EcoSEC® GPC Workstation Software Multiprocessing Feature

EcoSEC GPC System
APPLICATION NOTE

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Introduction

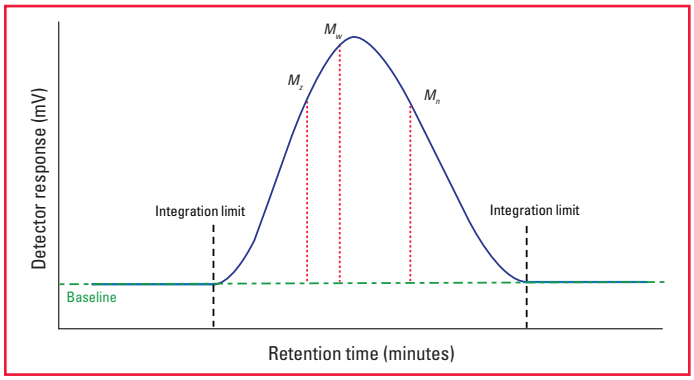
The Tosoh Bioscience EcoSEC GPC System has rapidly gained acceptance as the state-of-the-art instrument for the characterization of the molar mass averages of polymers. Temperature controlled pumps eliminate fluctuations in ambient lab temperature as a source of pump flow rate variability and the unique dual flow RI detector provides unmatched baseline stability. Here we report on reproducibility improvements enabled by the multiprocessing feature of the data processing software (EcoSEC GPC Workstation Software).

Common practice in the analysis of polymers via size exclusion chromatography is to perform multiple injections of several different sample dispersions to ensure repeatability and reproducibility in the molar mass averages obtained. Traditionally, the repeatability and reproducibility of molar mass averages is investigated through the analysis of inter- and intraday precision of these values by a series of round-robin tests.[1,2] Over the years round-robin tests have shown that molar mass averages are influenced by both hardware and software parameters between instruments and laboratories. Hardware parameters include column stability, flow rate precision, temperature precision of all components, detector baseline stability, and injection volume precision. Software parameters include precision of calculation procedures and baseline settings, location of start and end markers for chromatograms (integration limits), the number of data points, internal standard corrections for flow rate changes, and variations between operators. It is widely accepted among those who regularly analyze size exclusion chromatography results that the reproducibility for the same sample, injected on the same instrument, with the same experimental conditions, *e.g.* concentration, injection volume, flow rate, etc., depends on the consistency of the signal from the detector, the long term accuracy of the pumping system, and data processing parameters.[3-5] The consistency of the signal from the detector and the long term accuracy of the pumping system are both dictated by the suitability of instrument design for precise solvent flow rates and stable RI detection, the wear and tear of the physical instrument as well as the routine maintenance of the chromatography system. On the other hand, the data processing parameters can vary from injection-to-injection and user-to-user.

The ambiguity in data processing lies in defining the baseline and the integration limits within a chromatogram, *Figure 1*. The baseline for a chromatogram should always begin prior to the elution of the sample peak and end after the solvent peak once the baseline has fully recovered. Arbitrary selection of integration limits is one of the most significant and restrictive aspects of accurate molar mass analysis by size exclusion chromatography. Variations in the assignment of integration limits on the low molar mass, late elution volume portion of a chromatogram results in dominant errors in the value of the number average molar mass M_n, while variations in the assignment of the integration limit on the high molar mass, early elution volume, portion of a chromatogram results in errors in the value of the *z*-average molar mass M_z.[3,5] Conversely, the integration limits have been shown to have little effect on the value of the weight-average molar mass M_w as the errors occurring on both the low and high molar mass portions of the chromatogram tend to cancel themselves out.[5]

To eliminate the errors caused by variations in the assignment of baselines and integration limits from injection-to-injection for the same sample, instrument, experimental conditions, and operator; some size exclusion chromatography specific software now include a multiprocessing feature which allows for simultaneous determination of baselines and integration limits for duplicate samples injections.

Figure 1. Example of baseline and integration limits selection for a chromatogram and the relative location of each of the molar mass averages, M_n, M_w, and M_z.



