CORDOUAN
Technologies

Molecular weight measurement using Dynamic light Scattering equipment

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Abstract

This note presents the basic concepts of average molecular weight determination based on light scattering measurements.

Introduction

Aboslute molecualr weight (M_w) measurement is of primary importance for anyone that deals with macromolecules such as proteins or polymers; Indeed molecular weight determines many important physical properties of matter like stifness, strength, viscosity, transition temperature from liquids to gels, etc [1].

Today, there are many known ways to estimate or determine average molecular weight such as liquid chromatography, mass sperctrometry, ultra centrifugation, and in particular optical methods such as Dynamic Light Scattering (DLS) and Static Light Scattering (SLS). Indeed the intensity of light scattered by molecules depends on their size and on their polarizability which is related to their molecular weight. These optical methods are the ones used in cordouan intruments to measure M_w. We present their principle hereafter.

SLS Molecular weight measurements: principle and theory

Static Light Scattering is a well known characterization technique used in chemistry and macromolecules research which principles has been described many decades ago [2,3]. Basically, a laser is focused into liquid (solvent) containing macromolecules/particles (see figure 1); as it propagates, the laser light is scattered in all direction of space because of the refractive index contrast between the solvent and the molecules. This scattered light intensity depends on several parameters like the observation angle, the distance between the detector and the sample cell, the quantum efficiency of the detector, the scattering volume, etc.
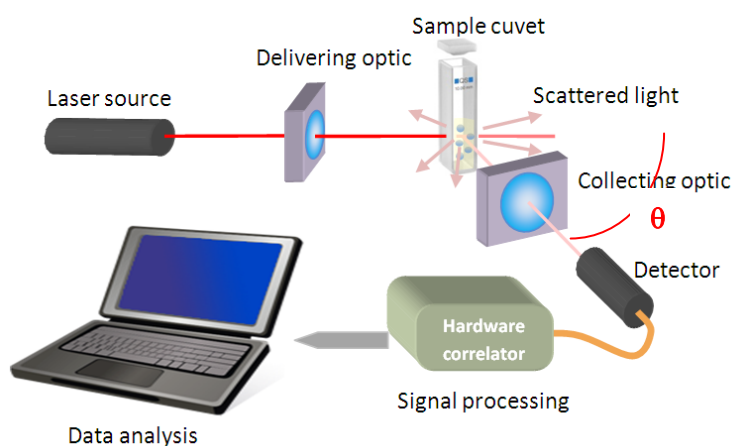

Figure 1: "typical" light scattering measurement configuration

In order to circumvent the tedious determination of these instrumental factors, one usually measures intensity ratios with respect to a reference sample, usually Toluene. Indeed it has been shown that, for a given observation/scattering angle θ with respect to the incident light direction, the ratio of the scattered intensity to the incident intensity can be defined by the Zimm/Rayleigh equation:

$$\frac{KC}{R(\theta)} = \frac{1}{M_W \, P(\theta)} + 2 \, A_2 C \tag{1}$$

Where

$$K = 4\pi^2 n_0^2 \left(\frac{dn}{dC}\right)^2 / N_A \lambda^4 \,, \tag{2}$$

$$R(\theta) = \frac{I_A(\theta) n_0^2 R_T}{I_T n_T^2 N(\theta)} \,, \tag{3}$$

M_w is the **weight average molecular weight**, ie, the quantity to be measured; n_0 is the refractive index of the solvent, λ is the laser wavelength, $\frac{dn}{dC}$ is the refractive index gradient of the solution with respect to the macro molecules/particles concentration, it can be measured with a differential refractometer; N_A is the Avogadro Number (6.023×10^{23}), and C is the particle concentration. R(θ) is called the Rayleigh ratio. N(θ) is an instrument angular dependence correction factor; for measurements at single scattering angle, one sets N(θ) = 1. P(θ) is a corrective shape factor for big particles/molecules with respect to the laser wavelength; it is given by :

$$P(\theta) = 1 + \frac{q^2 R_G{}^2}{3} \,, \tag{4}$$

where q is the scattering vector given for a vertically polarized light by:

$$q = 4\pi \, n_0 \sin\left(\frac{\theta}{2}\right) / \lambda \tag{5}$$

R_G is the gyration radius of the particles, it depends on the shape and the hydrodynamic radius of the particles (see Annex 1) ; A_2 is the famous second virial coefficient that is a corrective factor for non ideal solution and which accounts for the interaction strength of the particles with the solvent[1]. $I_A(\theta)$ is the difference of the intensity scattered by the analyte (solvent+ particles) minus the intensity scattered by the solvent only in the same conditions. R_T, n_T are respectively the Rayleigh ratio and the refractive index of the Toluene used as a reference solvent; these two values are tabulated and can be found in handbooks: $R_T = 1.35 \times 10^{-5}$ cm-1, $n_T = 1.49$. I_T is the measured scattered intensity for Toluene.

