



Reproducibility and Accuracy in Modern Size Exclusion Chromatography

Xavier Villarreal, GPC Product Manager, Tosoh Bioscience LLC

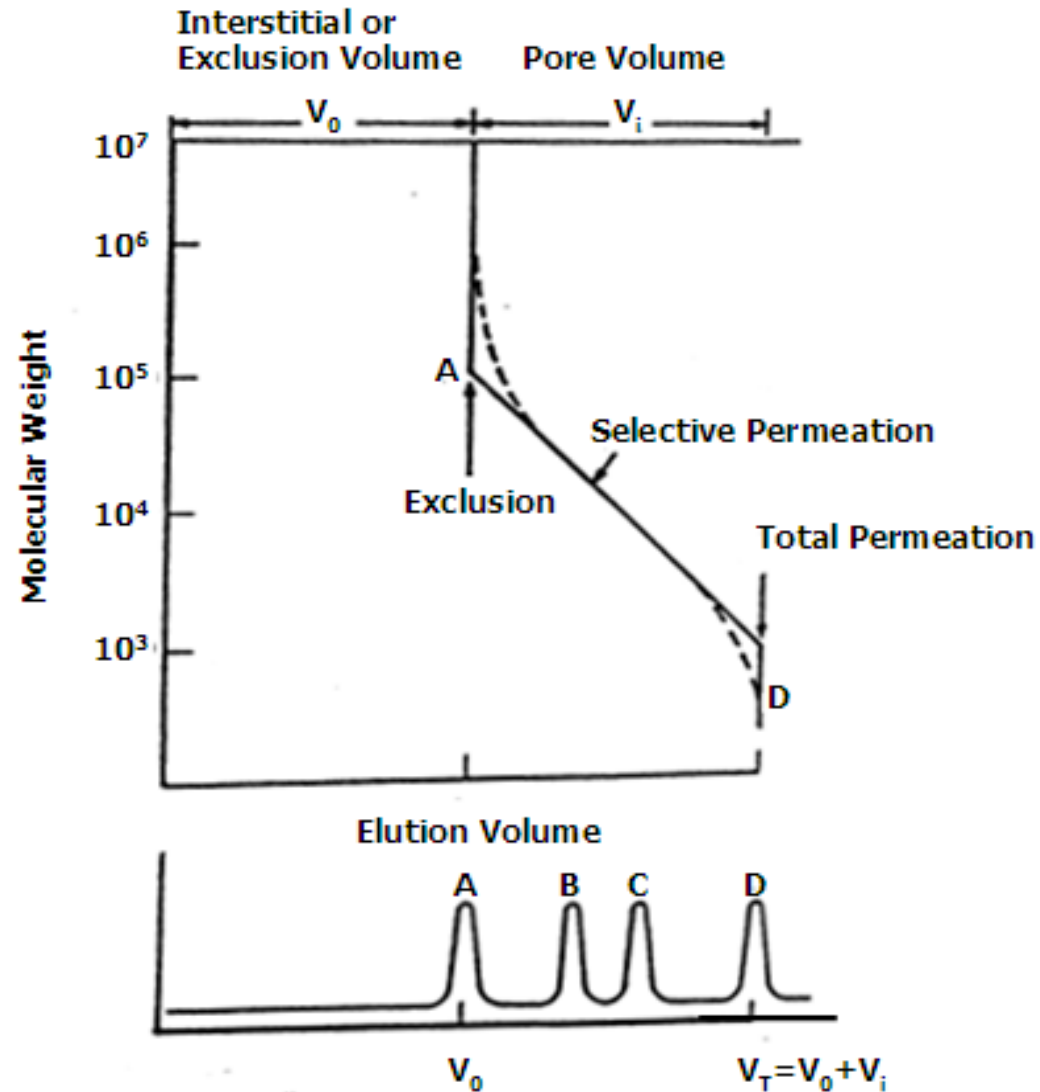


Agenda

- 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

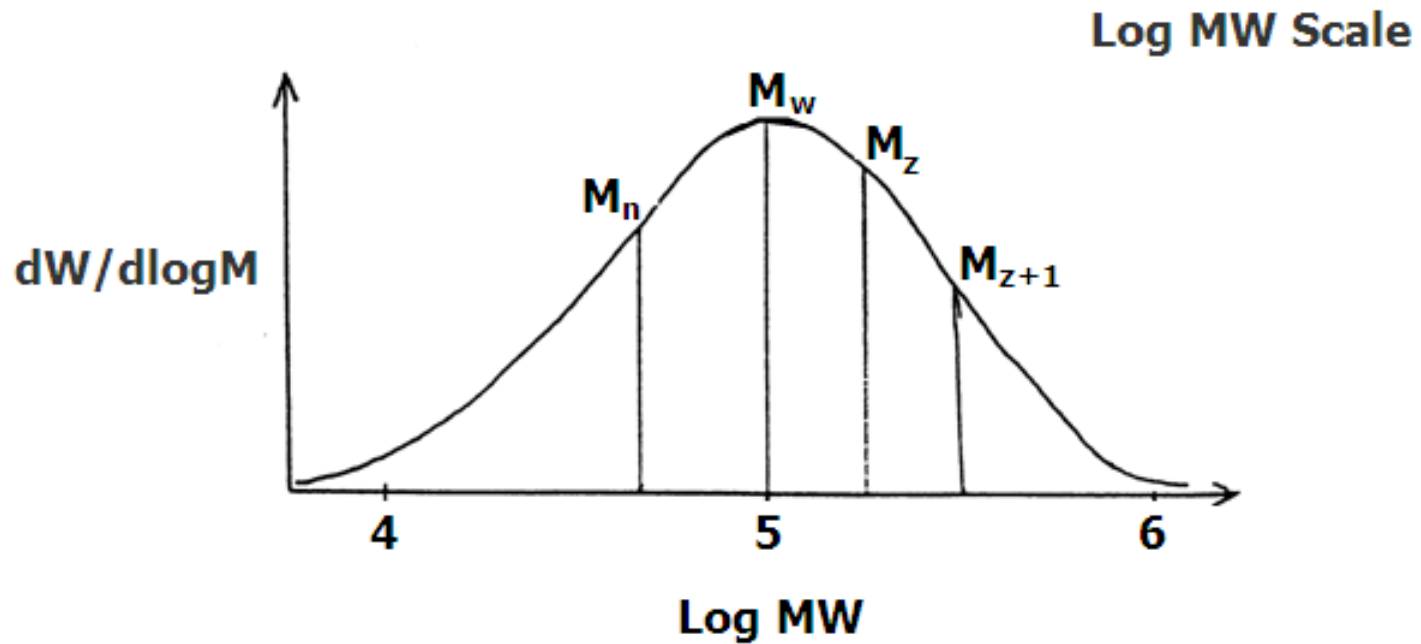


SEC Terminology





Mass Distribution of Polymers





Guidelines for Improving MW Accuracy

