Introduction to the calculators in the Zetasizer software

PARTICLE SIZE

ZETA POTENTIAL
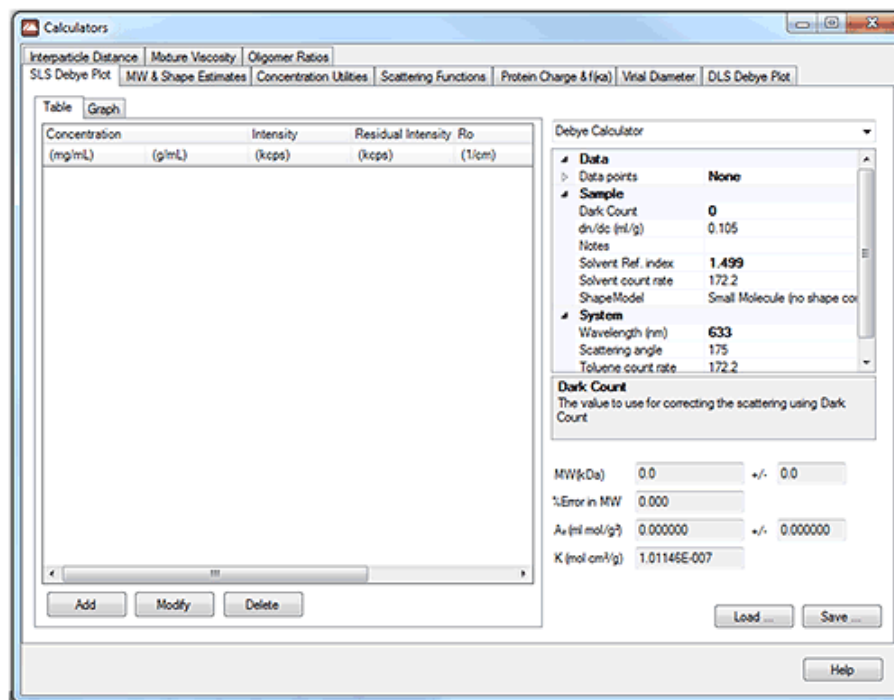
MOLECULAR WEIGHT

MOLECULAR SIZE

PROTEIN AGGREGATION

Introduction

The calculators are a series of tools in the Zetasizer software that allow the user to make the most of the available data by calculating a number of extra parameters such as protein charge and the DLS interaction parameter, k_D. Although not all are exclusively for proteins, most of the calculations are designed with proteins in mind.

This technical note describes these calculators, shown below, and gives a brief explanation of the theory behind them.

SLS Debye Plot

The SLS Debye plot uses the Zimm equation to create a Debye plot and, from it, calculate molecular weight and the second virial coefficient, A_2.

The Zimm equation is:

$$\frac{KC}{R_\theta} = \frac{1}{Mw P_\theta} + 2A_2 C$$

Where, K is a constant dependent on the sample dn/dc, C is the sample concentration, R_θ is the Rayleigh ratio (the ratio of scattered light intensity to incident light intensity), Mw is the weight average molecular weight and A2 is the second virial coefficient. P_θ is an angular dependent term (by default, assumed to be 1 for small molecules <15nm radius). The software will estimate a value of P_θ if a shape model is selected based on a simple relationship between Rh and Rg. If this is used, a sample radius must be input into the calculator.

A Debye plot is a plot of KC/R_θ as a function of concentration. If P_θ is assumed to be 1, the slope of this line is $2A_2$ and the intercept is 1/Mw.

The user inputs a series of values of measured light scattering intensity at different sample concentrations and the graph is plotted. Other parameters such as the sample dn/dc etc. are input in the table on the right hand side. By selecting 'chart properties' from the drop-down menu in the top right hand corner of the tool, the graph can be customized.

For information on why A_2 is a useful value to measure, see the application note MRK1845, "Using A_2 and k_D to assess protein interactions in formulations".

MW & Shape Estimates

The shape estimates calculate the Perrin factor and prolate and oblate axial ratios of a molecule based on the molecular weight, specific volume and measured hydrodynamic radius. The Perrin factor is a measure of how the particle deviates from being spherical in shape. The prolate axial ratio is the length divided by the thickness of an ovoid shape whereas the oblate axial ratio is the width divided by the thickness of a disc shaped object.

