Multi-Detector Size Exclusion Chromatography (SEC)

(Also Multi-Detector Gel Permeation Chromatography, GPC)

Along with many other groups and companies, we have been involved for many years in the coupling of multiple detectors to GPC. We hence don't claim any major advances of our own in this area, but feel that we still speak from a position of long experience and authority, as the list of our references below attests.

Traditionally, GPC columns are calibrated by measuring the elution behavior of polymer standards of known molecular mass. It is then assumed that other, unknown polymers will elute in a similar way so that their mass distribution can be determined by reference to the calibration established by the standards. The problem, of course, is that a GPC column does not separate according to mass, but rather via other mechanisms; when separation occurs according to hydrodynamic volume the Size Exclusion Chromatography mechanism is operative, meaning that, in principle, a 'universal calibration' can be applied, in which the calibration mass is multiplied by the intrinsic viscosity (hydrodynamic volume per unit mass), yielding hydrodynamic volume. Where the SEC mechanism is operative, this procedure often works well. There are, however, many cases where other mechanisms, such as enthalpic (e.g. electrostatic) and affinity effects control elution behavior. In this case 'universal calibration' can break down completely. This is very often the case in aqueous systems.

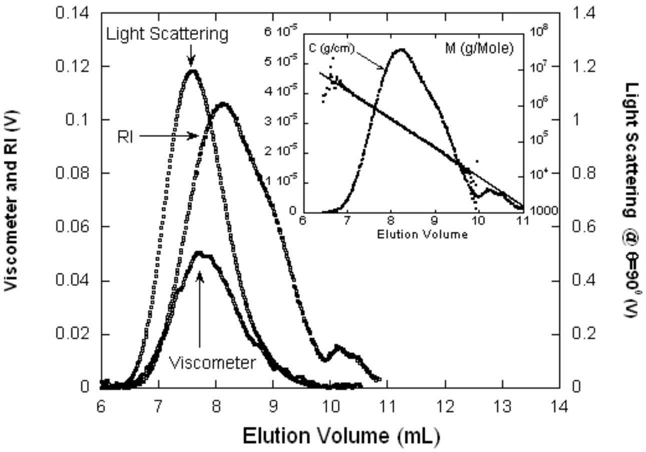
In order to remove mass distribution determinations from the assumptions and vagaries of column calibration, people began adapting static light scattering as a detector for GPC. Static light scattering (SLS) is sometimes claimed to provide an 'absolute M_w'.* The Chromatix laser light scattering instrument used a single, low scattering angle, and was the first and most widespread detector used with GPC for many years. The use of simultaneous multi-angle detection US patent #3,850,525 "Simultaneous Multiple Measurements in Laser Photometers", was filed in 1973 by Wilbur Kaye, opening the door to full angular extrapolation of GPC light scattering data to zero scattering angle ($q=0$). Later, companies, such as Wyatt Technologies, Precision Detectors, Inc., and Brookhaven Instruments Corp., began to commercialize multi-angle detectors for GPC use.

SLS detection for GPC is now commonplace. In addition to absolute molar mass at each elution slice, multi-angle SLS can also give the mean square radius of gyration $<S^2>$, if the RMS value of this (the root mean square radius of gyration, R_g) is larger than about 10nm. This allows the scaling laws in polymer Physics relating R_g and M to be determined, $R_g=aM^\alpha$, and can also be useful in determining branching, and conformational information about the polymer.

