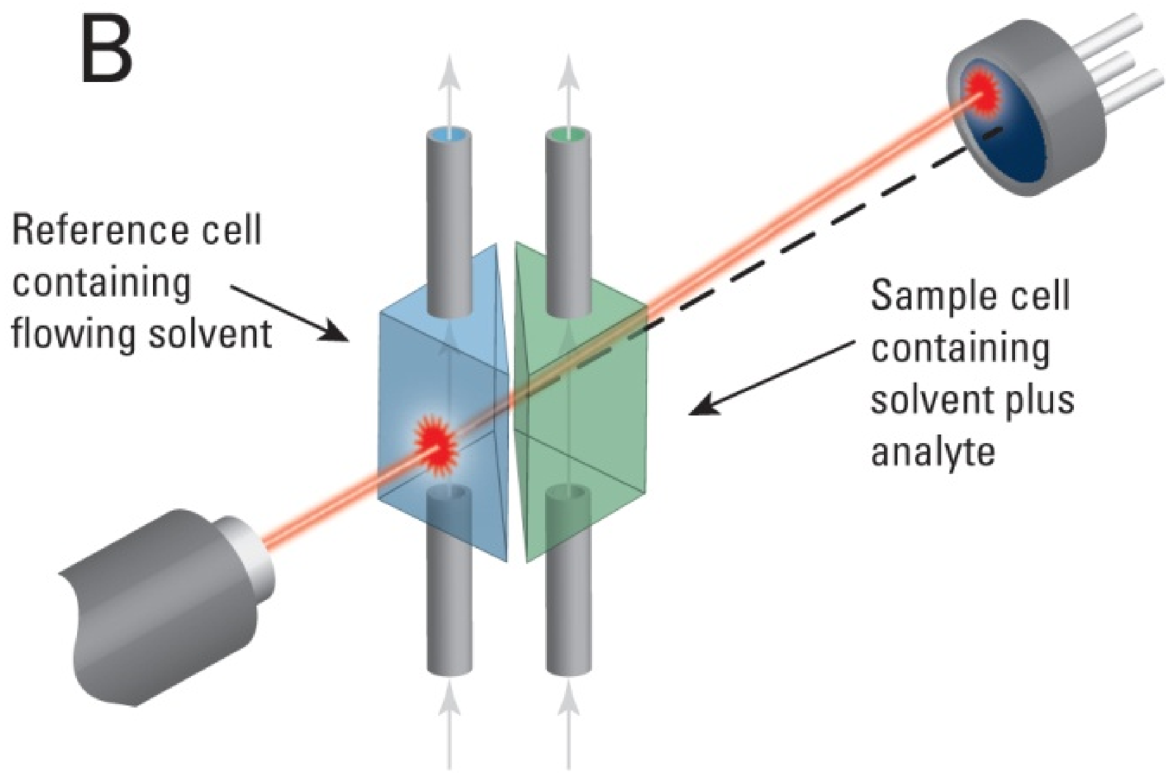
Depiction of a conventional RI detector flow cell showing the effects of THF degradation in the reference cell. Over time, the reference side consisting of stagnant pure solvent will slowly change resulting in baseline drift.

Dual-flow RI Detector



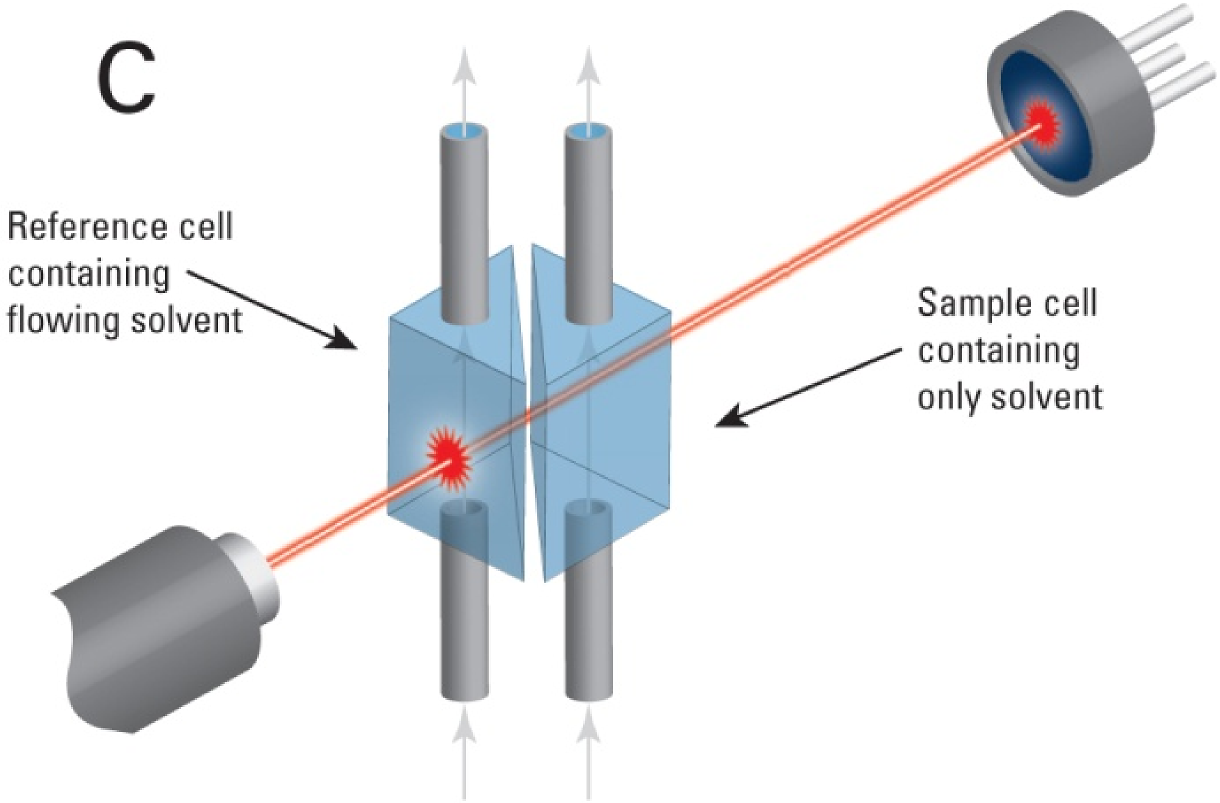
Depiction of a dual-flow RI detector flow cell when the contents of the reference and sample sides have the same refractive indices as each other, i.e., both sides contain a flowing stream of pure solvent only.

Dual-flow RI Detector Continued



Depiction of a dual-flow 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 a flowing stream of pure solvent and the sample cell contains a dilute polymer solution.

Dual-flow RI Detector Continued



Depiction of a dual-flow RI detector flow cell showing the compensation of the changes in refractive index of the solvent over time.

Experimental

Materials:

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

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)

Experimental Continued

- For equal comparison between the dual-flow and conventional RI detectors, all experiments were performed for both semi-micro and conventional SEC 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 $\times$ 15 cm) and conventional (7.8 mm ID $\times$ 30 cm) SEC columns.
- The conventional RI detectors are coupled to a modular HPLC or SEC system optimized for the use of conventional SEC columns.