Experimental Conditions

Sample analysis was performed on a system consisting of an EcoSEC GPC System (HLC-8320) equipped with a refractive index detector (RI). Separation of unfiltered 20 µL injections occurred over a column bank consisting of two 6.0 mm ID × 15 cm, 3 µm particle size TSKgel® SuperH3000 columns preceded by the appropriate guard column (Tosoh Bioscience LLC). The solvent and mobile phase were tetrahydrofuran (THF) (Fisher Chemical) at flow rates of 0.3 mL/min. Detector, pump oven, and column oven were maintained at 35 °C. Sample dispersions were prepared by diluting Polymer A (modified polyurethane prepolymer) (99% purity) with THF for a final sample concentration of approximately 10 mg/mL. Samples were shaken manually for a minute and allowed to sit for 3 hours before analysis was performed. Data was processed with the EcoSEC GPC Workstation software version 1.11.

A calibration curve was created using PStQuick Kit-L polystyrene standards (Tosoh Bioscience LLC) ranging in molecular weight from 266 to 37,900 g/mol. Calibration curve data for 0.3 mL/min were fitted with a cubic function and error values were no greater than 5%.

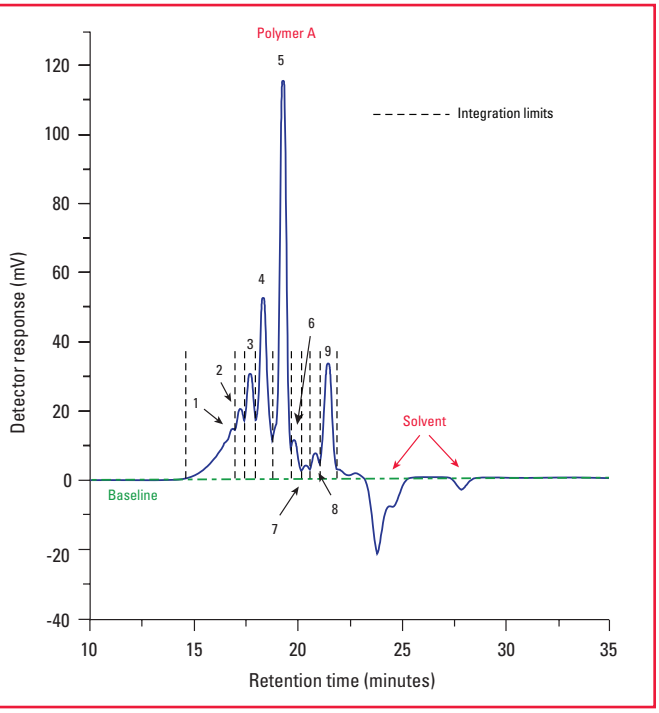
For all chromatographic determinations, results are averages of three injections from two separate sample dispersions. The three replicate injections of each sample dissolution were processed separately using two independent methods. The two methods used were individual processing and multiprocessing. For individual processing, the assignment of baselines and integration limits were performed on each individual injection independent of the previous injection. For multiprocessing the assignment of baselines and integration limits for the three injections from a given sample dissolution were determined simultaneously.

Procedure

Individual Processing

Figure 2 shows the relative assignment of the baseline and integration limits that was applied individually to each injection. For each individual injection, the baseline was drawn from a location just prior to the elution of the first chromatographic peak to a location where the baseline had fully recovered, just past the elution of the solvent peaks. The chromatogram was determined to have nine peaks. Since baseline resolution was not obtained between peaks, the integration limits of each peak were defined as the center of the valley occurring between any two peaks. The start marker for peak one was defined as a location just prior to the fronting of the peak. The end marker for peak nine was defined as the center of the valley occurring between peak nine and the peak corresponding to the peroxide inhibitor in the THF.

Figure 2. Relative assignment of the baseline and integration limits as applied individually to each injection.



Multiprocessing

The multiprocessing feature found in the EcoSEC GPC Workstation software was used to process replicate injections of the two sample dispersions simultaneously. For this particular sample set, three injections of Polymer A from the same dispersion were processed, i.e. application of a calibration curve, baseline assignment and integration limits, using the multiprocessing feature. The use of the multiprocessing feature allowed for the replicate injections to be processed in the amount of time typically required for the processing of one injection, thus decreasing data processing time by more than 60%.

