Using Uncertainty Analysis to Optimize GPC System Performance

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Introduction

GPC when coupled with Static Light Scattering (SLS) is a fairly modern and powerful technique for determining absolute molecular weight values without the need to rely on traditional column calibration. In GPC-SLS the signal intensity levels rather than elution times are evaluated quantitatively. In addition, unlike in polymer standard calibration, details of the accurate sample concentration, injection volume and therefore accurate mass, is required for accurate interpretation of light scattering signals. Quantitatively analyzing sources of uncertainty in experimental

Objective

Quantitatively show the effect of column loading on molecular weights determined by GPC-LS. Column loading reflects the coupled errors in sample concentration and injection volume.

1. In standard calibration the GPC instrument is calibrated using a number of polymer standards and a calibration curve is constructed. The molecular weight of an unknown sample is obtained by observing the elution volume of the unknown sample and using the calibration curve. For column calibration, it is notable that the actual concentration and injection volume of standards, e.g., column loading, used for the calibration is not significant as long as they do not cause artifacts.

2. In universal calibration, the GPC instrument is calibrated using a number of polymer standards with known molecular weights. A calibration curve for the column is constructed by plotting the log of the known molecular weight times the intrinsic viscosity of the standards as a function of elution volume. The molecular weight of an unknown sample is obtained by observing the product of elution volume and intrinsic viscosity of the unknown sample and using the calibration curve.

3. In GPC-SLS the signal intensity levels rather than elution times are evaluated quantitatively. In addition, unlike in polymer standard calibration, details of the accurate sample concentration and injection volume and therefore accurate column loading, is required for accurate interpretation of light scattering signals.

However the actual injection volume can be significantly in error due to an incorrectly assigned loop volume. And, the actual injection concentration can vary due to insufficient attention given to this area. Fortunately, once recognized, both issues are readily resolved and enhanced system performance is obtained. The level of this sensitivity and its implications for molecular weight determination are discussed here.

Experimental

Eluent: THF
Degasser: Agilent G1379A
Pump: Agilent G1310A, isocratic
Flow Rate: 1 mL/min
Autosampler: Agilent G1313A
Injection Volume: 100 µL
Columns: PSS SDV EasyValid + PSS SDV 500 A
UV Detector: Agilent G1314A @ 280 nm
LS Detector: BI-MwA multi-angle laser light scattering
RI Detector: BI-DNDC differential RI (620 nm)

Analysis Choices

There are three choices for analyzing obtained data. These choices can be organized according to what is known about the sample. There are two possible parameters about an unknown that may be known in advance: the injected mass and the sample dn/dc. The three possible conditions are listed below

Column Loading (injected mass * injection volume)	Refractinve index increment, dn/dc
Known	Known
Known	Unknown
Unknown	Known

Light Scattering Calculations

Calculating Molecular Weight (and SLS Calibration)
Light scattering data is typically analyzed with the Zimm equation:

$$\frac{Kc}{\Delta R} = \frac{1}{M_w}\left(1 + \frac{q^2 R_g^2}{3}\right) + 2A_2 c$$

Here, K is the Debye constant, a constant of the polymer/solvent system. For vertically polarized light,