Figure 3: Comparison of Baseline Drift of a Dual-flow Refractive Index Detector to that of Two Conventional Refractive Index Detectors using Semi-micro SEC Columns

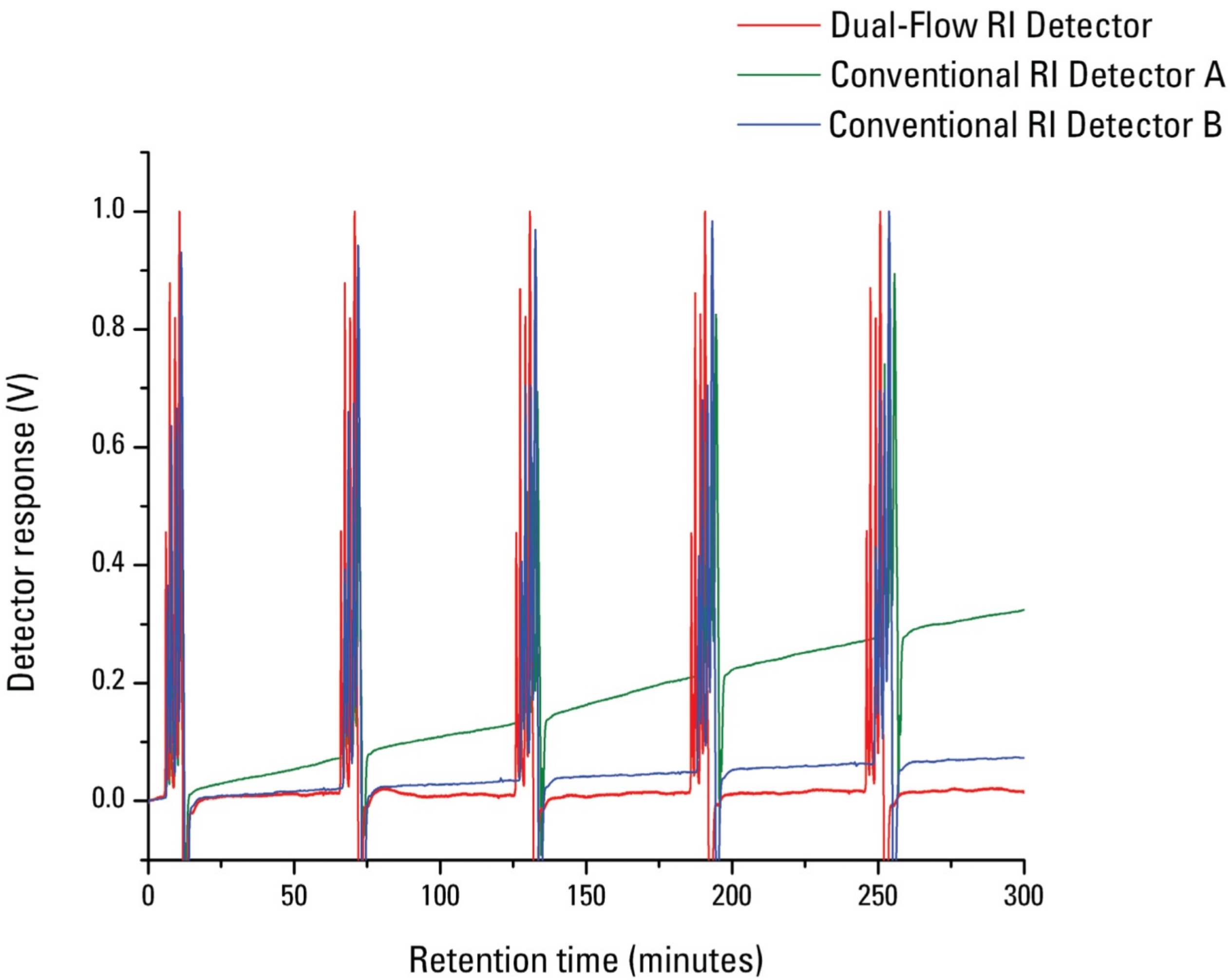


Figure 3: Conclusions

As shown in Figure 3, five consecutive injections of polystyrene standards, on semi-micro SEC 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 and a significantly drifting baseline on the two conventional RI detectors.

Figure 4A: Comparison of Baseline Stability of a Dual-flow Refractive Index Detector to that of a Conventional Refractive Index Detectors using Semi-micro SEC Columns