Results and Discussion

As previously discussed in the "Introduction," ambiguities in the processing of size exclusion chromatography data for the determination of molar mass averages lies in defining the baseline and the integration limits within a chromatogram. Inconsistencies in the selection of integration limits play a dominant role not only in the calculations of the molar mass averages and distributions but also in the standard deviations of these values. As can be seen by comparing standard deviations in the molar mass averages obtained via individual processing and multiprocessing for a complex polymer sample, *Table 1*, errors caused by variations in the assignment of baselines and integration limits for replicate injections of the same sample can be dramatically decreased by using the multiprocessing features.

Table 1. Molar mass averages, standard deviations and relative standard deviations using individual processing and multiprocessing of replicate injections.

Individual Processing

Peak	M_n	Rel Std Dev	M_w	Rel Std Dev	M_z	Rel Std Dev
1	4,218[a] ± 27[b]	0.63%	4,625 ± 26	0.56%	5,526 ± 25	0.48%
2	2,654 ± 15	0.57%	2,666 ± 14	0.51%	2,678 ± 11	0.42%
3	2,015 ± 29	1.43%	2,028 ± 30	1.50%	2,043 ± 29	1.41%
4	1,388 ± 9	0.65%	1,403 ± 11	0.75%	1,418 ± 12	0.81%
5	800 ± 1	0.07%	808 ± 1	0.12%	817 ± 2	0.19%
6	553 ± 5	0.91%	556 ± 5	0.91%	558 ± 5	0.92%
7	394 ± 4	1.02%	397 ± 4	1.01%	400 ± 4	1.01%
8	282 ± 4	1.24%	284 ± 2	0.54%	286 ± 2	0.53%
9	178 ± 1	0.56%	181 ± 1	0.83%	183 ± 2	0.83%

Multiprocessing

Peak	M_n	Rel Std Dev	M_w	Rel Std Dev	M_z	Rel Std Dev
1	4,205 ± 9	0.20%	4,630 ± 19	0.41%	5,526 ± 25	0.48%
2	2,648 ± 1	0.04%	2,659 ± 1	0.02%	2,670 ± 1	0.02%
3	2,006 ± 1	0.06%	2,021 ± 1	0.06%	2,036 ± 1	0.06%
4	1,382 ± 2	0.11%	1,396 ± 2	0.15%	1,411 ± 2	0.12%
5	798 ± 2	0.22%	807 ± 2	0.21%	816 ± 2	0.21%
6	545 ± 1	0.11%	548 ± 1	0.01%	551 ± 1	0.01%
7	388 ± 2	0.45%	390 ± 1	0.15%	392 ± 1	0.15%
8	281 ± 1	0.21%	283 ± 1	0.20%	285± 1	0.20%
9	179 ± 1	0.56%	182 ± 1	0.32%	184 ± 1	0.31%

[a] Average of three replicate injections from the same dispersion
[b] Standard deviation of three replicate injections from the same dispersion

By implementing the use of the multiprocessing feature for the data processing of replicate injections of the same dissolution the standard deviation in the molar mass averages decreased anywhere from 30 to 90%, depending on the peak in Polymer A. Significant decreases in the standard deviations associated with the molar mass averages obtained via multiprocessing rather than individual processing are a direct result of decreasing the variation in baseline and integration limit assignments from injection-to-injection. Additionally, the relative standard deviations for the peaks in Polymer A also decreased significantly using the multiprocessing feature. The added precision obtained by using multiprocessing allows for detailed studies of polymerization reactions and increases precision in determination of molar mass averages and distributions. Furthermore, in processing large sample sets with replicate injections, the use of the multiprocessing feature decreases the active operator time significantly, e.g. the processing time of three replicate injections decreases by more than 60%.

Conclusions

Molar mass averages of polymers determined via size exclusion chromatography are influenced by hardware and software parameters. Hardware parameters are dictated by the physical instrument while software parameters are dictated not only by the actual software package but also by assignment of the baseline and integration limits determined by the operator. Operator discretion in the baseline and integration limit assignments can decrease the precision of the molar mass averages and skew the values for both the number and *z*-average molar masses. The use of a multiprocessing software feature in lieu of individually processing for replicate injections of the same sample dissolution was shown to dramatically decrease the standard deviation within the injections. The precision in the molar mass values for the complex polymer examined here were shown to improve by 30 to 90% by implementing the use of the multiprocessing software feature in the EcoSEC GPC Workstation software. Finally, the use of the multiprocessing feature was also proven to significantly decrease the amount of hands on operator time needed for data analysis.

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