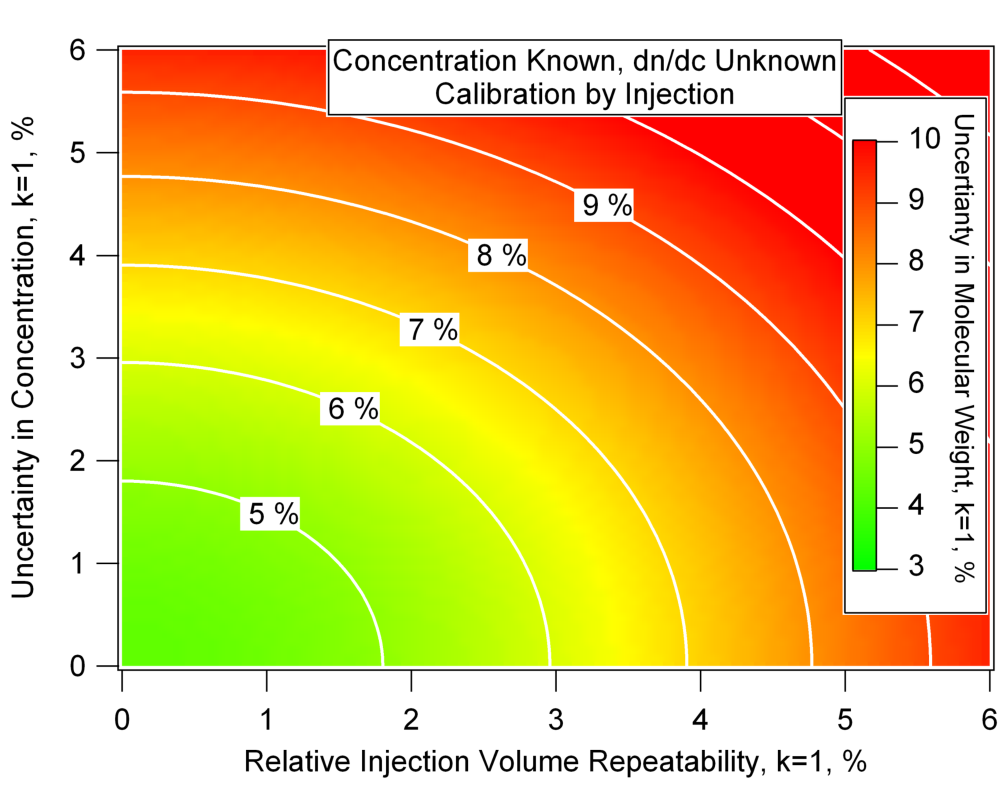
$$K = 4\pi^2 n^2 (dn/dc)^2/(N\lambda^4)$$

where n is the solvent refractive index, N is Avogadro's number, and λ is the wavelength of the laser.
Polymer concentration, c, is determined when sample solutions are prepared, and ΔR is proportional to the excess scattered intensity and measured.
For molecular weight determination, (dn/dc) and concentration must be known for each slice of the chromatogram

Example Calculations

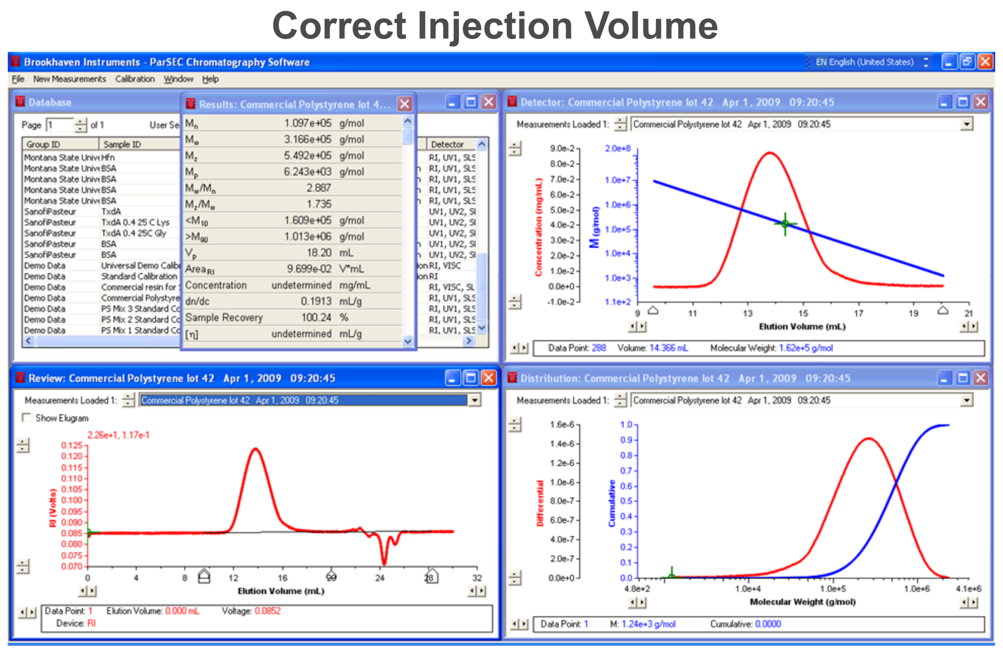
An example of the effects of injection volume variation is shown below. Data was collected with ParSEC software and analyzed in three ways. The first is with the injection volume set 1% too low The second is with the correct injection volume (the same as used for calibration), 100 microliters. The third is with the injection volume set 1% too high to 101 microliters. Analysis was performed for the case where concentration is known and dn/dc is unknown. Note that the determined molecular weight has also changed by about 5%. This illustrates the impact of varying the injection volume.

