

Light Scattering

1. Scattering of visible light by a single molecule (case of wavelength much larger than particle). Light can be considered as a wave with oscillating electric and magnetic fields. Since electrons are charged particles, the oscillating electric field of the light causes a corresponding oscillation of the electrons of the particle. Such oscillating charges act as miniature antenna, **scattering** some of the incident energy in all directions. According to electromagnetic theory, the amplitude of the electric field produced by an oscillating dipole arising from the use of polarized radiation, at a distance r from the dipole, and at an angle ϕ is given by:

$$E_r = \{(\alpha E_0 4\pi^2 \sin\phi) / r\lambda^2\} \cos 2\pi(r/\lambda - vt) \text{ where } \alpha \text{ is the molecular polarizability}$$

Since intensity depends on the square of the amplitude, we can equate the intensity of the scattered radiation (i) relative to the incident intensity (I_0) as:

$$i/I_0 = 16\pi^4 \alpha^2 \sin^2\phi / r^2 \lambda^4 \quad \text{for polarized radiation.}$$

For **scattering of unpolarized radiation**, this reduces to:

$$i/I_0 = [8\pi^4 \alpha^2 / r^2 \lambda^4] (1 + \cos^2\theta) \quad \text{for unpolarized radiation.}$$

Note: **Intensity falls off with r^2 and λ^4**
 Intensity depends on scattering angle

2. Rayleigh Scattering – scattering from N identical particles, each much smaller than λ , and dilute so interference can be neglected. **Amount of Light Scattered is directly proportional to product of molar mass and concentration.**

$$i/I_0 = N[8\pi^4 \alpha^2 / r^2 \lambda^4] (1 + \cos^2\theta) \quad \text{for unpolarized radiation.}$$

Substituting α in terms of dn/dC (spec. refractive index increment), C , and M

$$\alpha = (2n_0 / 4\pi)(dn/dC)(C/N) \text{ and noting that}$$

$$N = [C(\text{g/mL})/M] N^0.$$

$$\text{Yields: } i/I_0 = [2\pi^2 n_0^2 (dn/dC)^2 / r^2 \lambda^4 N^0] CM(1 + \cos^2\theta).$$

Define “*Raleigh Ratio*” as $R_\theta = (i_\theta / I_0)(r^2 / (1 + \cos^2\theta))$ and thus

$$R_\theta = [2\pi^2 n_0^2 (dn/dC)^2 / \lambda^4 N^0] CM \text{ or } R_\theta = KCM.$$

Note: This is frequently described in terms of the excess Rayleigh ratio, R_θ , expressed as light scattered per unit of solid angle in excess of that scattered by the pure solvent; C as conc. in g/mL, M_w is the weight-average molar mass, A_2 is the 2nd virial coefficient, and the constant $K^* = [4\pi^2 n_0^2 (dn/dC)^2 / \lambda^4 N^0]$, n_0 is the refractive index of the solvent and dn/dC the specific refractive index increment. P_θ is a form factor that describes the angular dependence from which the mean square radius $\langle r_g^2 \rangle$ may be determined.

$$\boxed{(K^*C / R_\theta) = 1/M} \text{ ideal solutions or } \boxed{(K^*C / R_\theta) = 1/(M P_\theta) + 2A_2C} \dots \text{for real solutions}$$

Note: Different experimental methods yield different types of experimentally arrived at “Molecular Weights.” Light scattering yields a “**weight average**” molar mass— solutions must be scrupulously clean since dust will contribute to average as very large molecules.

$$\Sigma C_i M_i^y / \Sigma C_i M_i^{y-1}$$

Number Average M_n	$\Sigma N_i M_i / \Sigma N_i$	$\Sigma C_i / \Sigma (C_i / M_i)$	$y = 0$	Osmotic Press / F.Pt.
Weight Average M_w	$\Sigma N_i M_i^2 / \Sigma N_i M_i$	$\Sigma C_i M_i / \Sigma C_i$	$y = 1$	Light Scatt. / Sed. Eq.
“Z” Average M_z	$\Sigma N_i M_i^3 / \Sigma N_i M_i^2$	$\Sigma C_i M_i^2 / \Sigma C_i M_i$	$y = 2$	Sedimentation Equil.

3. Dynamic Light Scattering – Measurement of Diffusion to get R_h

The movement of molecules in solution is related to their diffusion constants or frictional coefficients

$$D = kT/f = (RT)/(N_o f)$$

Consider the scattering from a small volume element of solution at some local concentration. As molecules move into and out of this volume element, the local scattering will vary with time. Dynamic Light Scattering measures these fluctuations with time.

$$\Delta i_t = i_t - \text{av.} \langle i \rangle$$

Do an autocorrelation analysis of variation of Δi_t at time t vs. $(t + \tau)$

$$A(\tau) = \text{average product of } (\Delta i_t \bullet \Delta i_{(t+\tau)})$$