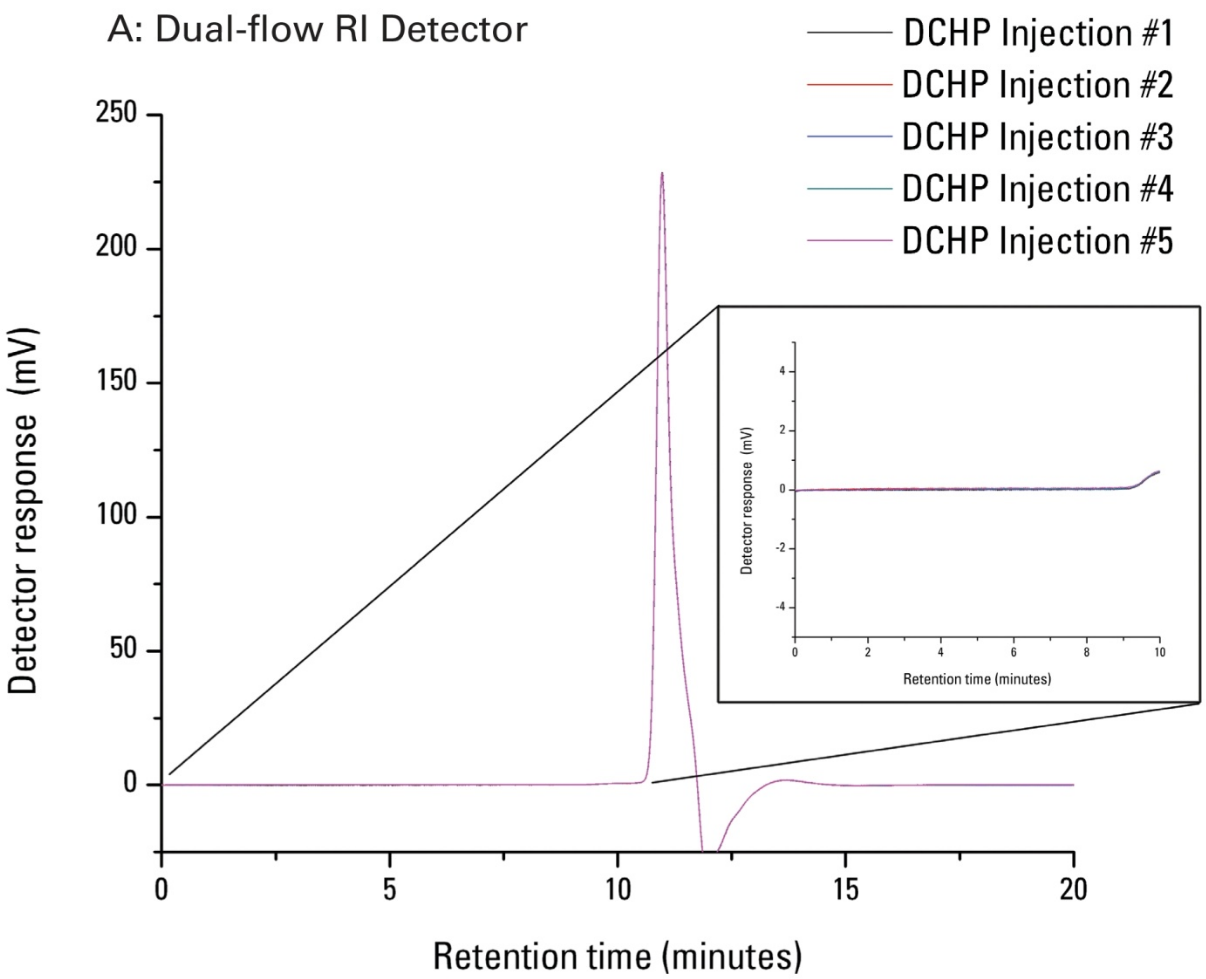
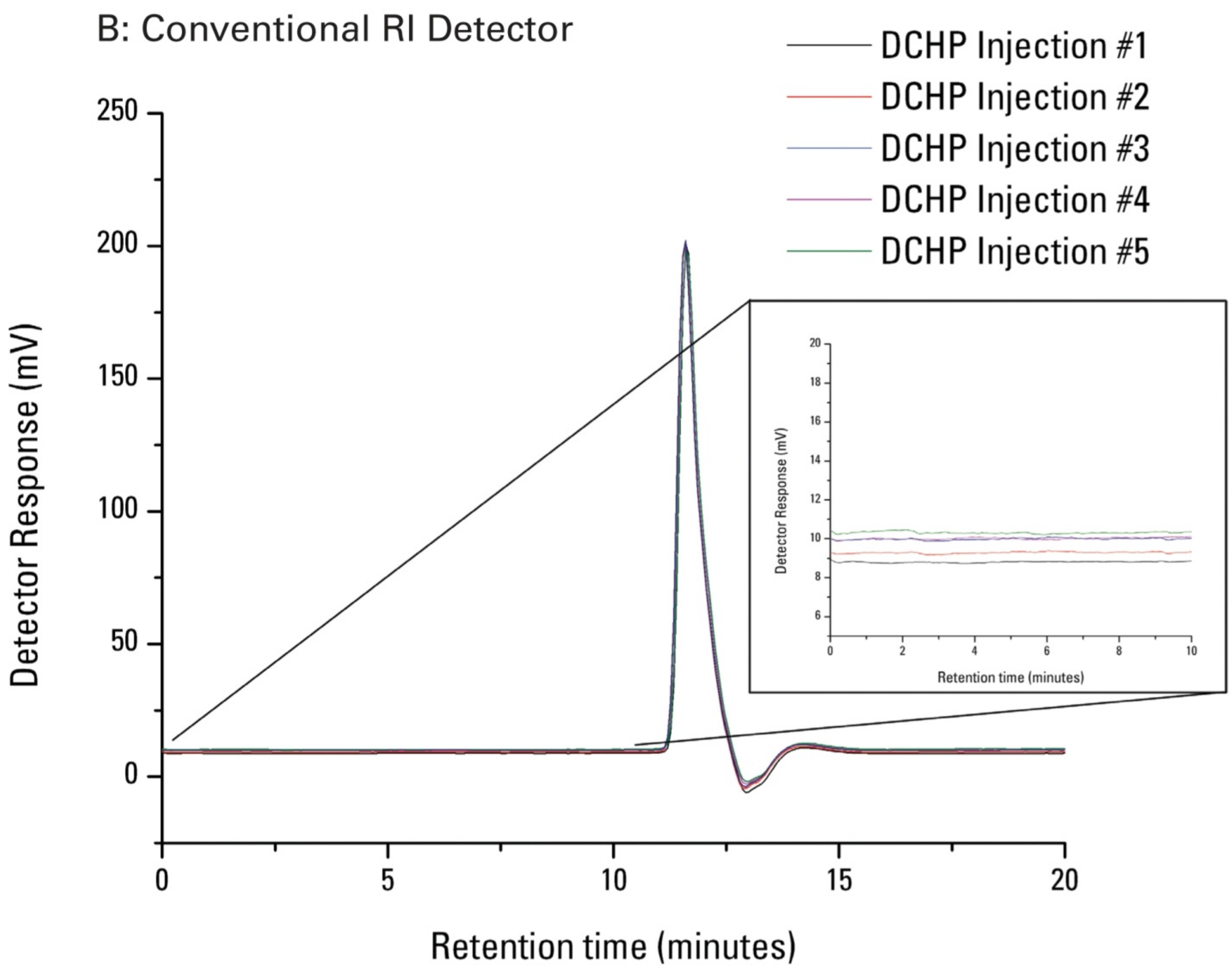


Figure 4B: Comparison of Baseline Stability of a Dual-flow Refractive Index Detector to that of a Conventional Refractive Index Detectors using Semi-micro SEC Columns



Figures 4A & 4B: Conclusions

- The chromatograms of five sequential injections of dicyclohexyl pthalate (DCHP) were overlaid and are shown in Figures 4A & 4B for a dual-flow and conventional RI detector.
- Superposition of five consecutive chromatograms obtained with a dual-flow RI detector using semi-micro SEC columns shows negligible baseline drift, compared to the same experiments repeated with a conventional RI detector.

Figure 5: Comparison of Baseline Drift of a Dual-flow Refractive Index Detector to that of Two Conventional Refractive Index Detectors using Conventional SEC Columns

