GPC From PeakSimple Data Acquisition

Introduction

The following is an outline of how PeakSimple data acquisition software/hardware can be used to acquire and analyze (in conjunction with an appropriate spreadsheet) gel permeation chromatography data. At this time, two different version of PeakSimple software were required for successful analysis. Version 2.08 was used to collect the data and obtain result tables for narrow polymer standard chromatograms, while version 2.09 was used to obtain the peak slice information for broad unknown polymers. That is, using 2.09, the voltage difference between the detector output and the subsequently drawn baseline was obtained for each data point and saved as an ASCII file, which was then imported into Excel for in-depth GPC analysis. Ultimately, it would be preferred to use only one version of PeakSimple. However, 2.09 (the latest version) was not stable while acquiring data. The program would crash after approximately 5 minutes. Furthermore, the time display in the upper right hand corner did not appear to work and retention windows were not visible on the screen although a component file was active. Thus, 2.09 was used only for obtaining slice information with non-active channels.

To illustrate how PeakSimple can be used for GPC analysis, I have included 3 narrow polystyrene standard chromatograms (4 standards per chromatogram) and two broad unknown polymer chromatograms. Chromatograms were obtained using a Waters 510 pump (U6K injector), an ethyl acetate mobile phase (1 mL/min), a series of Ultrastyrogel® columns (Waters 10^6, 10^4 and 500 Å) and Waters 2410 refractive index detector. All polymers were pre-dissolved in ethyl acetate and chromatograms were collected at 1 Hz. Polystyrene standard concentrations were 0.1 % w/w or less (50 μL injection volume) while broad unknown polymers were approximately 1 % w/w (75 μL injection volume). Also included are component files, containing the standard identities and expected retention windows, an event file for integration, and two ASCII data files containing slice information for the broad unknown polymers, and an Excel file with in-depth GPC analysis.

Obtaining a Calibration Curve

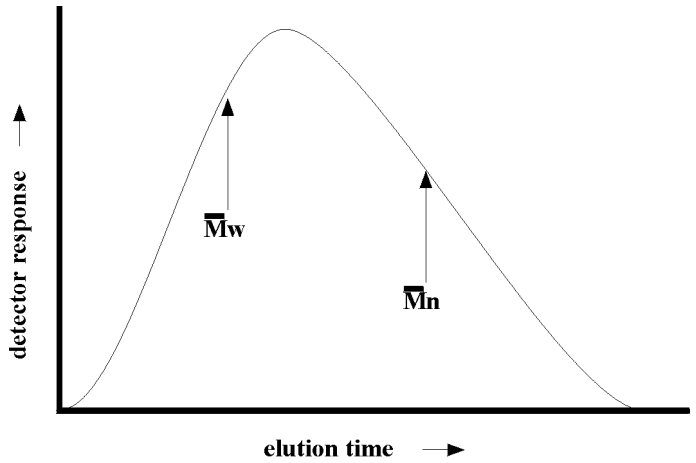
Polydisperse polymers in solution are fractionated according to size or hydrodynamic volume during GPC, which is also known as size exclusion chromatography. Molecular weight is related to the hydrodynamic volume. In GPC a dilute polymer solution is injected into a solvent stream which then flows through a series of columns packed with porous gel beads. Smaller molecules pass through and around the beads while larger molecules are excluded from all but the largest pores. Thus fractionation occurs with the largest molecules eluting first. The molecular weight of an eluting polymer molecule varies exponentially with eluting volume, the latter of which is proportional to time under constant flow rate conditions. To obtain molecular weight data and convert the GPC chromatogram into a molecular weight distribution, the relation between molecular weight and elution time is obtained from a series of polymer standards of known molecular weight. The calibration curve is thus obtained from a plot of the logarithm of molecular weight versus time. Given that GPC is a comparison of hydrodynamic volumes, unknown molecular weight determinations will be relative to the calibration standards. For a good introductory reference to polymer science, see R. J. Young and P. A. Lovell, Introduction to Polymers.

Using PeakSimple 2.08, the result table for each of the three polystyrene standard chromatograms was copied using DDE into Excel. The natural logarithm of molecular weight versus time was plotted and a best fit analytical approximation to the curve was obtained from a third order polynomial, $P(t_e)$. This is the calibration curve relating molecular weight to elution time.

