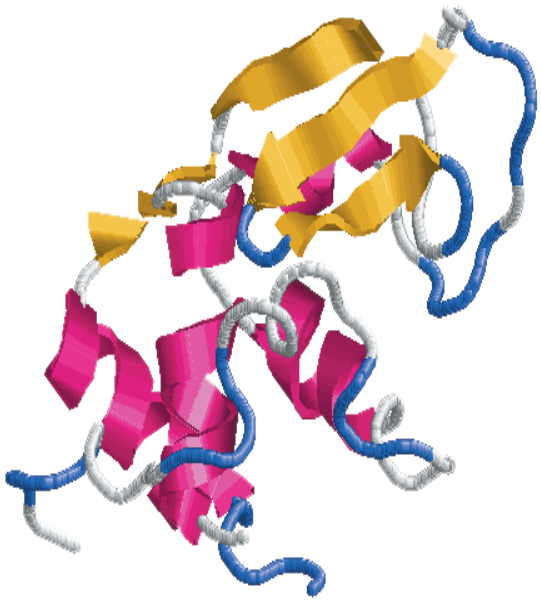
Molecular Weights
with the Brookhaven *90Plus Particle Sizer*



- **Proteins**
- **Synthetic Polymers**
- **Dendrimers**
- **PEGs, Polysacchrides, etc.**

In addition to using the **90Plus** for particle sizing and, optionally, zeta potential, you can now use it to determine molecular weights of macromolecules. There are two methods available: the standard Mark-Houwink-Sakurada (MHS) and the optional Debye Plot. The MHS method uses empirical constants to calculate the molecular weight from the diffusion coefficient determined from the autocorrelation function of the scattered light (DLS). The Debye Plot method is absolute and uses the intensity of light scattered at 90° (SLS).

With the MHS method, any of the following cells can be used: the plastic square cells, glass or quartz square cells, glass round cells, or the new, 40 μL quartz flow cell, the **90PFC**. With the Debye Plot method, the **90PFC** is required. In addition, given the weak scattering from small macromolecules like globular proteins, the **BI-APD**, avalanche photodiode detector, is required.

MHS Method of Molecular Weight Determination

In this method, the autocorrelation function of the scattered light intensity yields the diffusion coefficient, D, from which a hydrodynamic radius, R_H, is calculated. In addition, assuming the MHS empirical equation is appropriate, a diffusion-averaged molecular weight is calculated using $D = K \cdot M^{\alpha}$, where K and α are constants that depend on the macromolecule, the solvent, and to some extent on the solution temperature.

An analogous equation exists between viscosity-averaged molecular weight and the intrinsic viscosity. The molecular weight determined using the MHS equation is highly model dependent; yet, it very easy to determine given K and α. In the latest versions of the **90Plus** software, an extensive table of these constants is given along with the ranges of R_H and molecular weight over which they are useful. In particular, constants are tabulated in the software for globular proteins, linear and branched polysaccharides, and many synthetic polymer/solvent combinations.

Debye Plot Method of Molecular Weight Determination

In this method, the scattered light intensity is measured at several dilute polymer concentrations c. The following equation is used to fit the data:

