Reproducibility and Accuracy in Modern Size Exclusion Chromatography

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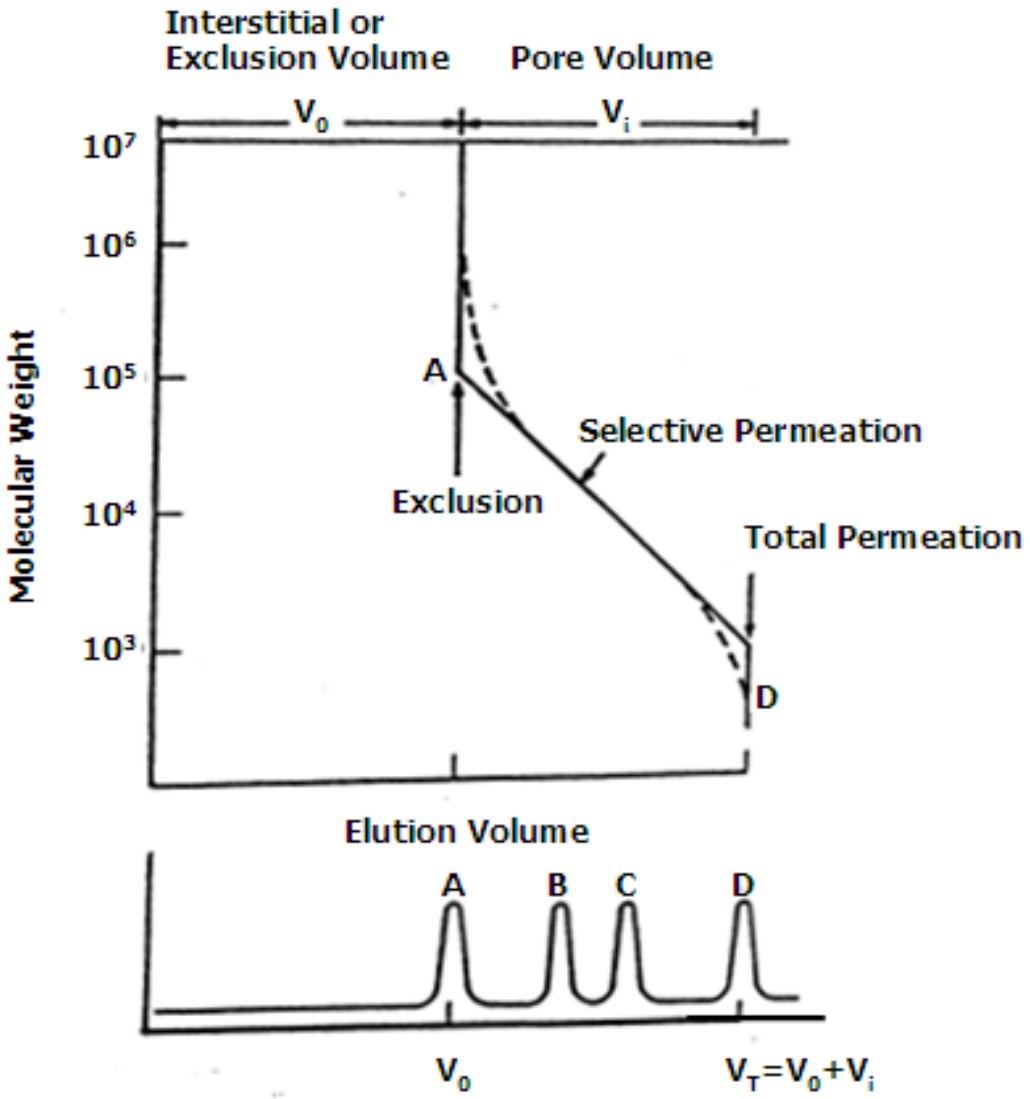
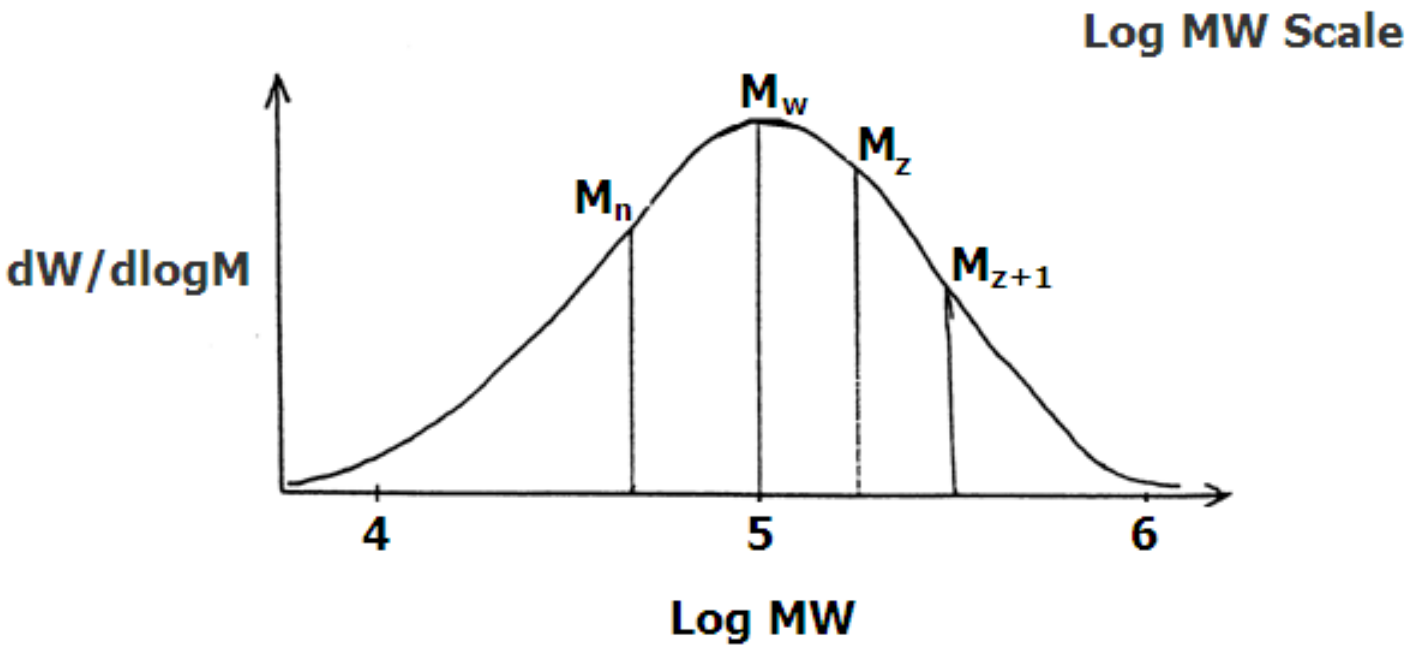
Agenda