Obtaining Molecular Weight Averages

The most common and convenient way to characterize a distribution of molecular weights making up a polymer sample is using molecular weight averages such as, number average molecular weight ($\overline{M}_n$), and weight average molecular weight ($\overline{M}_w$), as shown in the following figure for a typical polymer chromatogram. $\overline{M}_n$ is defined as a sum of products of the molecular weight of each fraction multiplied by its mole fraction. That is: $\overline{M}_n = \sum X_i M_i$ where X_i is the mole fraction of molecules of molecular weight mass M_i. The weight average molar mass is

defined as a sum of the products of the molecular weight of each fraction multiplied by its weight fraction, w_i. That is: $\overline{M}_w = \sum w_i M_i$. Additionally, it can be shown that the number average molecular weight, in terms of weight fraction, is equal to: $\overline{M}_n = 1/\sum(w_i/M_i)$. The ratio $\overline{M}_w/\overline{M}_n$ is known as the polydispersity or polydispersity index (PDI). The PDI is often used as a measure of the breadth of the molecular weight distribution. Polymers that are monodisperse (i.e. all chains have the same molecular weight) would have a PDI of 1.



A typical polydisperse polymer molecular weight distribution showing the approximate locations of $\overline{M}_n$ and $\overline{M}_w$.

Using PeakSimple 2.09, polymers p000604 and p000606 were integrated (using the GPC event file) and the results saved in ASCII files. The ASCII files were imported into Excel and the corresponding sample times were added as a third column of data starting at time equal to zero. Only slice and time data corresponding to the major peak of interest were retained (columns A,B and J,K respectively). For each time slice, a corresponding molecular weight, M_i, was calculated using the analytical equation fitted to the calibration curve (columns C and L, respectively). Note that extrapolation of a few minutes outside of the last standard (MW = 1,000,000) is usually not a problem. Furthermore, the refractive index response of the detector is proportional to the weight concentration of eluting polymer, independent of molecular weight. Thus, the weight fraction, w_i, of polymer in any slice is equal to the detector voltage response or height (baseline subtracted) divided by the sum of detector voltage responses for each polymer

elution slice (i.e. $w_i = \text{height}_i / \sum \text{height}_i$, columns D and M respectively). $\overline{M}_w$ was obtained by multiplying w_i and M_i and summing the appropriate columns (see bottom of columns E and N) $1/\overline{M}_n$ was obtained by dividing each w_i by M_i and summing the appropriate columns (see bottom of columns F and O). Thus, the molecular weight averages for the two polymers were obtained and are summarized in the following table.

Polymer Molecular Weight Averages

	P000604	P000606
$\overline{M}_w$	143,000	299,000
$\overline{M}_n$	69,500	99,300
PDI	2.06	3.01

Obtaining Normalized Molecular Weight Distributions

As mentioned the polydispersity index (PDI) is often used as a measure of the breadth of the molar mass distribution. However it is a often a poor substitute when compared to a graphical representation of the complete molecular weight distribution curve, especially when comparing polymer distributions. To a first approximation, the raw chromatogram (a graph of detector response, $f(t_e)$, versus elution time, t_e) is a graphical representation of the distribution. However, the chromatogram height is injection concentration dependent, making comparisons difficult, and t_e is often non-linear with $\ln(M)$, as evidenced by a third order calibration curve.

A normalized molecular weight distribution function is given by $w(M) = -\mathrm{d}w/\mathrm{d}\ln(M)$. Conversion of $f(t_e)$ versus t_e to a normalized molecular weight distribution plot (i.e. $w(M)$ versus M or $\ln(M)$), is obtained by considering that the weight fraction, $\mathrm{d}w$, of polymer which elutes between t_e and $t_e + \mathrm{d}t_e$ is given by: $\mathrm{d}w = f(t_e)\mathrm{d}t_e \Big/ \int_0^\infty f(t_e)\mathrm{d}t_e$ where the integral in the denominator is simply the area under the chromatogram. Thus, an analytical approximation of dw at the i^{th} slice is w_i, the weight fraction of polymer

A normalized analytical approximation to the distribution function, $w(M_i)$, is thus obtained from: $w(M_i) = -w_i / \mathrm{d}\ln(M_i)$. Given that M decreases as t_e increases, the same weight fraction, $\mathrm{d}w$, of polymer that exists between t_e to $t_e + \mathrm{d}t_e$ also exists between $\ln(M) - d\ln(M)$. $\mathrm{d}\ln(M_i)$ was obtained by evaluating the first derivative of the analytical equation fitted to the calibration curve, $\mathrm{d}P(t_e)/\mathrm{d}t_e$ and multiplying by the time interval (i.e. the 1 Hz sampling frequency ~ $\mathrm{d}t_e$). $(w(M_i))^2)^{1/2}$ was evaluated point by point (columns H and Q) and plotted against molecular weight to give a normalized distribution that is injection concentration and calibration curve independent, as seen in the Excel file.