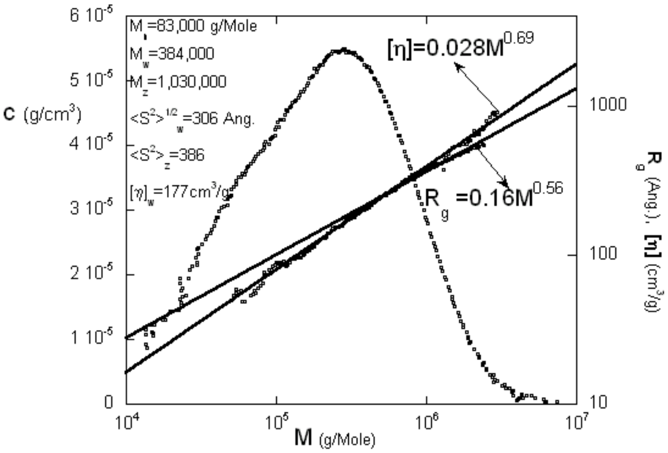
Viscosity detection is a very useful complement to SLS. Single capillary and bridge capillary viscometers are widely available, the former being extraordinarily inexpensive (around US$ 1k), whereas the latter typically give superior signal to noise ratios but usually cost upwards of US$ 20k. We made an extensive, simultaneous comparison of both types of viscometers several years ago (ref. 3, below). The intrinsic viscosity obtained from GPC can also be related to branching and conformation, and is especially powerful when related to the values of M and R_g obtained from SLS. It also permits the determination of the scaling behavior $[\eta]=bM^\beta$. The viscometer can continue to measure polymers whose effective hydrodynamic radius is much smaller than the approximate 10nm lower limit for R_g of SLS, giving extra range to GPC systems equipped with both SLS and viscosity detection.

A note of caution is the effect that small uncertainties in interdetector dead volumes can have on the results. These effects range from non-existent for quantities such as M_w and $[\eta]_w$, to extremely sensitive for the scaling exponents α and β defined above.

Here, an example of multi-detector GPC is shown; Raw GPC data for polyacrylamide showing RI, viscometer and LS at 90^0 . The inset shows M both as determined directly from the LS and RI data, which includes the scattered points at each extreme of the elution spectrum, as well as the semi-logarithmic fit to these points, and the concentration data, for reference. (GPC data courtesy of Alina M. Alb).



Analysis of above data, including the concentration, R_g and [η] vs. M distributions. Also shown are the various average quantities from the analysis.



[*] The assertion that an 'absolute M_w' is measured is true in the sense that an absolute Rayleigh scattering ratio from a polymer sample can be measured by comparing a photodetector's response to the scattering to the response obtained from a substance (e.g. pure toluene), whose Rayleigh ratio is well known. From this, via the fundamental properties of Maxwell's electromagnetic equations, and the Claussius-Mossotti equation that links molecular polarizability to the differential index of refraction, dn/dc, an 'absolute M_w' is determined.

Our references to multi-detector GPC

1. W.F. Reed, "Data Evaluation for Unified Multi-detector Size Exclusion Chromatography; Molecular Mass, Viscosity and Radius of Gyration Distributions" Macromolecular Chemistry and Physics, 196, 1539-1575, 1995

2. W.F. Reed "Evaluation of Coupled Multi-Angle Light Scattering and Viscosimetric Detectors for SEC, with Application to Polyelectrolyte Characterization", invited chap. for ACS symposium series 635 “Strategies in Size Exclusion Chromatography”, Ed. M. Potschka and P. Dubin, ch. 2, pp. 7-34, 1996

3. D.P. Norwood and W.F. Reed "Comparison of Single Capillary and Bridge Viscometers as Size Exclusion Chromatography Detectors", Int. J. Polym. Ana. and Char., vol. 4, no.2, pp. 99-132, 1997

4. W. F. Reed, "Automatic, Continuous Mixing Techniques for Online Monitoring of Polymer Reactions and for the Determination of Equilibrium Properties", ch. 20, pp. 589-

622, Handbook of Size Exclusion Chromatography and Related Techniques", 2nd Ed., Chi-san Wu, Ed., Marcel Dekker, 2003

5. Wayne F. Reed, "Fundamentals of static light scattering and viscometry in SEC and related methods", Lead chapter for ACS Symposium Series 893 "Multiple Detection Size-Exclusion Chromatography", A. M. Striegel, Ed. American Chemical Society: Washington, DC