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



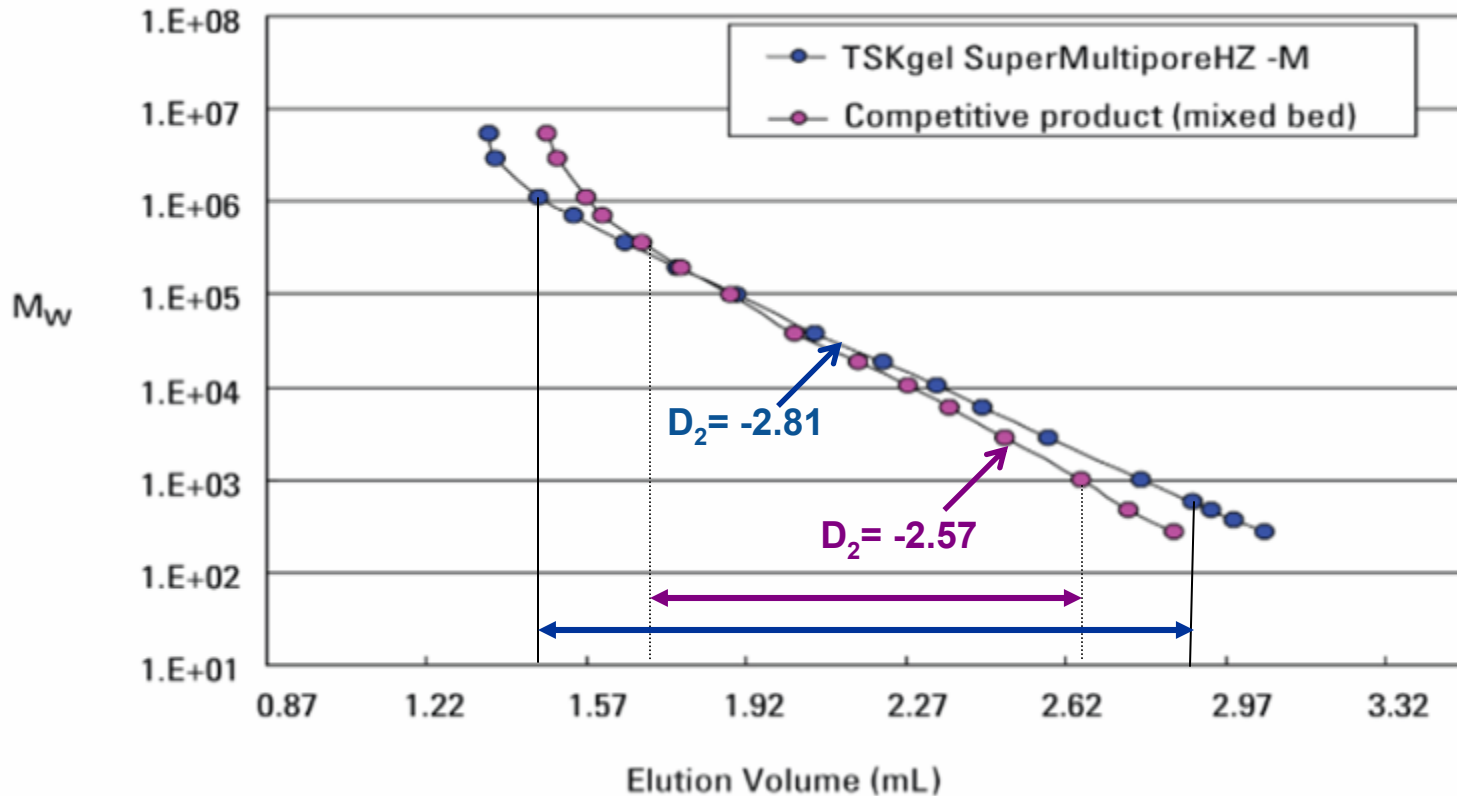
Guidelines for Improving MW Accuracy

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 (10 μ l) injected onto TSK-GEL SuperMultiporeHZ-M column (4.6mm ID x 15cm, 4 μ m) 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.

Std MW	Flow Rate	% Error		
		M_n	M_w	M_z
2.89 x 10⁶	0.355	+10.6	+12.4	+13.4
	0.345	-6.6	-3.5	-2.6
9.64 x 10⁴	0.355	+18.4	+18.5	+19.5
	0.345	-9.6	-9.6	-9.6
5.97 x 10³	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; 10 μ L; 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:
< 100 μ L for 7.8 mm ID columns
< 10 μ L 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