- Improving MW accuracy
  - Factors contributing to baseline instability
  - Effect of temperature on SEC measurements
- State-of-the-art dedicated EcoSEC® GPC system
- High efficiency semi-micro SuperMultiporeHZ columns
- Conclusions

SEC Terminology



Mass Distribution of Polymers



Guidelines for Improving MW Accuracy

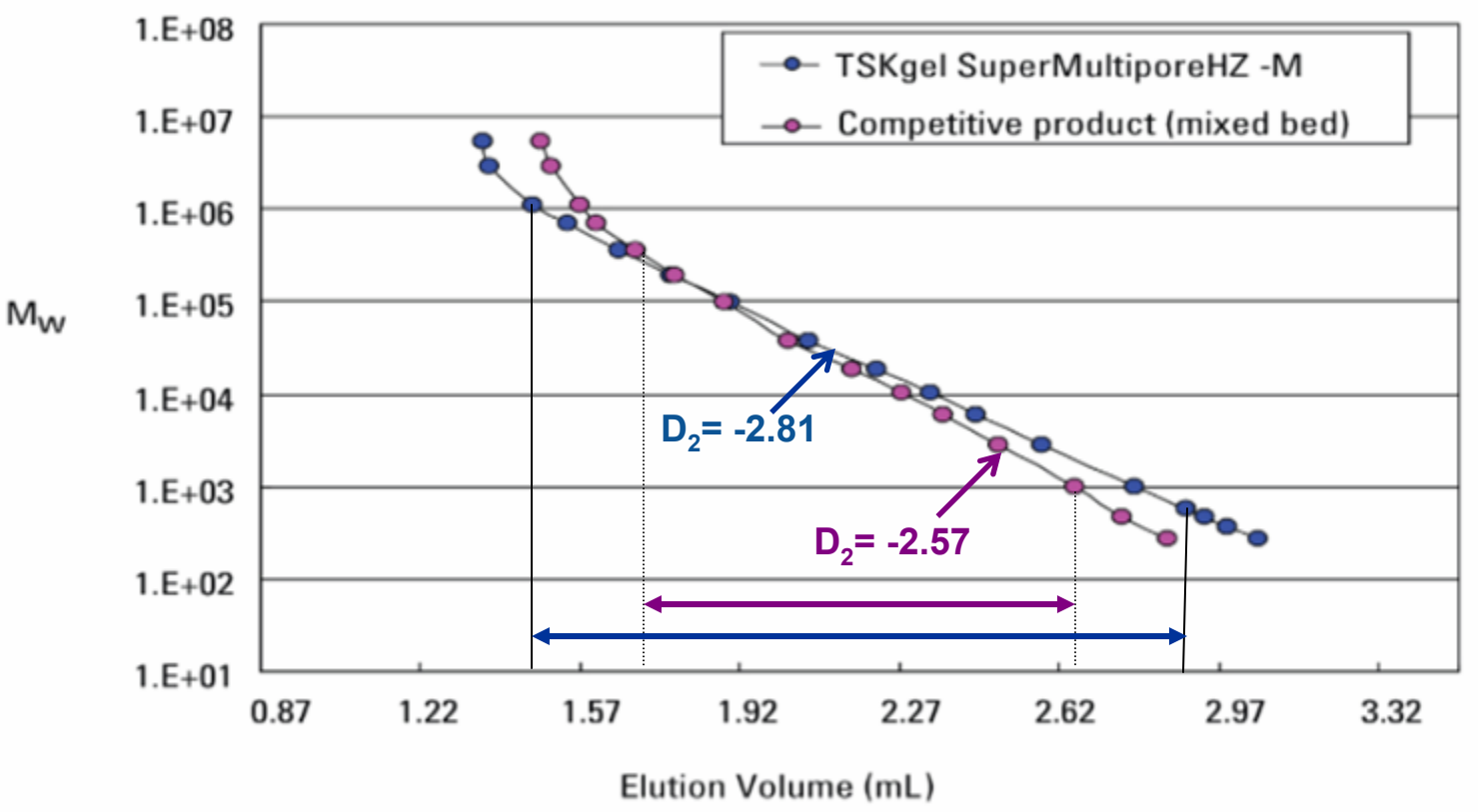
- SEC column selection
- Multipoint calibration
- Flow rate
- Mobile phase selection
- Sample concentration and volume

Guidelines for Improving MW Accuracy

SEC Column Selection

- Shallow slope of calibration curve (D_2)
- High column efficiency (N)
- Broad, linear MW range of calibration curve

Linearity of TSK-GEL SuperMultiporeHZ-M Columns



Calibrants (10µl) injected onto TSK-GEL SuperMultiporeHZ-M column (4.6mm ID x 15cm, 4µm) x 4, 25°C, 0.35mL/min,THF mobile phase, UV@254nm. Series of narrow MW polystyrene standards calibrants.

Guidelines for Improving MW Accuracy – Calibration

Multipoint calibration

- At least six calibrants must be used with two anchor points to identify V_0 and V_t
- Standards must be nearly monodisperse with known M_n and/or M_w values
- Calibrants and samples must be analyzed under the same conditions (solvent, F, T)

Guidelines for Improving MW Accuracy – Flow Rate

Flow rate

- Operate at or near minimum of the van Deemter plot (HETP* vs. linear velocity)
- Precise flow rate control
  - Peak width, peak area, and elution volume depend on flow rate consistency

** height equivalent of a theoretical plate*

Effect of Flow Rate on MW Measurements

Flow rate variability (+/- 1.43%) between running calibration curve and performing SEC analysis and its effect on MW averages.