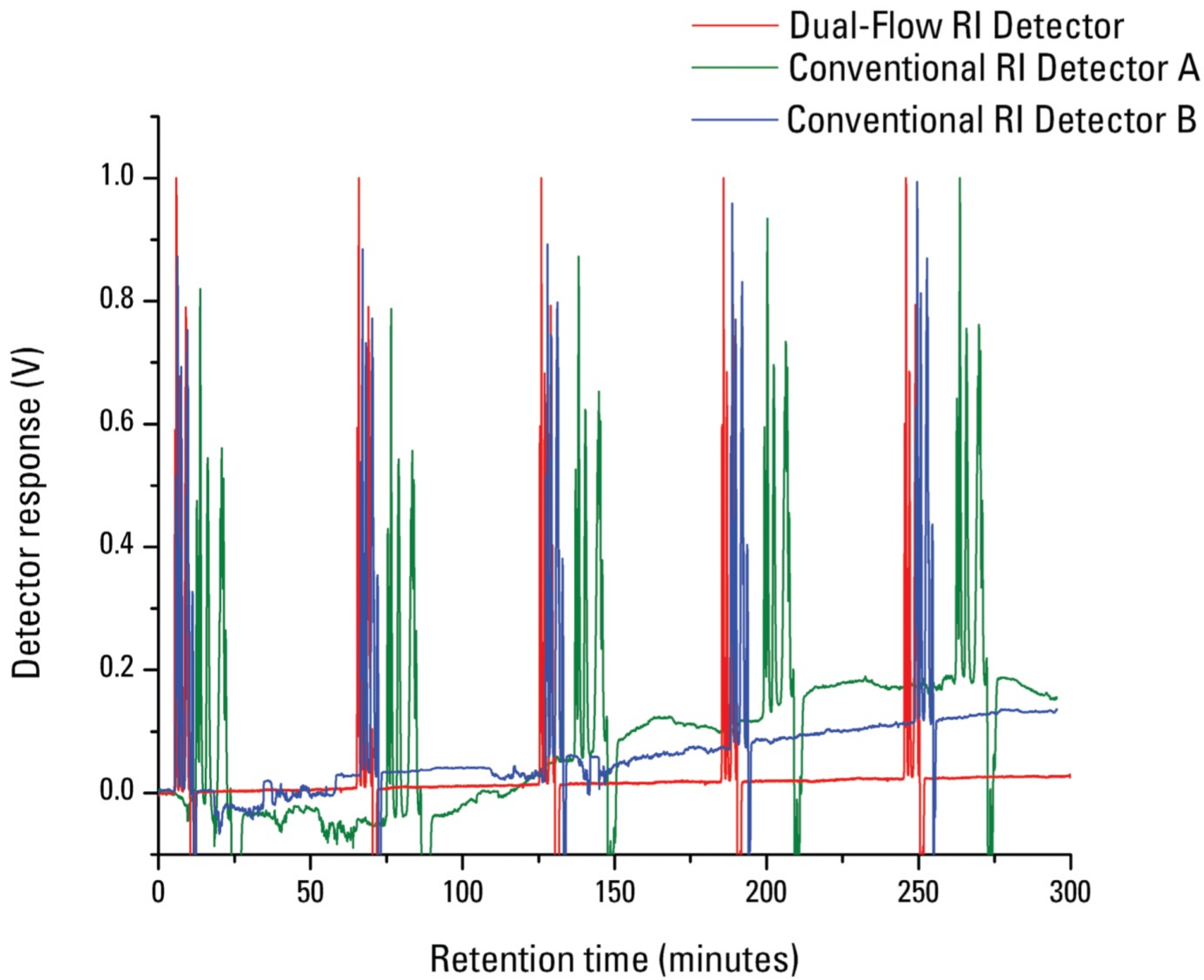


Figure 5: Conclusions

As shown in Figure 5, five consecutive injections of polystyrene standards, on conventional SEC 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 stable baseline with low baseline drift on the dual-flow RI detector and a significantly drifting and inconsistent baseline on the two conventional RI detectors.

Figure 6A: Comparison of Baseline Stability of a Dual-flow Refractive Index Detector to that of a Conventional Refractive Index Detectors using Conventional SEC Columns

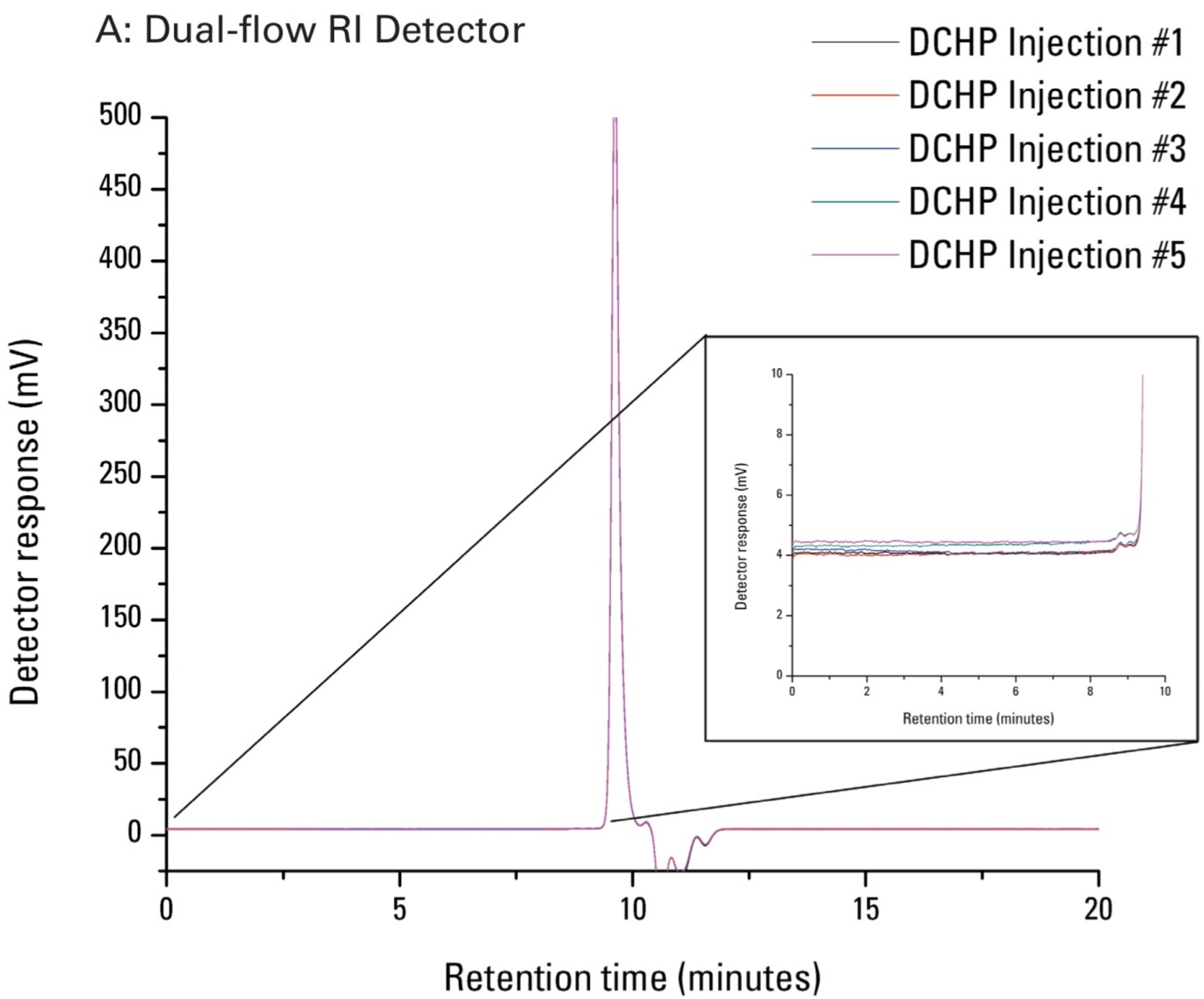


Figure 6B: Comparison of Baseline Stability of a Dual-flow Refractive Index Detector to that of a Conventional Refractive Index Detectors using Conventional SEC Columns