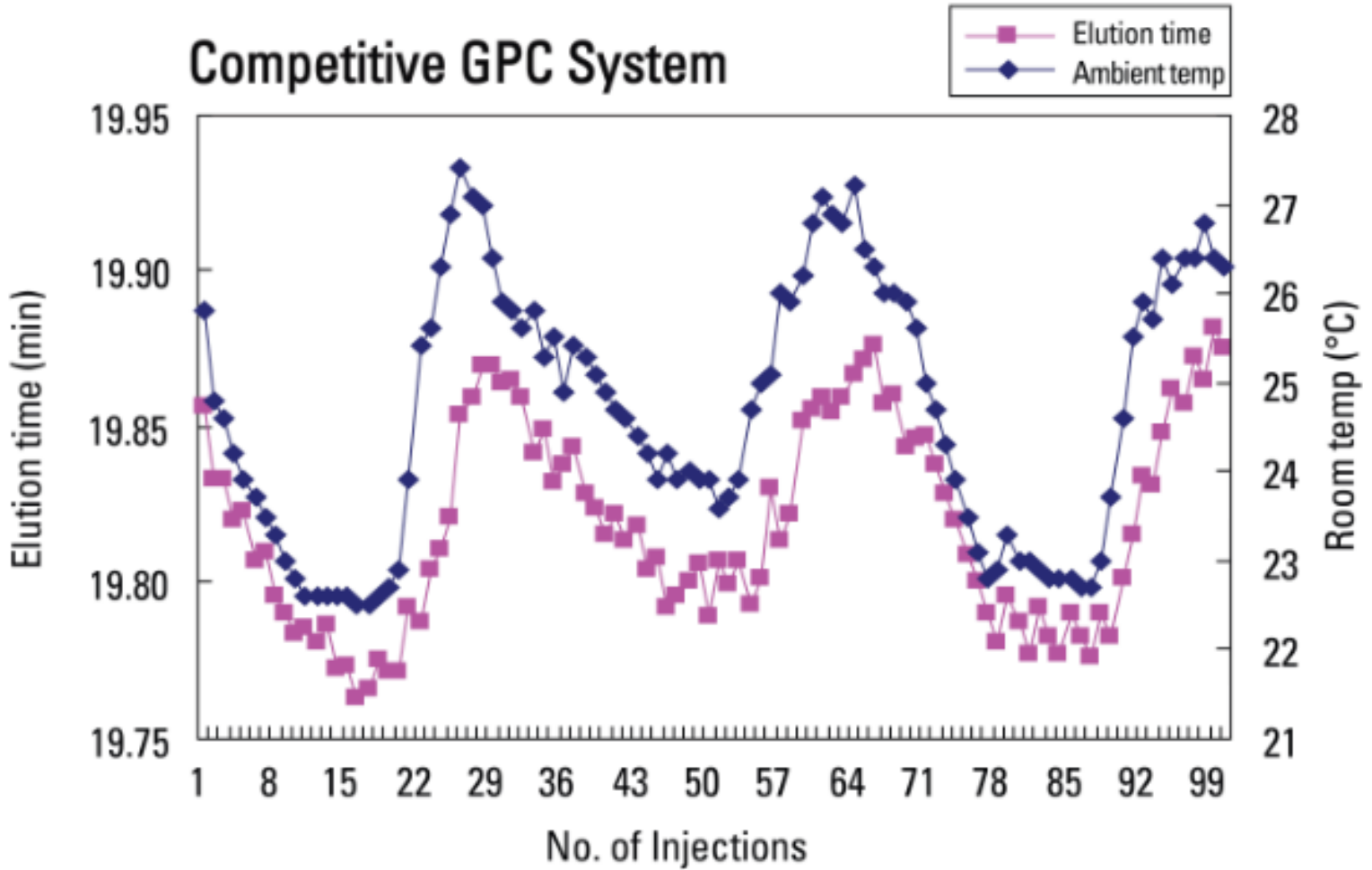
Packing pore structure

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

Retention volume

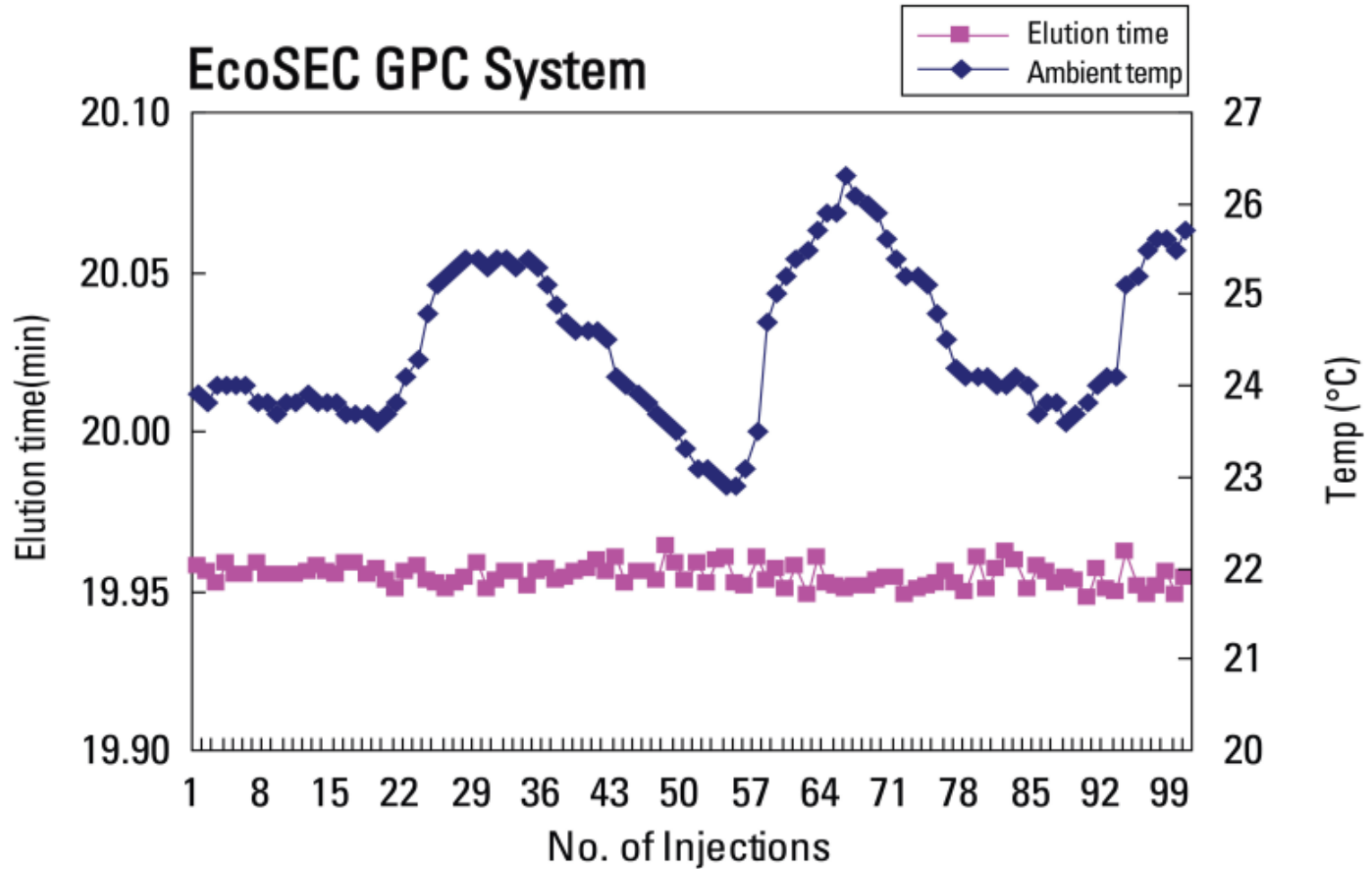


Temperature Fluctuation



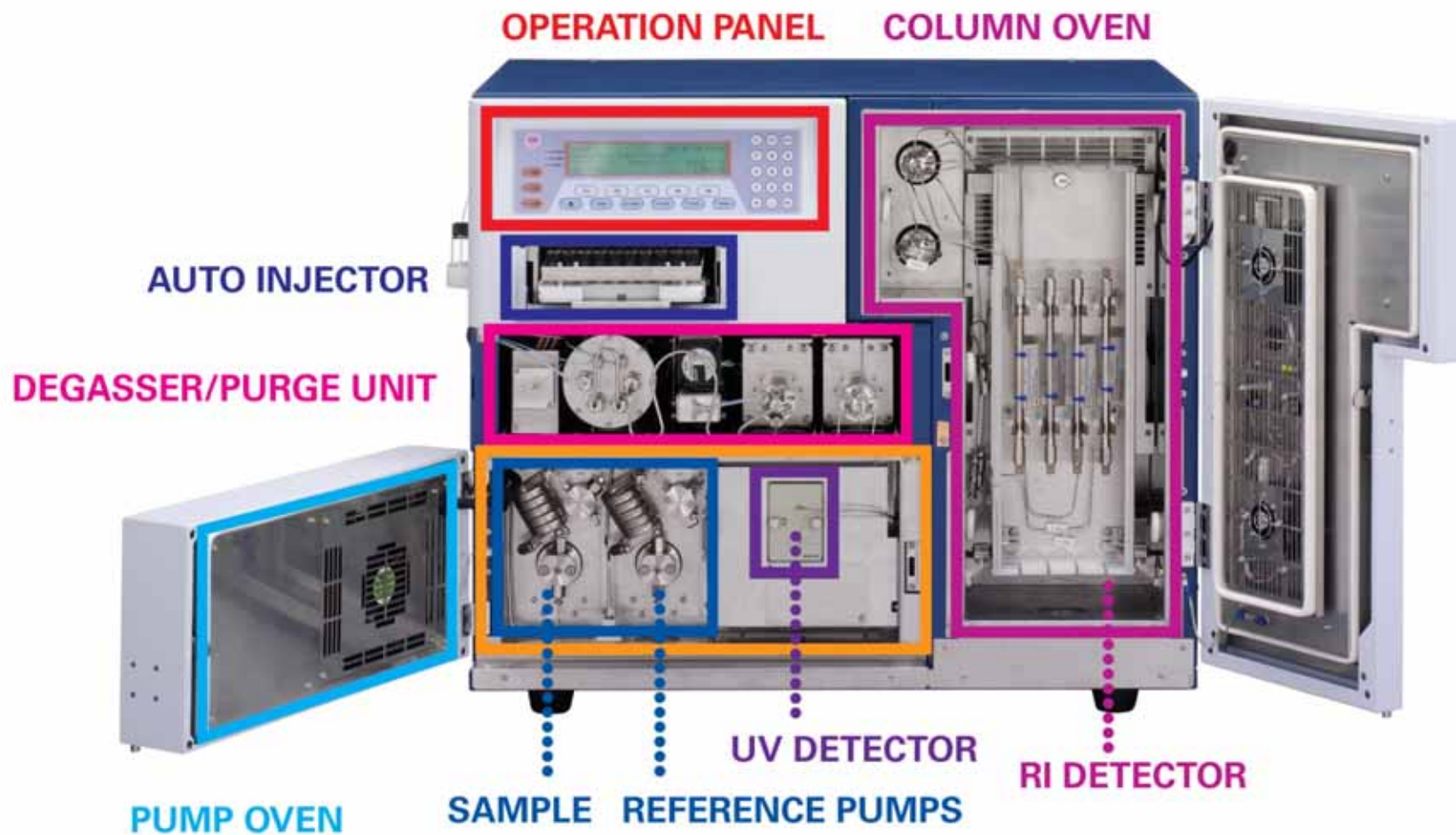


Temperature Fluctuation