		% Error		
Std MW	**Flow Rate**	**M_n**	**M_w**	**M_z**
2.89×10^6	0.355	+10.6	+12.4	+13.4
	0.345	-6.6	-3.5	-2.6
9.64×10^4	0.355	+18.4	+18.5	+19.5
	0.345	-9.6	-9.6	-9.6
5.97×10^3	0.355	+23.4	+25.0	+25.0
	0.345	-12.3	-12.2	-12.1

Conditions: TSK-GEL SuperHZ1000 and SuperMultiporeHZ-M column set; 0.350mL/min; 40°C; THF mobile phase; RI; 10µL; 1mg/mL PS.

Guidelines for Improving MW Accuracy – Mobile Phase

Mobile phase selection

- No polymer-packing interactions ($\Delta H = 0$)
- If ΔH is not zero
  - MW will be underestimated if ΔH is negative
  - Peak width will be overestimated
  - SEC calibration curve will not be valid

Guidelines for Improving MW Accuracy – the Sample

Sample concentration & volume

- To prevent macromolecular crowding,
  i.e., increased elution volume:
  $c \leq 1/[\eta]$

- To eliminate viscous fingering,
  i.e., distorted peak shapes:
  $\eta_r < 1.1\,\eta_{mp}$

- To prevent excess peak broadening, keep injection volume:
  < 100µL for 7.8 mm ID columns
  < 10µL for 4.6 mm ID columns

- Match injection volume of standards with samples

Factors Contributing to Baseline Instability

Peroxide Buildup

- Peroxides in THF build up in reference cell
  - Continuous flow-through RI reference cell

Pulsating flow

- Depending upon RI construction, cell may flex, changing cell path length
  - Effect is dampened by packed columns and is reduced when reference cell experiences same effect

Schlieren effect

- Caused by density/temperature gradients, or turbulent flow within RI cell
  - Reference RI cell lessens this effect