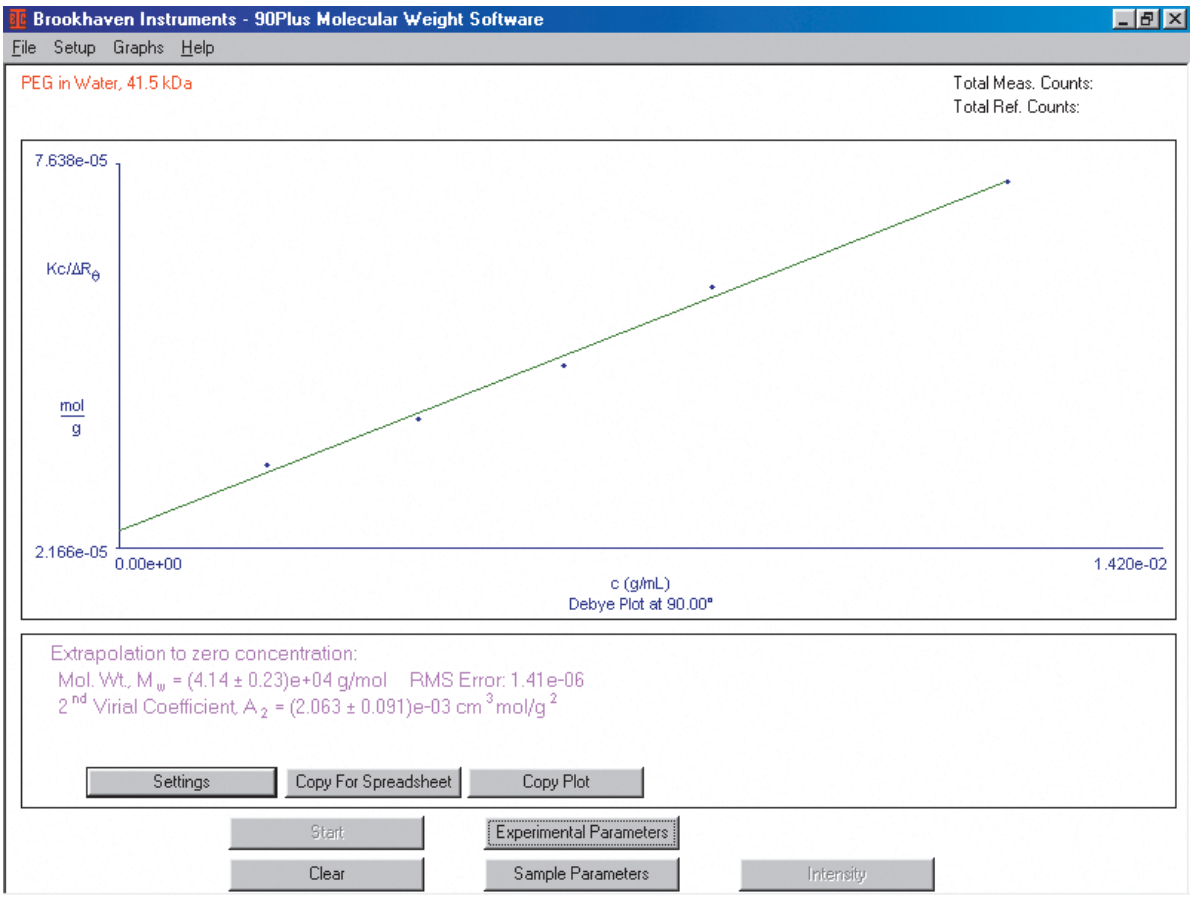
$$\frac{K \cdot c}{\Delta R} = \frac{1}{M_w} + 2 \cdot A_2 \cdot c$$

Here K is the Debye constant and ΔR is the difference in Rayleigh Ratio between the solution and solvent. Rayleigh Ratios are obtained from scattered intensities. M_w is the weight-average molecular weight, and A_2 is the second virial coefficient. The sign and magnitude of A_2 is useful in determining whether a protein solution will form crystals. A slightly negative A_2 favors crystallization.

The intercept yields the inverse of the weight-average molecular weight and the slope yields the second virial coefficient. The measurement on the next page was made on a GPC (Gel Permeation Chromatography) standard with a labeled value of 41.5 kDa for M_w. The result using the **90PDP** option, consisting of the **90PFC** flow cell and **9k90MW** software is 41.4 kDa and A_2 = 2.06E-3 $cm^3 \cdot mol/g^2$.

These values are in excellent agreement with those obtained using the **BI-MwA** Molecular Weight Analyzer, a flow-thru, 7-angle, detector for GPC and with the results from the **BI-200SM** multiangle DLS/SLS light scattering instrument.

Debye Plot Using 90Plus



Applications

Debye plots are most accurate when applied to any macromolecule with $R_g < 12$ nm, including globular proteins and dendrimers. In addition, such plots are generally accurate for random coil polymers with M_w < 100 kDa.

Picture from Berman, et. al., The Protein Data Bank 1LYZ, Nucleic Acids Research, 28, 235 (2000). Rasmol V2.7.1.1©H.J. Bernstein.

Specifications

Software: **9k90MW** Debye Plot
Cell: **90PFC**, 40 µL flow cell
Fits into 90Plus square cell holder.
For use with **90Plus** or **BI-MAS** options on **ZetaPlus** or **ZetaPALS.**
Tubing: 0.50 mm (0.020") I.D. PEEK tubing, changes on request.
External Fittings: Luer Lock
Wetted: PEEK, Quartz or Glass

Detector: **BI-APD,** avalanche photodiode. High sensitivity, avalanche photodiode, requires return of instrument for retrofitting.

For instruments that already have the APD or high power lasers, order software, cell and tubing only.

A policy of continual improvement may lead to specification changes

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