The user enters the values in the box on the left and the results are displayed on the right. If desired, a solvent layer can be subtracted from the hydrodynamic size value. It is possible for the user to define this value. The default is 2.5 nm.

The molecular weight estimate tool calculates an estimated molecular weight for a particle based on an input hydrodynamic size. The results are calculated for four models which are globular proteins, linear polysaccharides, dendrimers and branched polysaccharides.

The hydrodynamic size estimate tool calculates an estimated hydrodynamic size based on an input molecular weight. The same four models are used as in the molecular weight estimate.

For more information on these factors, consult the Malvern FAQ "Can you get shape information from DLS?"

Reference on Perrin Factor: Cantor, C.; Schimmel, P (1980) "Biophysical Chemistry Part II: Techniques for the Study of Biological Structure and Function", Pub: W.H. Freeman and Co, New York

Reference on globular protein molecular weight estimate: Nobbmann, U. "Characterization down to Nanometers: Light Scattering from Proteins and Micelles", Chapter 4 in "Mesoscale Phenomena in Fluid Systems" edited by Case, F. H. and Alexandridis, P., ACS Books & Oxford University Press, (2003), pp.22-59

Concentration Utilities

The concentration and scattering calculator uses a series of input values such as size, sample volume and concentration to calculate a number of other values. This includes the number of particles in the scattering volume and the estimated light scattering count rate for a real measurement of the sample.

The minimum concentration calculator estimates the minimum concentration of a protein required to perform a particular measurement on a particular instrument type. The user enters the protein molecular weight and the boxes will be populated with an estimated minimum concentration required to successfully perform the measurement.

Scattering Functions

The scattering function tool uses different light scattering theories, including Mie theory, to plot particle scattering as a function of laser wavelength, angle, solvent refractive index and particle properties.

Protein Charge & f(κa)

The protein charge and f(κa) calculator tool calculates f(κa) for the Henry equation using the Ohshima equation for monovalent salts. Measured electrophoretic mobility, hydrodynamic radius, ionic strength, solution viscosity and temperature are input by the user in the box on the left and the calculated values for f(κa) are displayed in the f(κa) results box.

The Debye length (the thickness of the electrical double-layer), $1/\kappa$, is calculated using the equation:

$$\frac{1}{\kappa} = [1.989 * 10^{-3}] \left[\frac{\varepsilon_r T}{I}\right]^{\frac{1}{2}}$$

Where, ε_r is the solution dielectric constant, T is the temperature, and I is the solution ionic strength.

κa is calculated using the equation:

$$\kappa a = \kappa \cdot R_h$$

Where R_h is the hydrodynamic radius.

f(κa) is calculated using the equation:

$$f_1(\kappa a) = 1 + \frac{1}{2}\left[1 + \left(\frac{2.5}{\kappa a[1 + 2e^{-\kappa a}]}\right)\right]^{-3}$$

Zeta potential (ζ) is calculated using the equation:

$$\xi = 1.328 \frac{\eta \cdot \mu_E}{\varepsilon_r \cdot f(\kappa a)}$$

Where η is the solution viscosity and μ_E is the measured protein electrophoretic mobility.

Finally, protein valence (Z) is then calculated using the equation:

$$Z = [5.986 * 10^{-3}]\varepsilon_r \cdot T \cdot \kappa a^2 \left[2sinh\left(\frac{5801.4\xi}{T}\right) + \frac{4}{\kappa a}tanh\left(\frac{2900.7\xi}{T}\right)\right]$$

Reference: Loeb, A.L, Wiersema, P.H. and Overbeek, (1961) "The Electrical Double Layer around a Spherical Colloid Particle" MIT Press, Cambridge Mass

Reference: H. Ohshima. J. Colloid Interface Sci. 168, 269 (1994)

Virial Diameter

The virial diameter calculator tool calculates an apparent size, also known as the "thermodynamic diameter", based on molecular weight and the 2nd virial coefficient.