The Effect of Temperature Fluctuations on SEC Measurements

Peak broadening

- Decreases with increasing temperature
- Efficiency increases

Flow rate

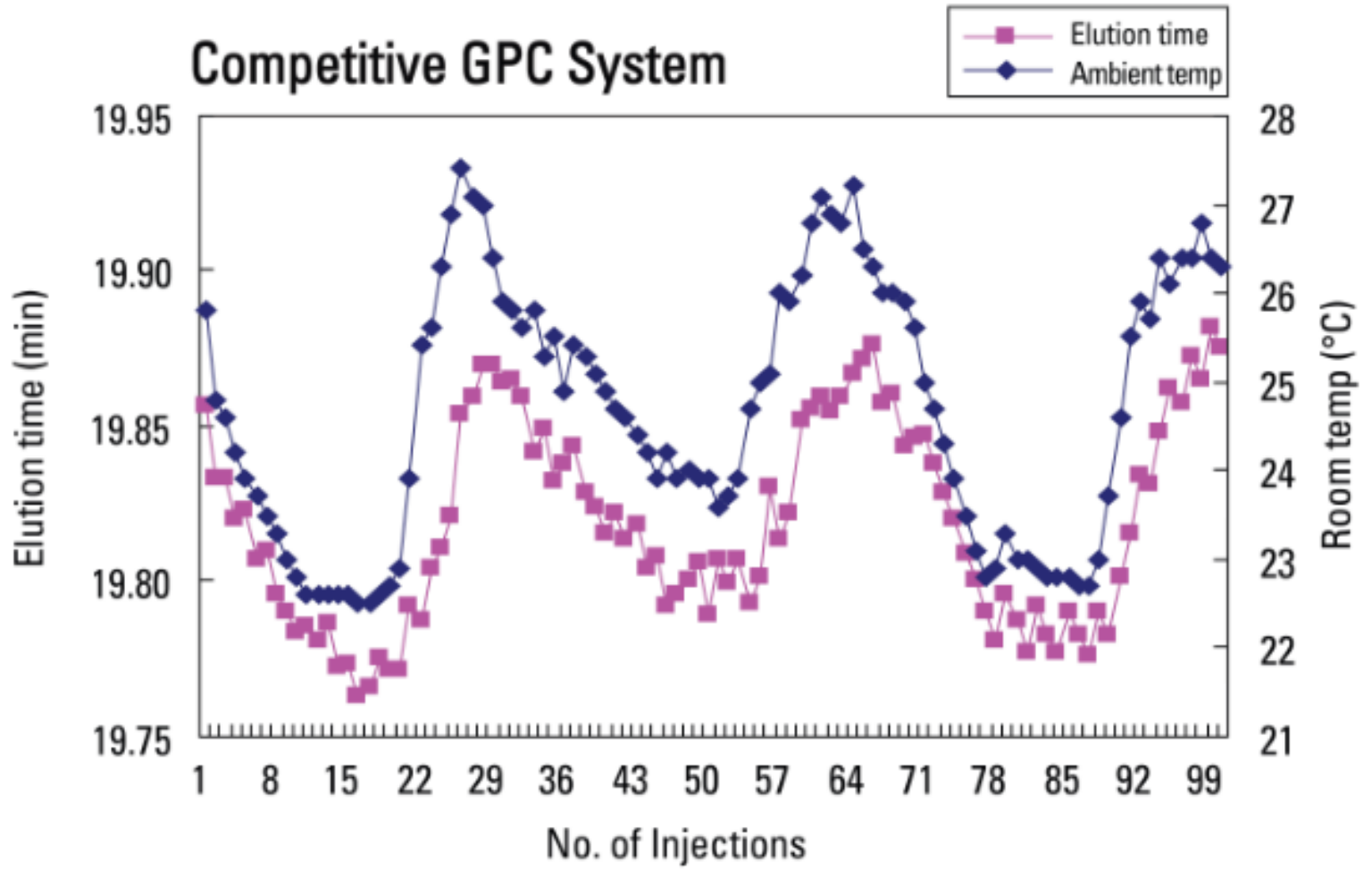
- Will lead to mobile phase contraction and expansion, will contribute to short-term noise