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 3 μ m, cross-linked PS/DVB.*

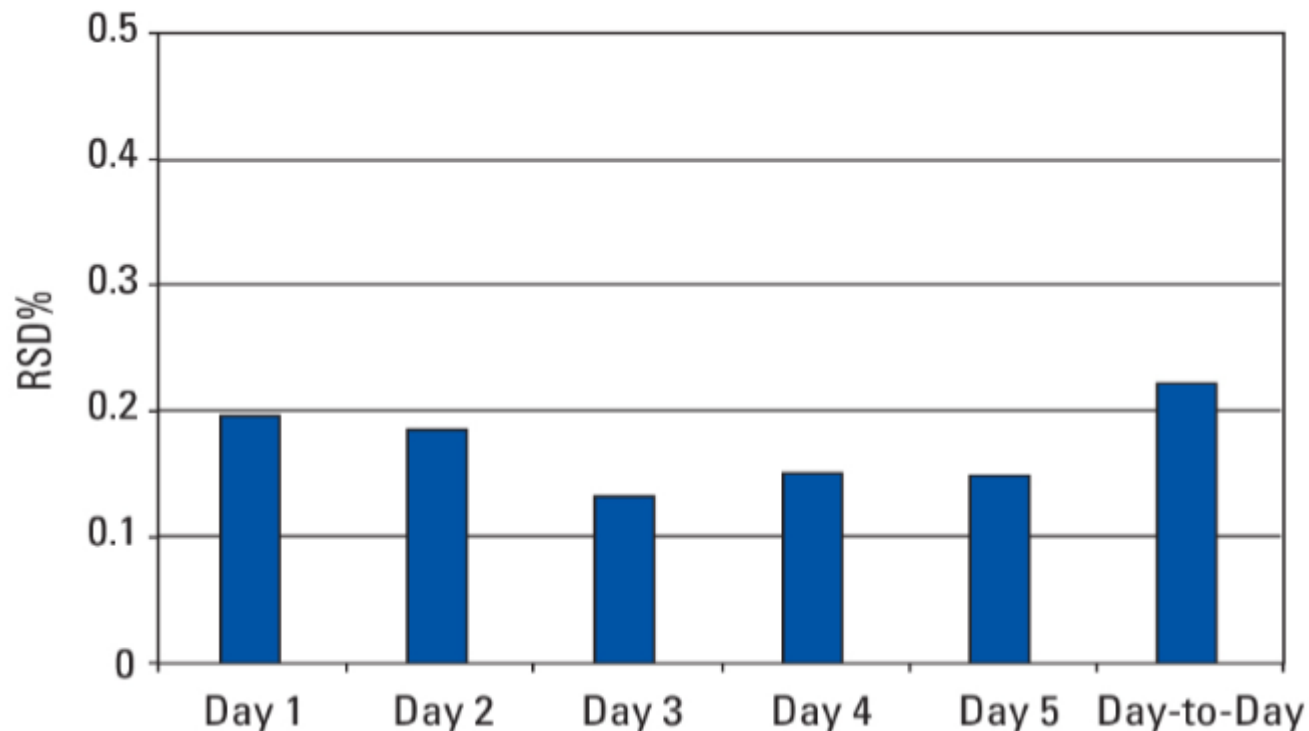


EcoSEC GPC System Accuracy

M_w^1	M_w^2	M_n^3 1.02 PD	M_n^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) ($M_w = 30,000$); TSKgel SuperHZ-M column (4.6mm ID x 15cm, 4 μ m) x 2; 0.35mL/min, injection volume of 5 μ L; 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, 4 μ m) 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 GPC 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