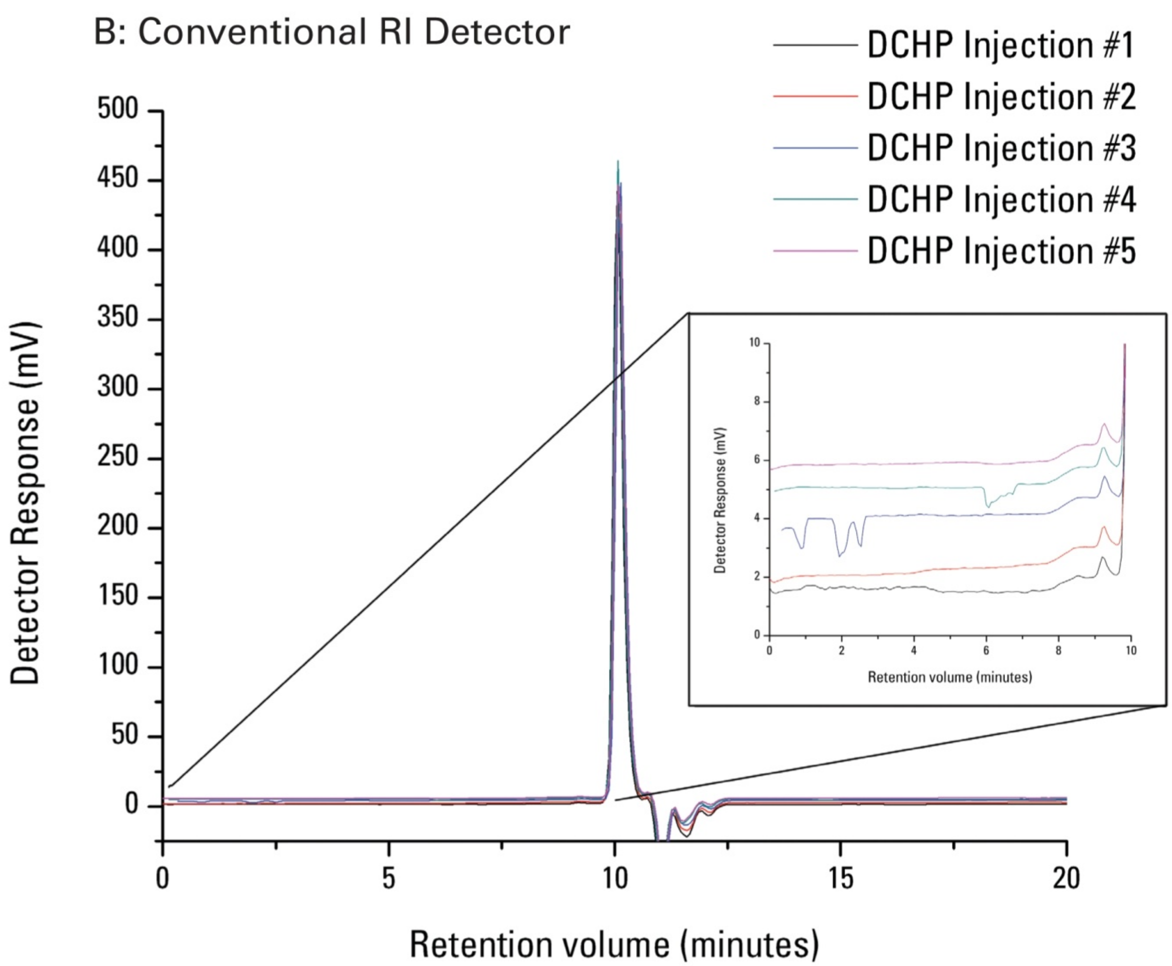
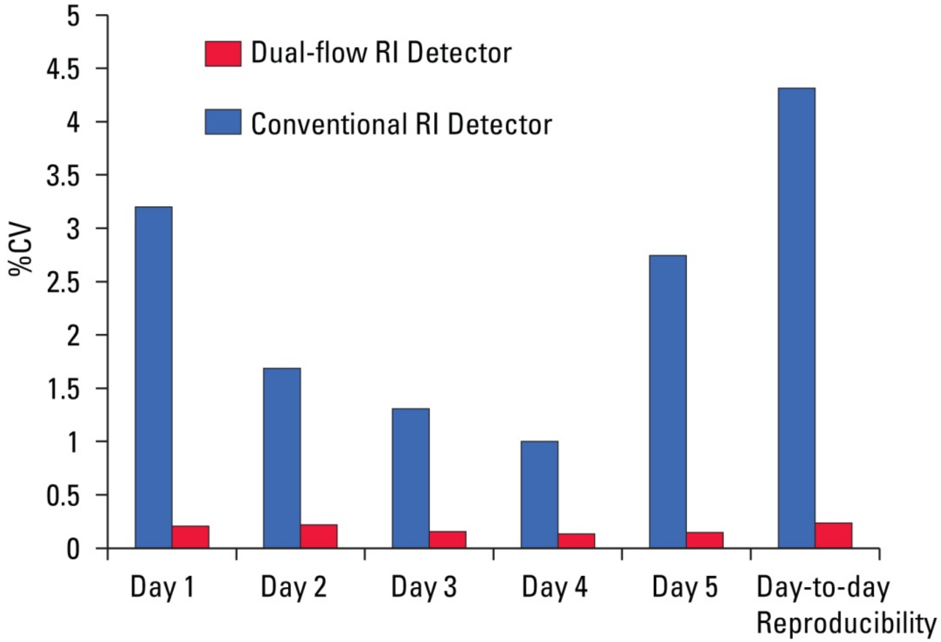


Figure 6: Conclusions

The comparison of the superposition of five consecutive chromatograms of dicyclohexylpthalate (DCHP) as obtained using dual-flow and conventional RI detectors with conventional SEC columns, as shown in Figures 6A & 6B, shows significantly less baseline drift occurs using a dual-flow RI detector compared to that of a conventional RI detector.

