HORIBA Scientific

Technical Note

Molecular Weight

TN176

The SZ-100V2 can be used to determine the molecular weight of proteins, starches, polymers, dendrimers, and other large molecules. This data can be obtained by two different methods: dynamic light scattering and static light scattering. These methods are discussed below.

Dynamic Light Scattering

There is a well-known empirical correlation between the diffusion coefficient of a macromolecule and its molecular weight known as the Mark-Houwink-Sakurada equation.

$$D_t = kM^{\alpha}$$

where:
D_t is diffusion coefficient
K is an empirical constant
M is molecular weight
α is an empirical constant

The values for K and α are found empirically for polymer/solvent pairs. That is, they must be specified for the polymer, solvent, and temperature. These values can be found in the literature. Note that Mark-Houwink-Sakurada were quite active in polymer dynamics. There are two similar equations with constants that bear their names. Be careful to choose constants that come from the DLS equation.

The disadvantage of this technique is that it relies on empirical constants and the nature of the average molecular weight. The advantages of this technique are that polymer concentration need not be well known and it is very fast.

Static Light Scattering (Debye plot)

The SZ-100V2 can also be used in a static light scattering mode to measure the molecular weight of proteins, small particles, and polymers. These results are generated using a Debye plot created by measuring the scattered light at a single angle (90°) at multiple sample concentrations. The intercept of the Debye plot is used to determine the molecular weight and the slope is used to calculate the second virial coefficient.

Molecular weight determination from static light scattering experiments uses the Rayleigh equation given below:

$$\lim_{\theta \to 0} \frac{Kc}{\Delta R_\theta} = \frac{1}{M_w} + 2A_2c$$

where:
K is the Debye constant
c is the sample concentration
R_θ is the Rayleigh ratio
M_w is the weight average molecular weight
A_2 is the second virial coefficient

The Debye constant is given by $K=4\pi^2n^2(dn/dc)^2/(\lambda^4N_A)$ where n is the refractive index of the liquid, (dn/dc) is the refractive index increment, λ is the wavelength of light in vacuo, and N_A is Avagadro's number. In most cases, all of these values are independent of molecular weight.

The limit given in equation (1) above deserves special attention. The equation only works at the limit of zero angle. One practice required for larger macromolecules is to use a multi-angle scattering instrument and extrapolate the result to zero angle. For smaller molecules (Rg<20 nm), this is not necessary and data at a single angle can be used. However, this does introduce a systematic error that increases with angle used. That is, measurement results using back angle have about twice the systematic error compared to results obtained using scattering at right angle (90°). For this reason, the SZ-100V2 collects static light scattering data at 90°.

The disadvantages of this technique are that it requires careful sample preparation and is a time consuming measurement. The advantages of this technique are that the results are well-defined and do not rely on empirical correlations.

An example of Debye plots for several samples is shown in Figure 1.

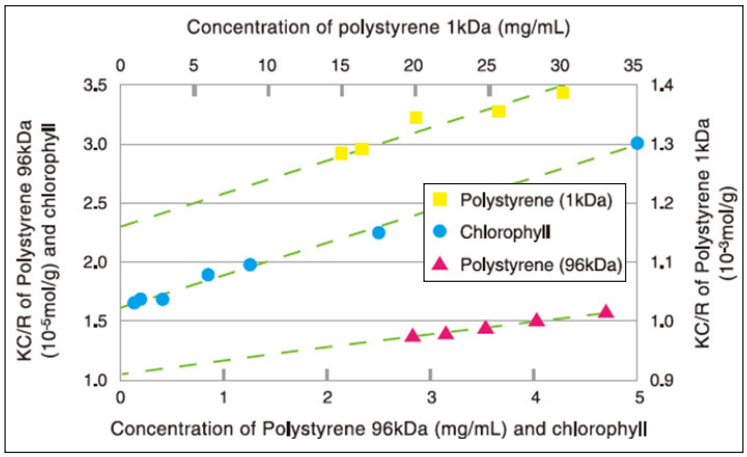

Figure 1: Debye plots to measure molecular weight

Comparison

If you need absolute molecular weight, you will need to use the Debye plot method. As mentioned above (and detailed in the section below) this is arduous.

Where does that leave the analyst who is running a polymerization and wants a fast and cheerful molecular weight measurement. Well, molecular weight is correlated to molecular size. High molecular weight means a larger polymer. For a linear polymer size goes up with the 0.5 to 0.6 power of molecular weight. And the DLS can determine particle and molecular size. So you determine size, feed in constants relating size and obtain an average molecular weight. No more messing with precision concentration. Just ensure you have a true solution (the polymer is dissolved). Often dust is less of an issue. All of the tricks and shortcuts in DLS work. And that is the Mark Houwink method above.

What do you give up? Well, unlike GPC, but like molecular weight analyzers, only the average molecular weight is reported. And it is based on cumulants. One note of caution about terminology, to a polymer scientist, "z-average" is NOT the same as z-average for a DLS person. The reported molecular weight comes from a correlation and is not considered an absolute molecular weight like from a Debye plot or Zimm plot.

If you need a QA measurement to compare lot-to-lot, DLS is much easier. The customer can build the correlations based on some Zimm plot measurements, if required.

With a DLS instrument, the Mark-Houwink-Sakurada method is the method of choice. Sample prep is easier and the measurement is much faster than measurement by a static light scattering approach (either a Zimm plot or Debye plot).

Outline of Debye Plot Procedure

Materials:
About 10 mL of spectrophotometric grade toluene (or benzene).
About 10 mL of solvent used for the polymer solvent systems.
Five solutions of polymer in solvent with accurately known (to with 1%) concentration as mass of polymer per volume of solution. All polymer must be completely dissolved. Typically, this means mixing the sample overnight. An initial measurement will use the concentrations of 2, 4, 6, 8, and 10 mg/mL of polymer.

Syringe:
Syringe filters, generally 0.1 micron pore size.
Scrupulously clean and dust free glass cell with a mark at top for orientation.

Procedure:
1. Load cell with toluene and cap. Use a laser pointer to look for the telltale speckles of dust. If they are apparent, then the toluene will need to be filtered. Follow software procedure to measure toluene, noting the orientation of the cell in the instrument.
2. Clean and dry cell using particle free or filtered THF and then alcohol.
3. Load syringe with 5 mL of solvent. Pass 3 mL of solvent through the filter. Fill cell with remaining 2 mL. Use a laser pointer to look for the telltale speckles of dust. If they are apparent, then further filtration is needed. Follow software procedure to measure solvent, ensuring the cuvette orientation matches that used for the toluene.
4. Remove solvent from cell using a syringe and needle.
5. Load syringe with 5 mL of lowest solution concentration. Filter 2 mL of solution into cell. Remove solution from cell using a syringe and needle. Filter 2 additional mL of solution into cell. Remove from cell. Load syringe again (or use 10 mL syringe from the start) and filter 2 mL into the cell. Now the cell has been rinsed twice with solution. The third fill is measured; again ensuring the cell alignment matches earlier measurements.
6. Repeat step 5 for each increased concentration.

Note that for a completely unknown molecular weight, the choice of concentration is quite likely incorrect. If the signal is too weak, repeat with higher concentrations. If the signal is too strong, repeat with lower concentrations.

Note that the value of dn/dc is required. It is not optional. Do not use the default. If you need to guess, use 0.15 for synthetic polymers in organic solvent and 0.185 for biomolecules (e.g., protein) in water. The value of dn/dc depends on the polymer/solvent pair for a particular wavelength (for the SZ-100V2 use 532 nm). Many, many values are in the literature. Alternatively, it can be determined with a differential refractometer.