Analysis and interpretation

Equation (1) shows that the Rayleigh ratio has a linear dependence with concentration C and with M_W; it can be written like f(C) = a x C + b; Indeed if one plots KC/R(θ) vs C at constant scattering angle θ, one expects a straight line with the intercept at C= 0 given by $\frac{1}{M_W P(\theta)}$, with a slope equal to $2A_2$ (see figure 2).
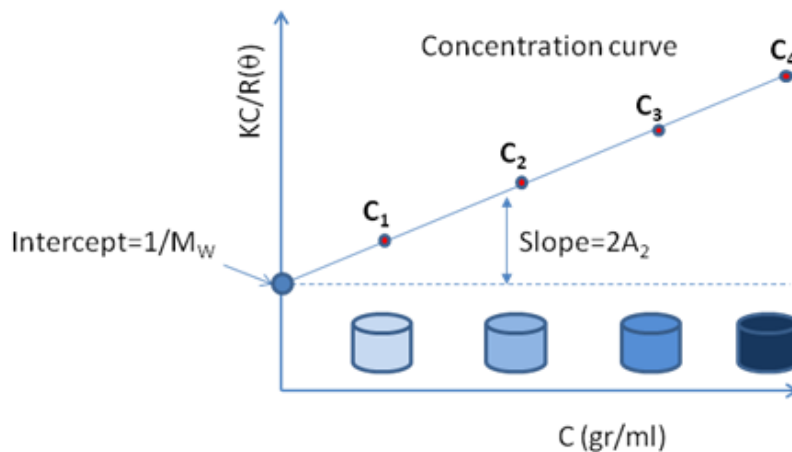

Figure 2: illustration of a Debye plot

[1] For A_2 >0, Particles have good affinity with the solvent and the colloid is stable. For A_2 <0, the particles have good affinity with themselves and then tends to aggregate. When A_2 =0, the strength of particle-solvent interaction is the same magnitude as that of Particle-particle interaction (Theta solvent)

Basically, the measurement principle is very simple; it consists in measuring the scattered intensity averaged over a period of time of 10 to 30 sec for different analyte concentration, for the solvent alone and for Toluene. Then for each concentration one can calculate the ratio $KC/R(\theta)$ given by (1). A linear regression fit allows determining the intercept and the slope of the straight line from which M_w and A2 are deduced.

Remark: with a multi-angle setup it is possible to measure the scattered intensity for various scattering angles and various concentrations; the graph obtained for $KC/R(\theta)$ as function of $\sin^2(\theta/2) + KC$ is called a Zimm plot. This approach allows determining M_w and A2 like for the Debye plot and Rg directly from the fit without any assumption on the particle shape like the models presented in Annex 2.

Example of measurement with the VASCO analyzer

The principle of M_w measurement is illustrated with the VASCO analyzer on Bovine Serum Albumin (BSA) proteins solutions diluted in DI water. The following BSA concentrations have been prepared: 10 mgr/mL, 5 mgr/mL, 2.5 mgr/mL , 1 mgr/mL. Prior to the measurements, filtration at 250 nm size cut off was achieved on all the solution. Scattered intensities were averaged over a period of 10 sec.
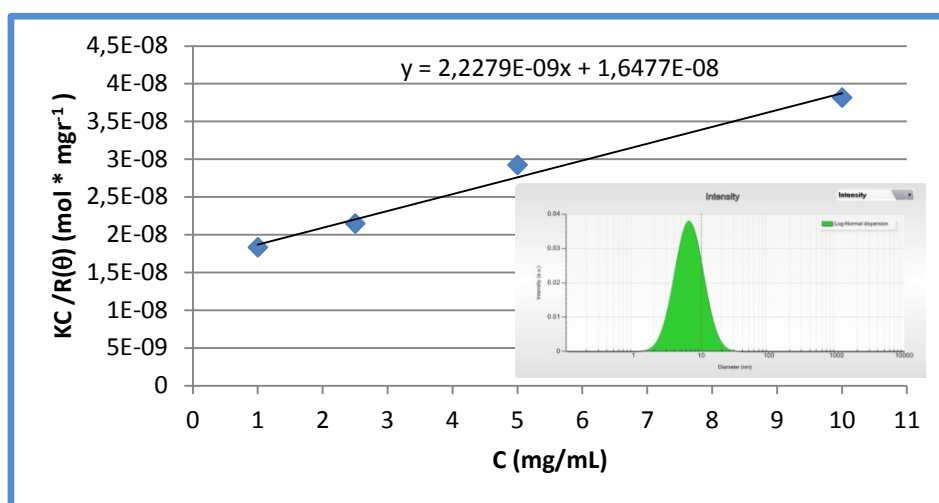

Figure 3: Debye plot on a BSA solution measured with the VASCO system

The hydrodynamic diameter of the BSA proteins has been measured with the VASCO at a value 7.3 nm (see insert of fig 3). The $P(\theta)$ value is thus assumed to be equal to 1.