Figure 7: Comparing M_w Reproducibility *of* a Dual-flow Refractive Index Detector to that of a Conventional Refractive Index



TSKgel SuperMultiporeHZ-M, 4.6 mm ID × 15 cm, x 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 7 Conclusions

- The repeatability and reproducibility of the molar mass averages as obtained via dual-flow and conventional RI detectors were also 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 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, 6 mm ID × 15 cm × 2 + guard column
Solvent/ mobile phase:	chloroform
Flow rate:	0.35 mL/min
Temperature:	40 ºC (pump and column ovens and RI detector in the EcoSEC GPC System)

Figure 8: Baseline Stability of a Dual-flow Refractive Index Detector using Semi-micro SEC Columns in Chloroform

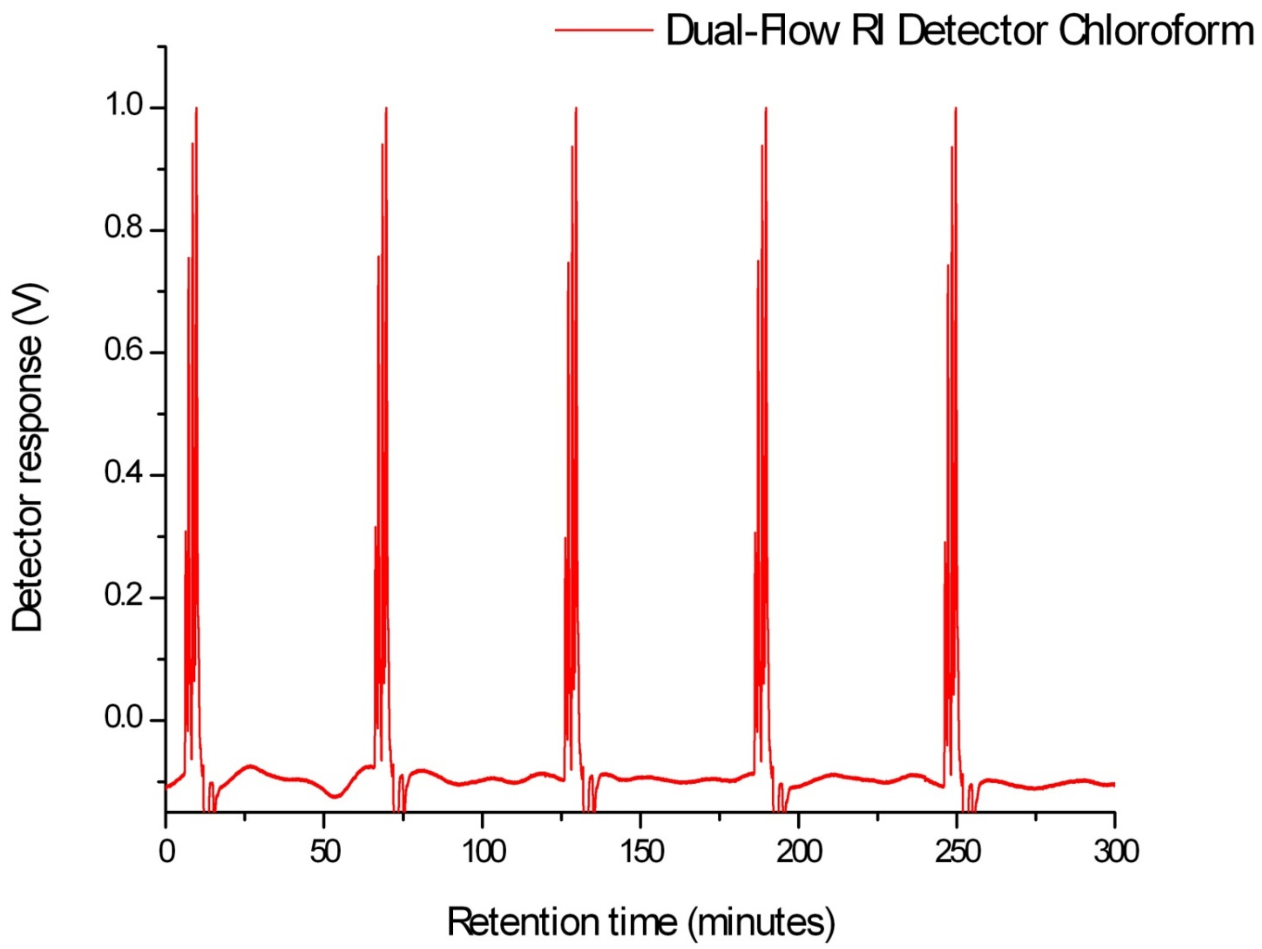


Figure 8: Conclusions

As shown in Figure 8, five consecutive injections of polystyrene standards in chloroform, on semi-micro SEC 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 9: Baseline Stability of a Dual-flow Refractive Index in Chloroform

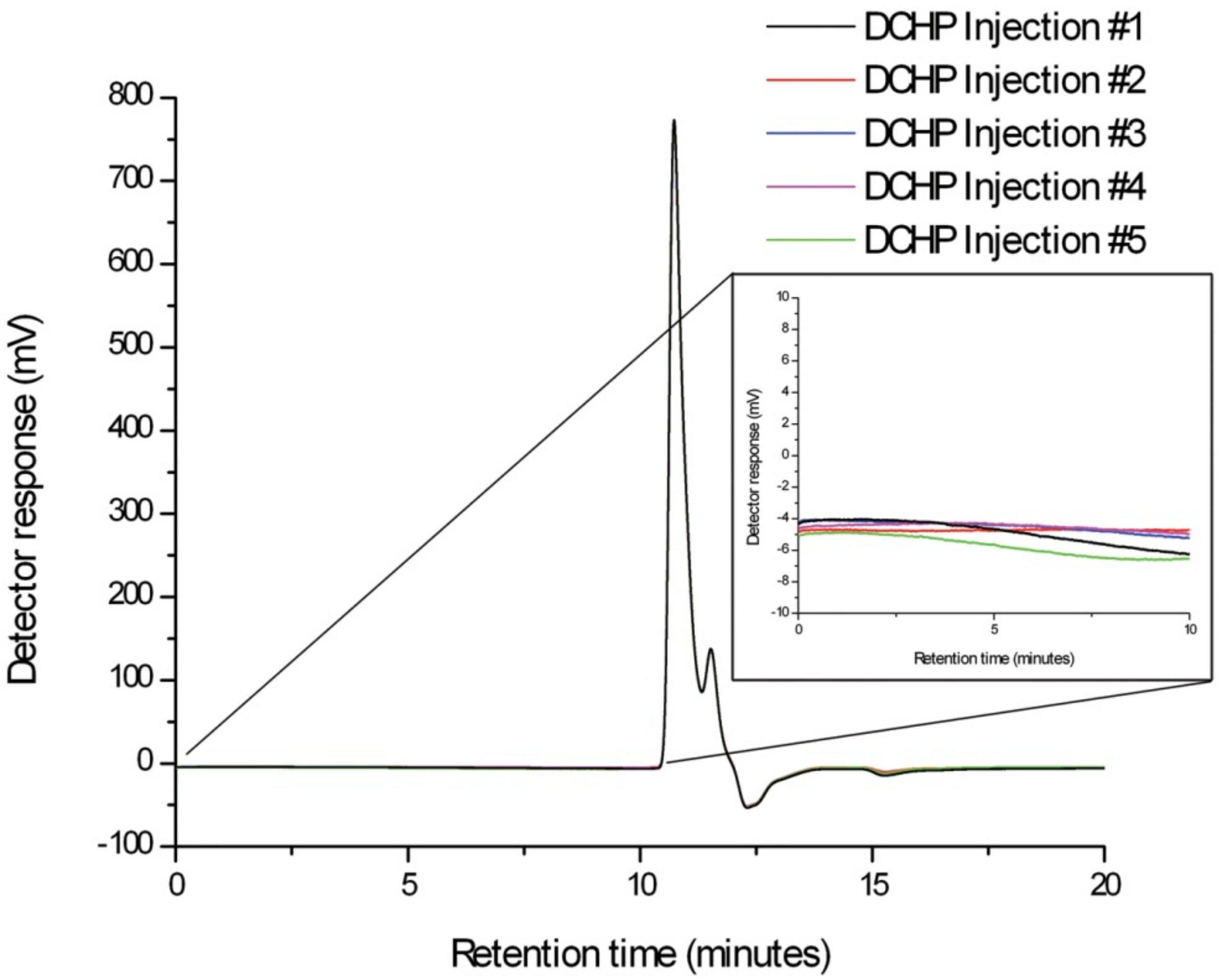


Figure 9: Conclusions

Chromatograms of five sequential injections of dicyclohexylpthalate (DCHP) obtained with a dual-flow RI detector using semi-micro SEC columns in chloroform were overlaid and are shown in Figure 9. The five injections of DCHP show negligible baseline drift in chloroform.