Packing pore structure

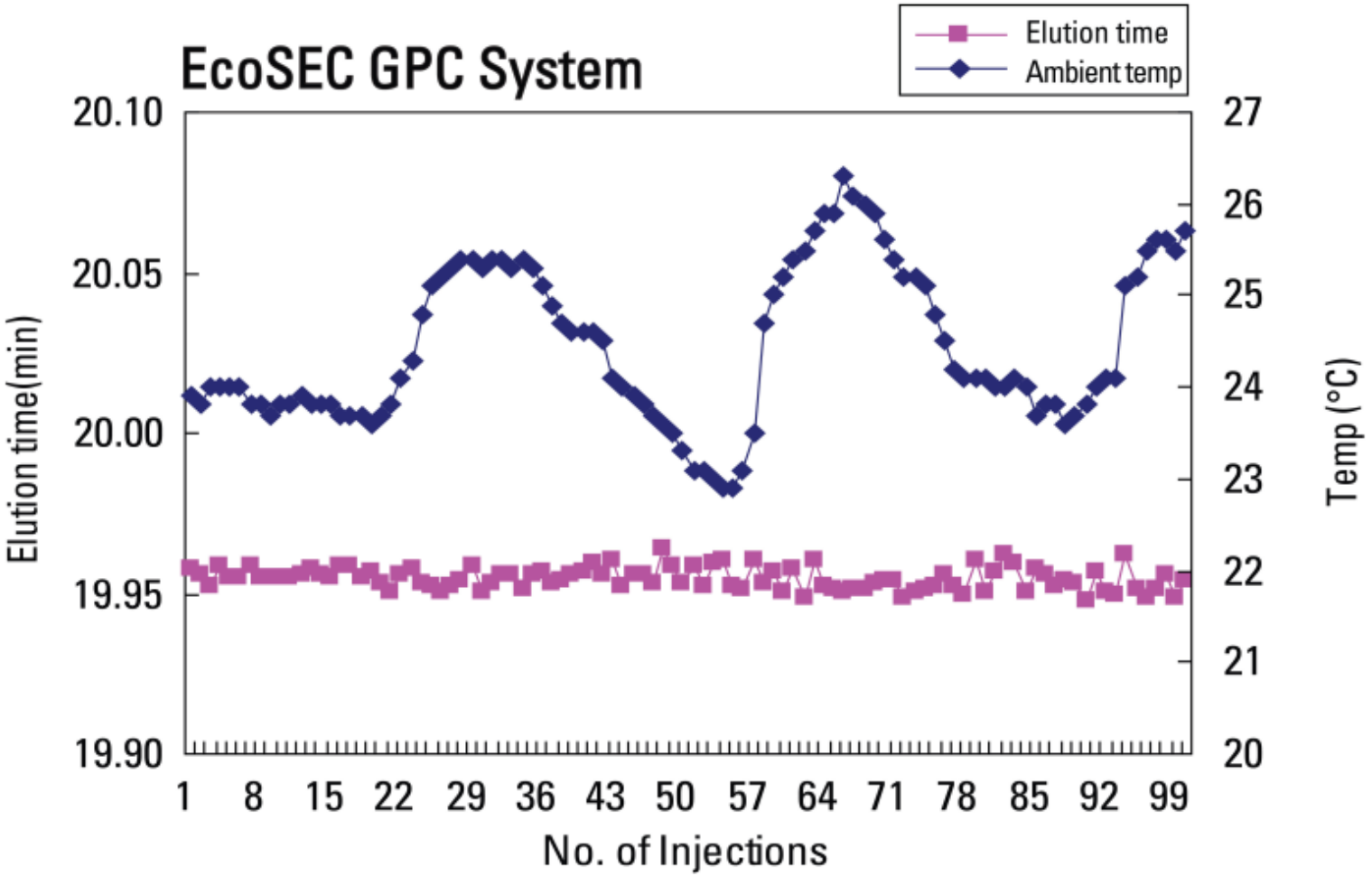
- Negligible with high performance packings, depending on degree of particle cross-linking