From the intercept value one obtain 1/Mw of $1.65 * 10^{-5}$ mol*gr^{-1}. This gives a molecular weight value **M_w= 63 kDa** which is consistent with hand book values. The slope of the linear fit give the value of the second virial coefficient **A2=$1.1 * 10^{-3}$ mol*mL*gr^{-2}.**

CONCLUSION

We have shown that it is possible to make average Molecular weight measurement with the VASCO DLS instrument used as single angle static light scattering set-up combined with the Debye plot approach. Measurement in the 1 x 10^3 to 2 x 10^7 g/mol range is acheivable with an accuracy of +/-10%.

References:

[1] C. Tanford (1961), "Physical Chemistry of Macromolecules". John Willey & Sons-ISBN 0 471 84447 0

[2] P. Debeye (1944), "light scattering in solutions". J Appl Phys. 15 (4) :338

[3] B.H. Zimm (1945). "Molecular Theory of the scattering of lights in fluids". J. Chem . Phys 13 (4) :141

Appendix 1 : Evolution of P(θ) as a function of hydrodynamic Radius

The graph below presents one example of the evolution of the shape factor P(θ) given by equation (4) as a function of the hydrodynamic radius of spherical particles for three different scattering angle of commercial instruments:

Evolution of 1/P(θ) vs RH as for spherical particles
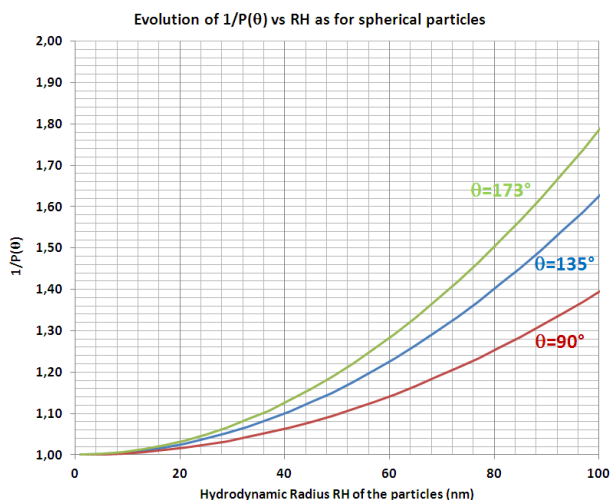
One can notice several points:

For small particles (ie, < 10 nm) like proteins, P(θ) has a small impact on the scattered signal which is almost isotropic, ie, weakly dependent on the scattering angle θ. In this case, equation (1) can be approximated by:

$$\frac{KC}{R(\theta)} \approx \frac{1}{M_W} + 2\,A_2 C \tag{6}$$

From equations (4) & (5) one can also notice that for θ=0°, P(0)=1 ; there is no shape factor correction whatever the size of the particles; in practical case, it is not possible to measure in the axis of the laser light. In commercially available instruments, measurements are made either at 90° or in back scattering configuration. For these later configuration, the P(θ) factor needs to be calculated and accounted for. This is illustrated on the graph above. The calculation of P(θ) requires the determination of the gyration radius of the particles (see Appendix 2)

Appendix 2: Gyration radius definition and calculation

Radius of gyration R_G of a macromolecule is basically associated with the overall spread of the molecule and is defined as the root mean square distance of the collection of atoms from their common centre of gravity:

$$R_G{}^2 = \int r^2 g(r)dr$$

Where g(r) is the pair distance function which contains information about the size and shape of the particles.

Different shapes models are used to describe the particles accounting for their symmetry and mass distribution; the most used ones are:

For a **Sphere** of hydrodynamic radius R_H:

$$R_G{}^2 = \frac{3}{5} R_H{}^2$$

For **cylindrical rod** of diameter R and length L

$$R_G{}^2 = \frac{R^2}{2} + \frac{L^2}{12}$$

For **disk** of hydrodynamic radius R:

$$R_G{}^2 = \frac{R^2}{2}$$

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