(Note: $A(\tau)$ will be large when $\tau = 0$ and $A(\tau) =$ some minimum at large τ , with the fall off depending on “D” – the diffusion coefficient)

$$\text{It can be shown that } \ln(A(\tau)) = \ln(A_o - ([8\pi^2 n^2 / \lambda^2][\sin^2(\theta/2)]) \bullet D \bullet \tau$$

Thus a plot of $\ln(A(\tau))$ vs. τ can yield the diffusion coefficient “D”

Normally report “**Hydrodynamic Radius – R_h** ” ; $D_o = kT/f = kT/(6\pi\eta R_h)$

4. X-ray Scattering – With shorter wavelengths, scattering occurs from particles that are comparable or smaller than the incident radiation. Radiation will induce dipoles within the large particle that are out of phase with other parts of the large molecule causing appreciable interference effects. This type of scattering information can yield information about size and shape as well as mass of the particle in terms of the R_G (Radius of Gyration) of the particle.

Define $P(\theta) =$ (scattering by real particle at θ) / (scattering by point particle at θ)

$$\text{At low angle; } P(\theta) \sim (1/n^2) \Sigma \Sigma 1 - ([16\pi^2 / \lambda^2][\sin^2(\theta/2)]/6n^2) \Sigma \Sigma r_{ij}^2$$

$$\text{or } P(\theta) = 1 - ([16\pi^2][\sin^2(\theta/2)]R_G^2 / 3\lambda^2) + ; \text{ where } \boxed{R_G^2 = (1/2n^2) \Sigma \Sigma r_{ij}^2}$$

For “real” solutions using wavelengths with large particles – and since $1/(1-x) \sim 1+x$, we have

$$\boxed{KC / R_\theta \sim [1/M + 2A_2C +] \bullet [1 + ([16\pi^2 R_G^2 / 3\lambda^2][\sin^2(\theta/2)])]}$$

Analyze the light scattering data as a function of concentration and angle, and use a “**Zimm Plot**” to get $1/M_w$ by extrapolating to both *low concentration and low angle*.

5. Other Types of Scattering: Low-Angle X-ray Scattering / Raman Scattering –

Some Applications – Wyatt Technologies Web Site (<http://www.wyatt.com/notes.html>)

Headline Summary for Light Scattering

Static Light Scattering (Classical or Rayleigh Scattering): Absolute Molecular Weight

- 1) Amount of Light Scattered is directly proportional to product of molar mass and concentration.

$$\boxed{K^*C / R_\theta} = 1/M \quad \text{ideal solutions or}$$

$$\boxed{K^*C / R_\theta} = 1/(M P_\theta) + 2A_2C \quad \dots \text{for real solutions}$$

- 2) For large or rod shaped particles or using smaller wavelengths, variation of scattered light with scattering angle is proportional to average size (R_G^2) of the scattering molecules. For classical light scattering

Size Range: $R_G > 10\text{nm}$ to 150nm

where $R_G^2 = (1/2n^2) \sum \sum r_{ij}^2$ for n identical particles and r_{ij} is distance between i and j
(You will also see the distribution of mass by mean square radius, $\langle R^2 \rangle = \sum r_i^2 m_i / \sum m_i$)

Dynamic Light Scattering (DLS or Photon Correlation Spectroscopy): Stokes Radius (R_h)

- 1) Measure variation of light scattering over short times (microseconds)
– look at autocorrelation function using Stokes-Einstein Equation \rightarrow “D”
– report “Hydrodynamic Radius – R_h ” instead of $D_o = kT/f = kT/(6\pi\eta R_h)$

Size range: R_h from 1 to 1000nm

- particularly good at *sensing very small amounts* of aggregates (<0.01% by wt.)

Practical Considerations:

- 1) Normally use SEC plus a 2-detector or 3-detector experiment
SEC/LS/RI or SEC/LS/RI/UV

Light Scattering - $LS = K_{LS}CM(dn/dC)^2$

Refractive Index – $RI = K_{RI}C(dn/dC)$

$(dn/dC) \sim 0.186 \text{ mL/g}$ for proteins; different if carbohydrate present

Determine M from ratio of the two detectors:

$$LS/RI = M [(K_{LS}/K_{RI})(dn/dC) = M K']$$

or $M = K'(LS)/(RI)$; (2-detector method valid when (dn/dC) is known)

(use protein standards like BSA to calibrate both RI and LS detectors)

(use 3rd UV detector for concentration when carbohydrate is present)

- 2) Polydispersity [(M_w/M_n) and (M_z/M_n)]

MW Averages - $\sum C_i M_i^y / \sum C_i M_i^{y-1}$ (or $M_w = \sum C_i M_i / \sum C_i$ for $y = 1$)

Note: Since $LS \sim MC$ and $RI \sim C$, when normalized and

checking for oligomerization – $LS = RI$ for monomer

$LS = 2 RI$ for dimer

$LS = 3 RI$ for trimer, etc.

MALS – Multi-angle Light Scattering

- with large particles (or shorter wavelengths) – can obtain information on size

- Angle and Conc. dependence \rightarrow **Zimm Plot** (K^*C/R vs. $\sin^2(\theta/2)$):

\rightarrow y-intercept gives $1/M$, slope gives R_G^2