Retention volume

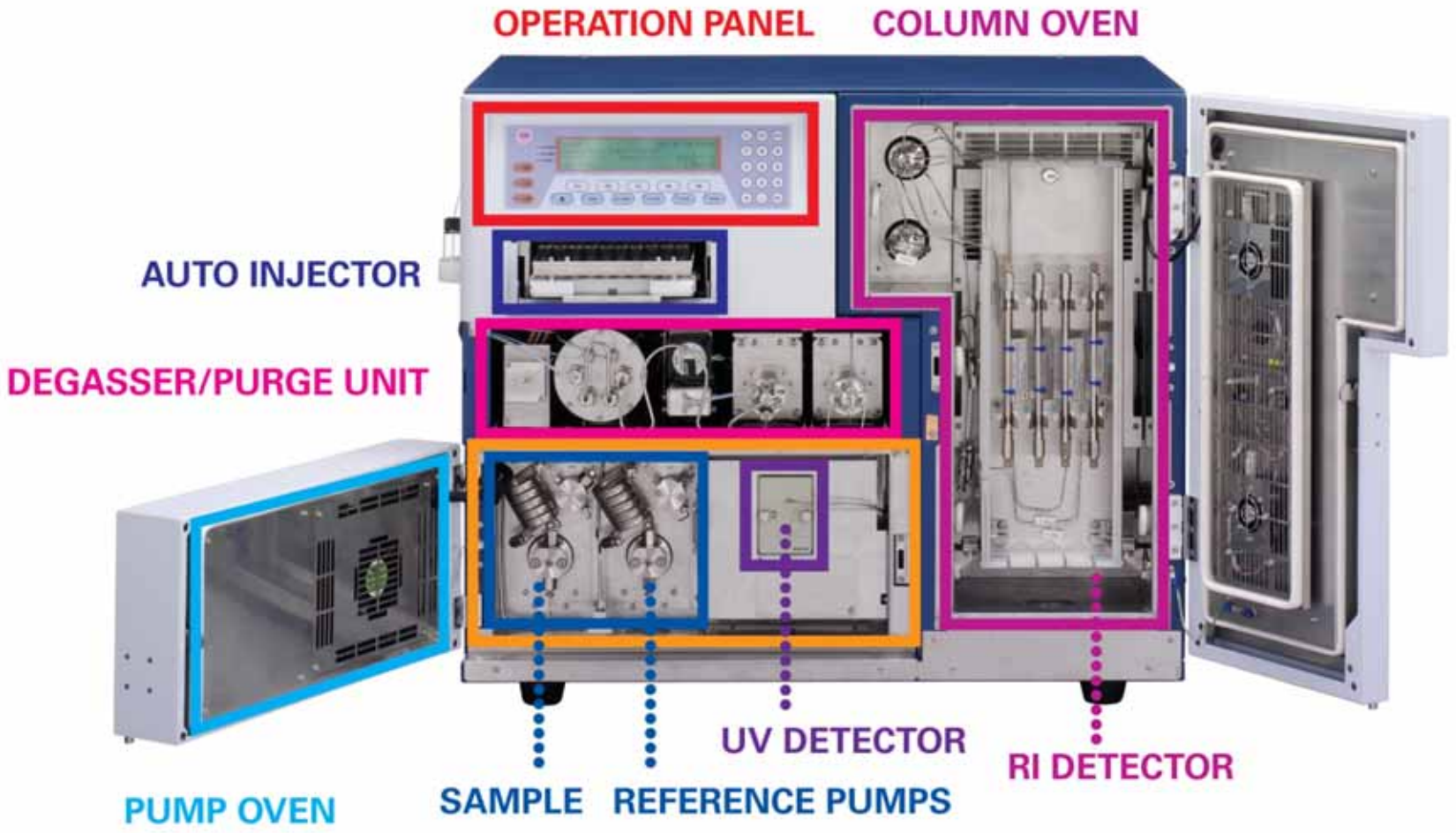
Temperature Fluctuation



Temperature Fluctuation



View of EcoSEC GPC System



Benefits of the EcoSEC GPC System

- Excellent retention time reproducibility
- Retention times are independent of temperature fluctuations in the lab
- Low system dead volume - allows the use of semi-micro columns
- A very stable RI baseline

Low RI Noise of EcoSEC GPC System

- Single-head pumps for sample and reference side reduce RI flow-rate pulsations
- To lower RI noise further, pumps, columns, UV, and RI are compartmentalized for precise temp control
- Sample is injected in temp controlled environment
- Reference side of instrument equipped with either reference* column or second column. Coupled with flow through RI reference cell to produce very stable baseline

