Light Scatter Theory

The amount of light scattered is directly proportional to the product of the weight-average molar mass and the macromolecule (solute) concentration, i.e., LS ~ $M_W \cdot c$ (Wyatt, 1993).

Based on Zimm's formalism, the Rayleigh-Debye-Gans light scattering model for dilute polymer solutions can be expressed as equation [1] (Wyatt, 1993):

$$\frac{K^* c}{R(\Theta)} = \frac{1}{M_w P(\Theta)} + 2A_2 c$$

where:

R(Q) is the excess intensity of scattered light at DAWN angle Q
c is the sample concentration

M_W is the weight-average molecular weight (molar mass)

A_2 is a second virial coefficient

K^* is an optical parameter equal to $4p^2n^2 (dn/dc)^2 / (l_o^4 N_A)$

n is the solvent refractive index and dn/dc is the refractive index increment

N_A is Avogadro's number

l_o is the wavelength of the scattered light in vacuum.

The function P(Q) describes the angular dependence of scattered light.

The expansion of 1/ P(Q) to first order gives:

$$1/ P(Q) = 1 + (16p^2/3l^2) \langle r_g^2 \rangle . \sin^2(Q/2) + f_4 \sin^4(Q/2) + \ldots$$

At low angles the angular dependence of light scattering depends only on the mean square radius $\langle r_g^2 \rangle$ (alternatively called radius of gyration) and is

independent of molecular conformation or branching. A plot of K*c /R(Q) vs.

$\sin^2(Q/2)$ (Zimm plot) yields a curve whose intercept gives M_w and whose slope at low angles gives $\langle r_g^2 \rangle$.

MW calculation based on the "Two detector" approximation

During gel permeation chromatography, the protein concentrations are so low that the viral coefficient term is negligible (Wen et al, 1996). In addition, the term $(16 p^2/3l^2) \langle r_g^2 \rangle \sin^2(Q/2)$ will be negligible if we measure the light scattering at a sufficiently small angle. Furthermore, this term will be negligible at all angles for $\langle r_g^2 \rangle$ smaller then 15 nm; thus, the scattered light signal measured at 90^o is commonly used in this approximation (Takagi, 1990; Wen et al, 1996). This approximation is true for folded polypeptides with molecular weight (MW) smaller than about $5x10^7$ Da. Under such conditions, Eq. [1] can be simplified to:

$(K^* c)/R(Q) = 1/M$ [2]

Substituting K^* with $4p^2n^2 (dn/dc)^2 / (l_o^4 N_A)$ and introducing K_{LS} (calibration constant for LS detector) the intensity of scattered light is given by:

$(LS) = K_{LS} c M (dn/dc)^2$ [3]

Similarly, the refractive index signal, (RI), can be expressed as:

$(RI) = K_{RI} c (dn/dc)$ [4]

where K_{RI} is the instrument calibration constant.

For proteins/protein complexes that contain only polypeptides (no carbohydrates) the dn/dc is constant (~0.19 ml/g) and nearly independent of amino acid composition. Thus, we can determine the concentration of protein from the RI signal and the MW from the ratio of the two detectors, (LS) and (RI):

$MW = K' (LS)/(RI)$ [5]

where $K' = K_{RI}/[K_{LS}(dn/dc)]$.

This is the so-called two-detector" method. This method is widely used but it is valid

only when dn/dc is known (Takagi, 1990, Wen et al, 1996). In this approach the MW is NOT determined from the absolute light scattering measurements but is calculated from Eq. [5] assuming K' is known. The instrument calibration constant, K', is derived from running protein standards.

Absolute MW Calculation based on the ASTRA software

Alternatively, the MW can be determined directly from the absolute light scattering measurements by solving Eq1 using the ASTRA software (note that the concentration needs to be measured independently). Click here for details on ASTRA calculations.

References:

1. Wyatt, P. J. (1993) *Anal. Chim. Acta*, **272**, 1-40

2. Takagi, T. (1990) *J. Chromatogr.* , **506**, 409-416

3. Wen, J., Arakawa, T. & Philo, J. S. (1996) *Anal. Biochem.*, **240**, 155-166