It is based on the equation:

$$\text{Virial diameter} = 2 \cdot \left(\frac{3M_w^2 A_2}{16\pi N_a}\right)^{\frac{1}{3}}$$

where M_w is the weight average molecular weight, A_2 is the second virial coefficient, and N_a is Avogadro's number.

Reference: Smith et. al. Analyst, 2007, 132, 455-460

DLS Debye Plot

The DLS Debye plot allows the user to manually create a 'dynamic Debye plot' of measured diffusion coefficient as a function of sample concentration. The graph is based on the following relationship:

$$D_m = D_o(1 + k_D C)$$

Where D_m is the measured diffusion coefficient, D_0 is the diffusion coefficient at a sample concentration of zero, C is the sample concentration and k_D is the DLS interaction parameter. In a dynamic Debye plot, D_m is plotted as a function of C. The

intercept of this graph is D_0 and the slope of the line is kD*D_0. k_D can therefore be calculated from the slope of this line.

As protein concentration increases, the individual molecules will interact with each other affecting their diffusion speed and subsequently the measured hydrodynamic radius. k_D is therefore a measure of this interaction. k_D has two components, a thermodynamic (k_T) and a hydrodynamic component (k_H). The value itself is defined as:

$$k_D = k_T - k_H$$

Where:

$$k_T = 2M_w A_2$$

$$k_H = k_f - 2v_2$$

The thermodynamic component is therefore related to the weight average molecular weight and the second virial coefficient. The hydrodynamic component is related to k_f, the first-order concentration coefficient in the virial expansion of the frictional coefficient and v_2 is the partial specific volume of the protein.

D_0 and k_D can therefore both be calculated from the dynamic Debye plot. When size is varying by concentration, the hydrodynamic size calculated from D_0 can be considered the 'true' hydrodynamic radius. k_D is, then, a measure of the interactions between the protein molecules and is related to A_2.

The user inputs a series of values of measured diffusion coefficients at different sample concentrations and the graph is plotted. Other parameters such as the viscosity etc. are input in the table on the right hand side. By selecting 'chart properties' from the drop-down menu in the top right hand corner of the tool, the graph can be customized.

For more information on the usefulness of k_D see the application note MRK1845 "Using A_2 and k_D to assess protein interactions in formulations".

Reference: Yadav et. al. J Pharm Sci, 2012, 101, 998-1011

Reference: Teraoka, I. (2002) "Polymer Solutions: An introduction to Physical Properties", Wiley & Sons, Inc.

Interparticle Distance

The interparticle distance calculator can be used to compare the physical distance between the molecules in a sample and the size of the electrical double layer on the molecules.
Concentration, protein valence (charge), ionic strength, protein molecular weight and temperature are input in the box on the left and the results are calculated on the right.

The separation distance between the molecules is calculated using the equation:

$$\text{Separation distance} = \left[\frac{\frac{4}{3}\pi R_h{}^3}{0.001 C \cdot 0.73} \right]^{\frac{1}{3}}$$

Where C is the sample concentration, R_h is the protein hydrodynamic radius and 0.73 is a commonly used estimate of protein partial specific volume.

The Debye length, 1/κ is calculated as in the 'protein charge and f(κa)" calculator.

The protein contribution to I, I_p, is calculated using the equation:

$$Ip = \frac{\left(\frac{C}{Mw} * |Z| \right)}{1000}$$

Where Mw is the protein molecular weight and Z is the protein valence.

The intended use of this calculator is to assess the difference between the Debye length and the separation distance between the particles. The conclusion that can be drawn is that if the Debye length is longer than the separation distance, then the molecules' electrostatic fields will be overlapping and therefore interacting.

Mixture Viscosity

The mixture viscosity calculator is a tool that calculates mean viscosity based on the volume fraction and individual viscosities of component solvents.
It should be noted that some combinations of solvents do not have a predictable viscosity so this tool can only be used as an estimate.

Oligomer Ratios

The oligomer ratio calculator is a tool that estimates the level of protein monomer and dimer in a single intensity peak. The known sizes of the monomer and dimer are input along with the intensity-weighted modal peak size. The calculator estimates the proportions of monomer and dimer, which could account for the measured size.

Reference: Nobbmann et al.Biotechnology and Genetic Engineering Reviews, 2007, 24, 117-128