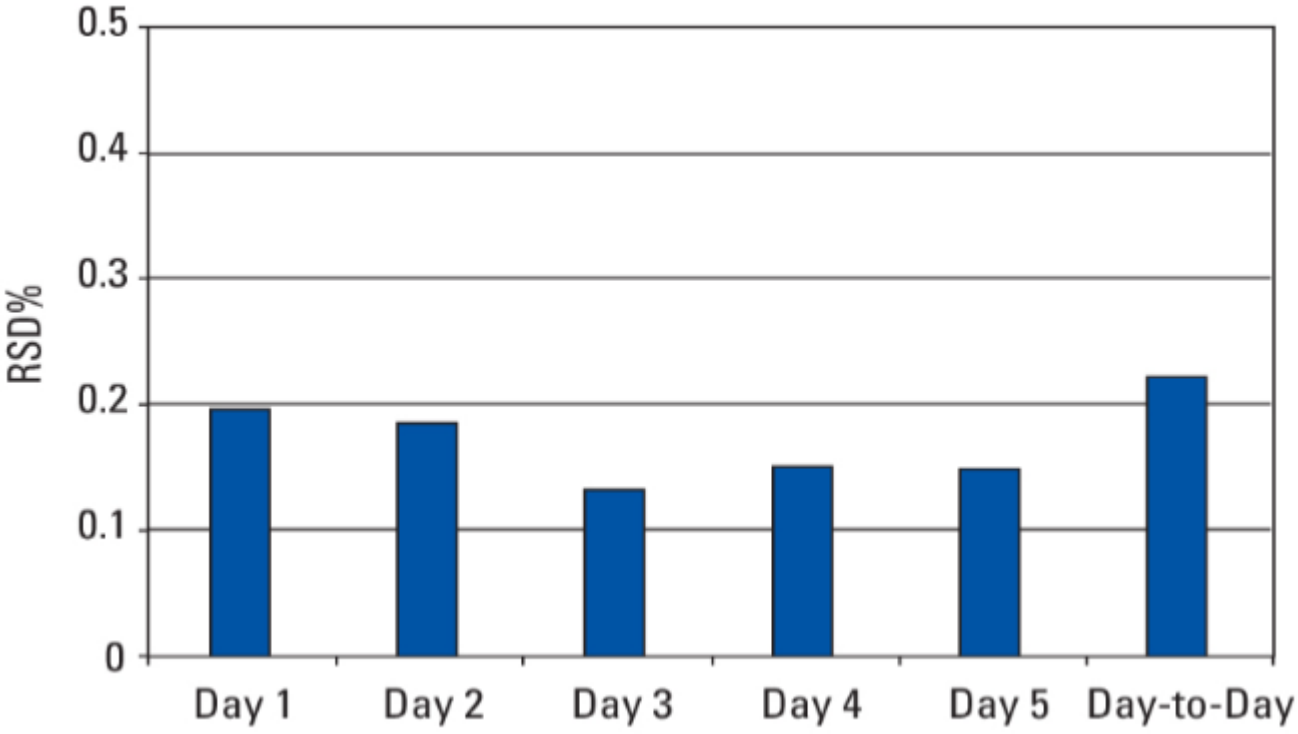
**Packed with 3µm, cross-linked PS/DVB.*

EcoSEC GPC System Accuracy

M_W[1]	M_W[2]	Mn[3] 1.02 PD	Mn[2]
5,970	5,730 (-4.0% error)	5,850	5,530(-5.5% error)
96,400	95,100 (-1.3% error)	95.4	92,900 (-2.6% error)

(1) from light scattering
(2) from EcoSEC GPC System
(3) based on 1.02 PD (poly-dispersity)

MW Reproducibility of a Terpolymer



Ten injections per day using poly(vinyl chloride-vinyl acetate-vinyl alcohol) (Mw= 30,000); TSKgel SuperHZ-M column (4.6mm ID x 15cm, 4µm) x 2; 0.35mL/min, injection volume of 5µL; THF mobile phase; measured using an EcoSEC GPC system RI detector.

Reproducibility at Four Different Sites with Four Different EcoSEC GPC Systems

A polydisperse poly(vinyl chloride-vinyl acetate) sample was run using a TSKgel SuperHZ-M column (4.6mm ID x 15cm, 4µm) x 2 at a flow rate of 0.35mL/min at 40 °C using THF as the mobile phase.

	M_n	M_w	M_z
Site A – System 1	13,800	29,800	53,700
Site B – System 2	13700	29,900	54,300
Site C – System 3	13,600	29,800	53,200
Site D – System 4	13,700	30,200	54,100
Average	13,700	29,900	53,800
Deviation	70	160	420
% RSD	0.52	0.55	0.78

Conclusions

- Flow rate, baseline drift, temperature fluctuations affect accuracy and reproducibility of MWD measurements.
- The EcoSEC GPS system is a high-sample throughput, high-performance instrument that rapidly characterizes polymers with unsurpassed efficiency, reliability and reproducibility.
- With the EcoSEC GPC system, methods developed at one location can be transferred to other plant sites.
- With low-dead volume engineering, the EcoSEC GPC system is optimized for semi micro technology:
    - analysis times are reduced by 50%
    - solvent consumption and disposal costs lowered by 1/6

Acknowledgments

- Bruce Kempf, Technical Services, Tosoh Bioscience LLC
- Hiroshi Tomizawa, Column Marketing Manager, Tosoh Corporation
- Dr. Howard Barth, Consultant, Polymer Characterization, Wilmington, DE

For more information visit our website at:
www.tosohbioscience.com