Results of Uncertainty Calculations

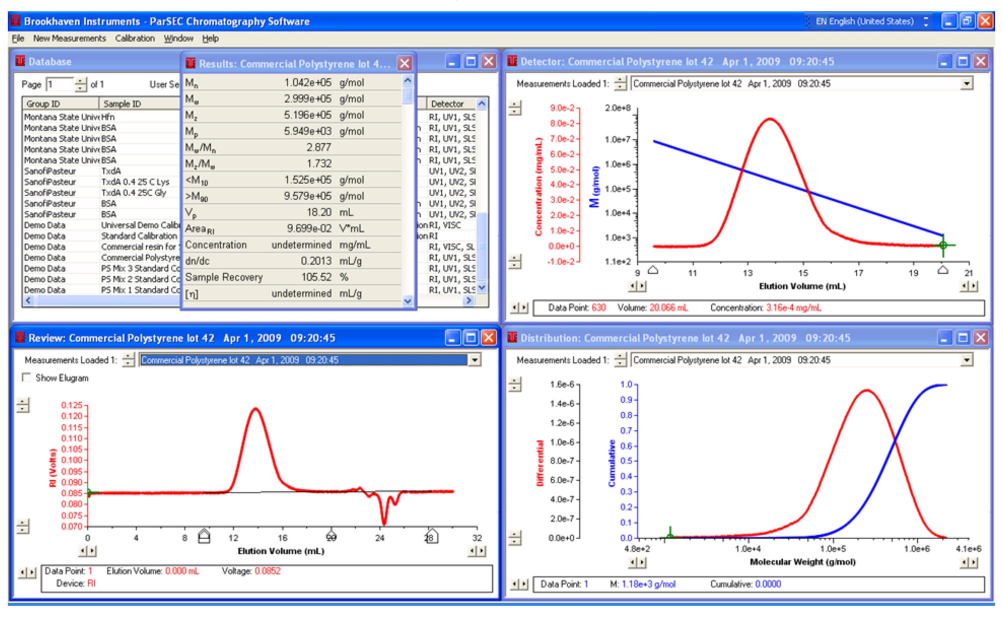


Results and Discussion

Correct Injection Volume



Incorrect Injection Volume



Stated Injection Volume (µL)	M_w (g/mol)	M_n (g/mol)	Poly-dispersity (M_w/M_n)	Comments
99	313,200	108,600	2.89	
100	316,600	109,700	2.89	Correct injection volume
101	319,900	110,700	2.89	

Conclusions

Reproducible column loading is critical for successful implementation of GPC-LS. This requires that both injector perfo

Choice of Analysis Method
If the injector repeatability is no better than 2%, analyzing a samples with an unknown injected mass (concentration and injection volume) and a known dn/dc value is preferred. The other choices are not.

Injector Performance Target
When analyzing samples with unknown values of dn/dc (the most common case), injector repeatability should be better than 1%. This specification is easy to meet.

Sample Concentration Performance Target
When analyzing samples with unknown values of dn/dc (the most common case), sample concentration should be accurate to better than 1%. This specification is easy to meet.