Baseline Stability for Polymers in Mixed Solvents

Experimental Continued

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 ^{o}C (pump and column ovens and RI detector in the EcoSEC GPC System)

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

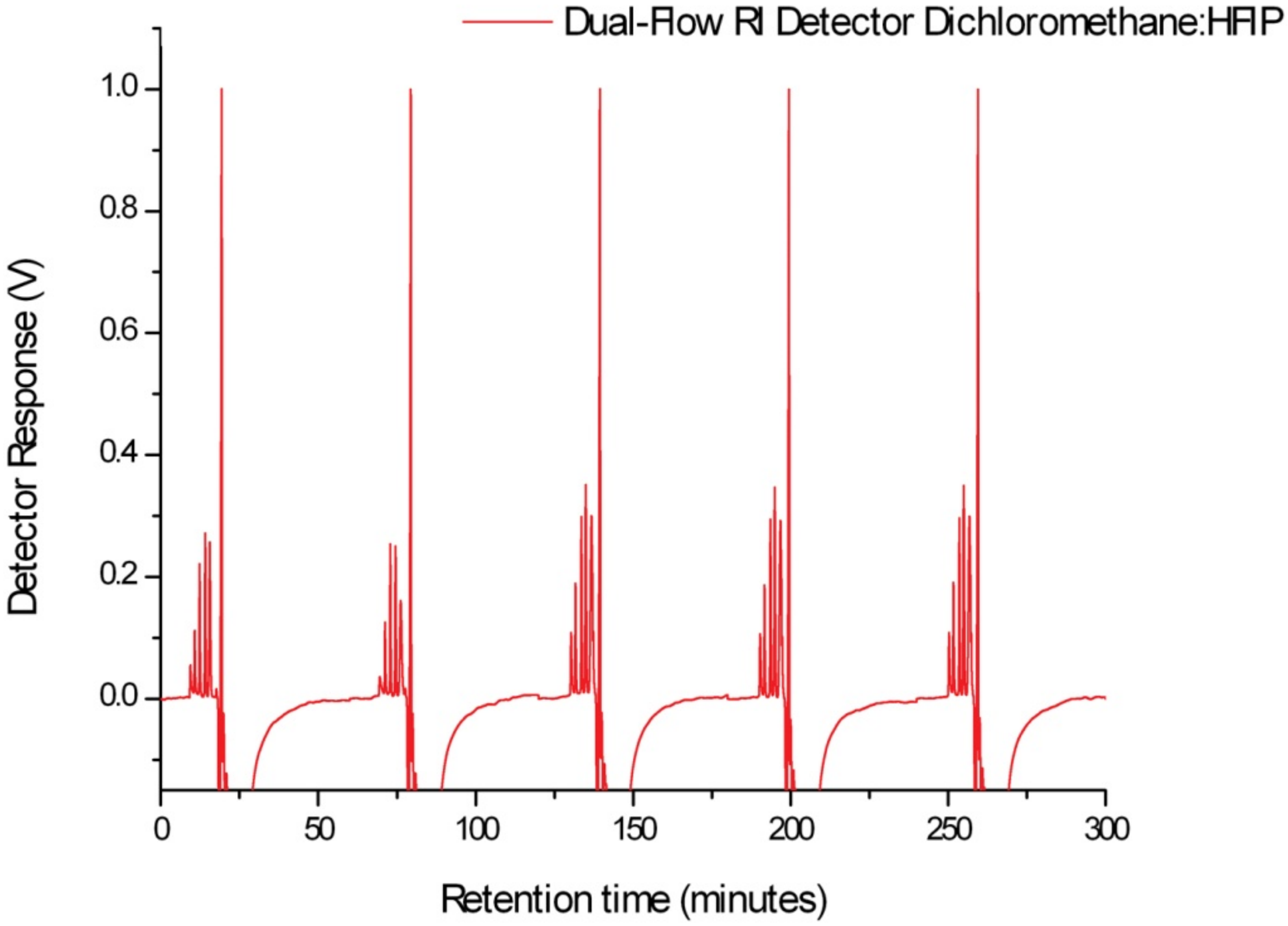


Figure 10: Conclusions

As shown in Figure 10, five consecutive injections of polystyrene standards in 95:5 Dichloromethane:HFIP with 5 mmol/L tetraethylammonium bromide, on semi-micro SEC 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.

Baseline Stability for Polymers in Complex Solvents

Experimental

Instrumentation: EcoSEC GPC System (HLC-8320) equipped with a dual-flow refractive index detector

Columns: TSKgel SuperHZM-M, 3 & 5 µm, 6 mm ID × 15 cm × 2 + guard column

Solvent/ mobile phase: DMAc 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 Stability of a Dual-flow Refractive Index Detector using Semi-micro SEC Columns in DMAc with 0.02 mol/L LiBr

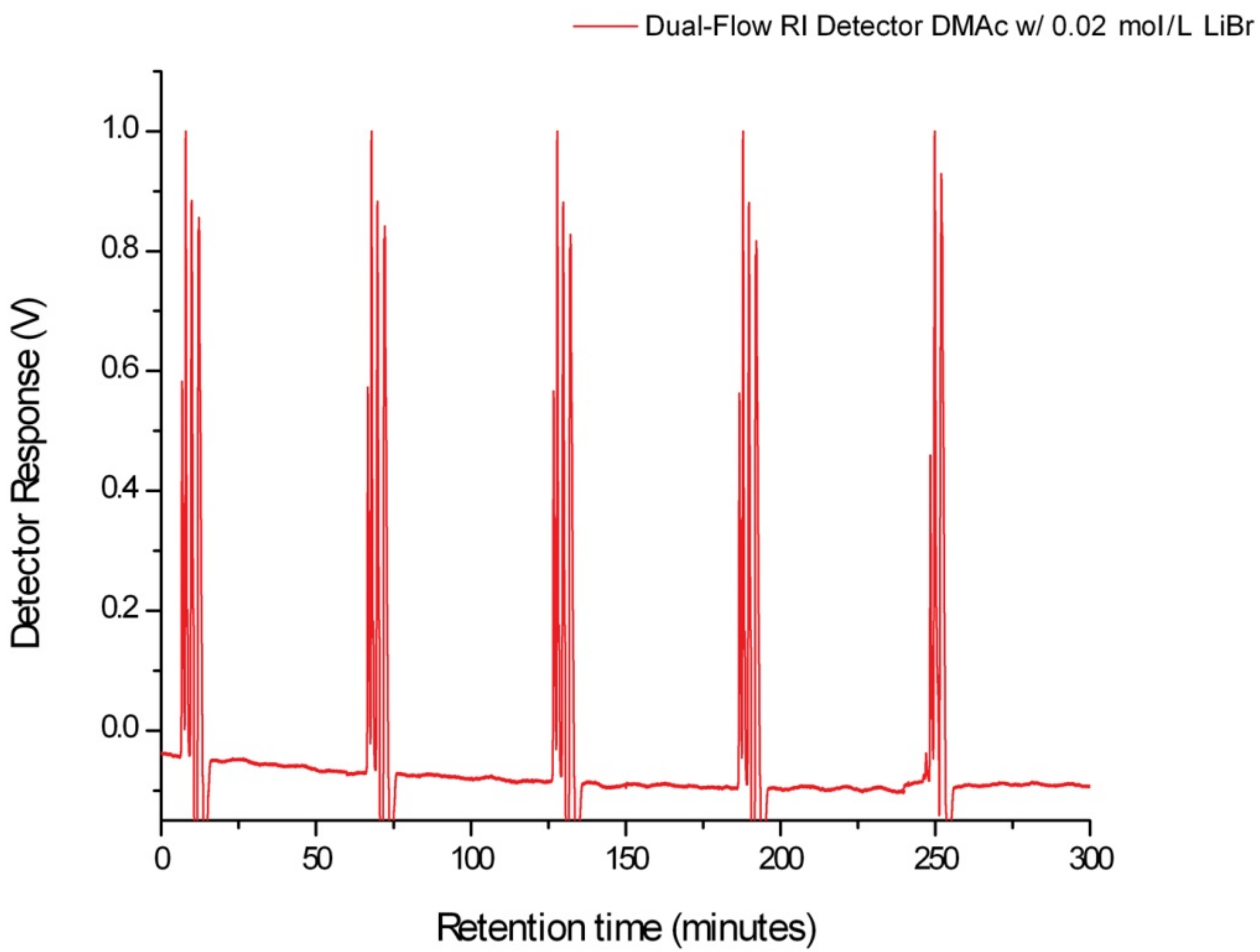


Figure 11: Conclusions

As shown in Figure 11, five consecutive injections of polystyrene standards in DMAc with 0.02 mol/L LiBr, on semi-micro SEC 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 DMAc with 0.02 mol/L LiBr

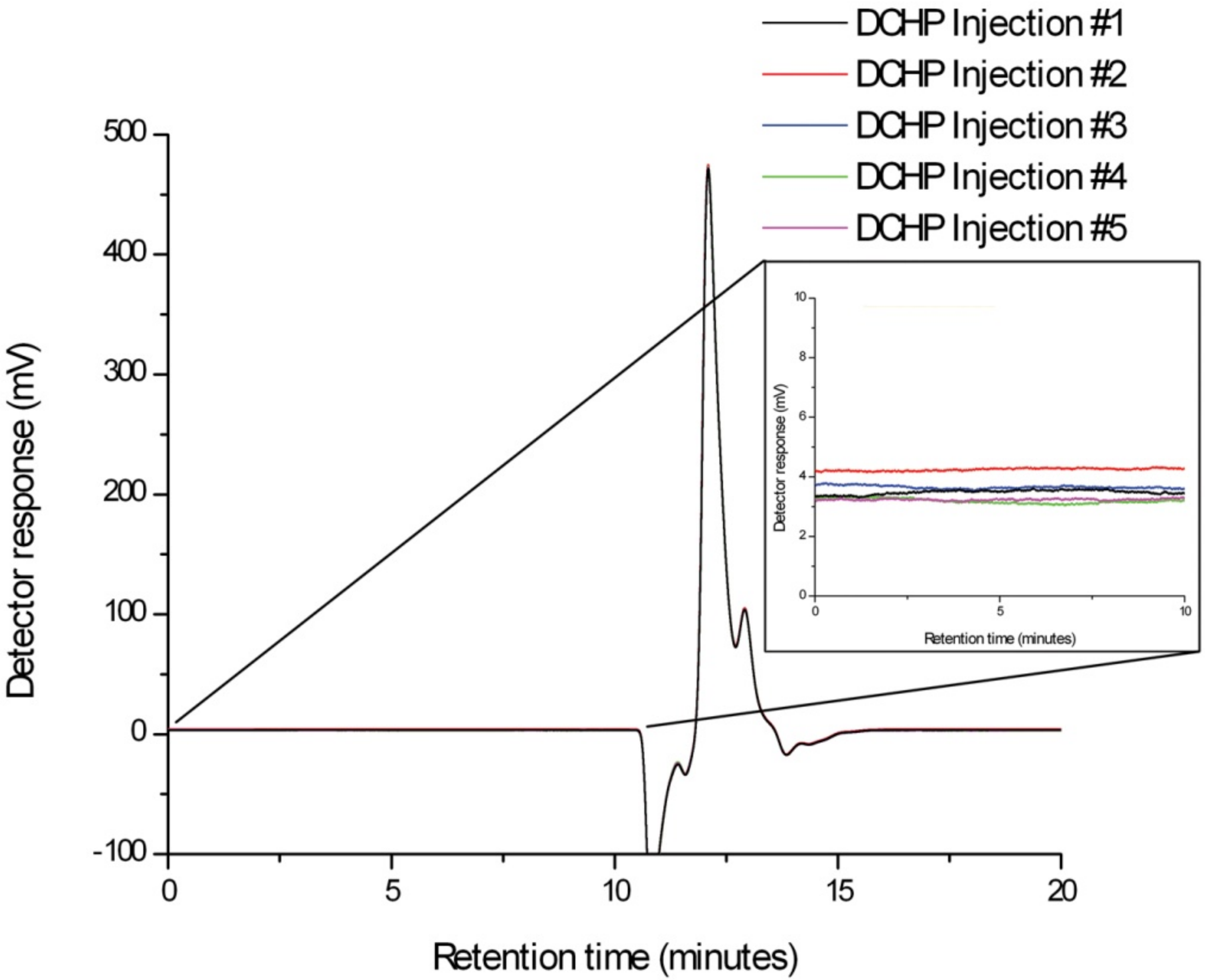


Figure 12: Conclusions

The superposition of five consecutive chromatograms of dicyclohexylpthalate (DCHP) as obtained using dual-flow RI detector with semi-micro SEC columns, as shown in Figure 12, shows a negligible baseline drift occurs between injections..

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 SEC 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 SEC experiments which rely on accurate and precise instrumentation and multi-detector SEC experiments which require excellent baseline stability and consistent instrumentation.

References

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