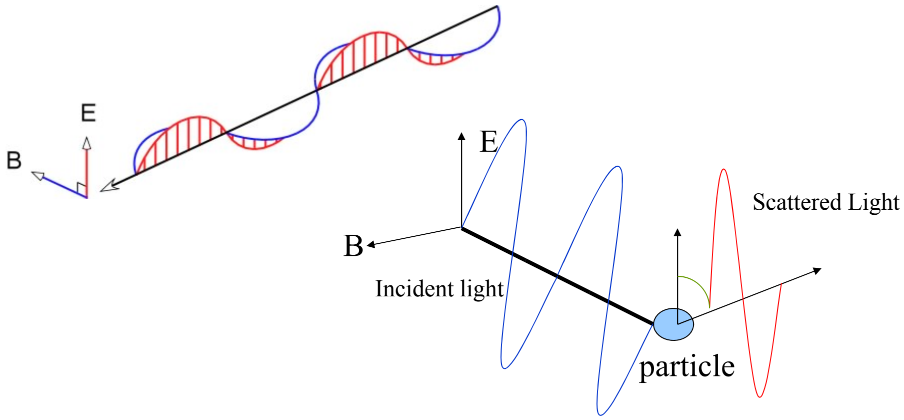
Scattering by macromolecules



Oscillating E field from light makes electronic cloud oscillate surrounding the particle

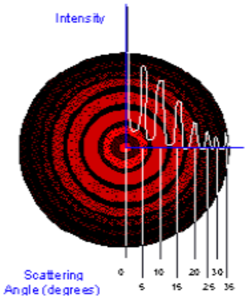
- Intensity: $I \propto |E|^2$

Accelerating charges means EM radiation is produced which radiates into different directions → **Scatter**

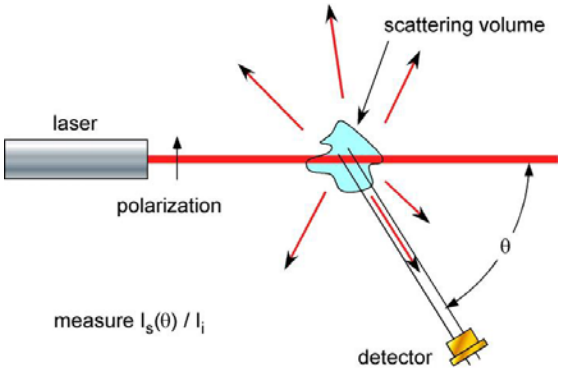
Light scattering
Small and large particles

- Small particles one scattering center < 10 nm
- Scatter intensity independent of scattering angle (Rayleigh scattering)

- Large particles multiple scattering centres
- Scattering depend on angle and gives diffraction pattern

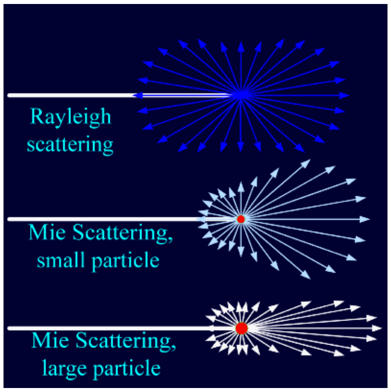


What is light scattering?



We can choose the wavelength (λ), polarization, and intensity (I_i) of the incident light. The size of the laser beam and the field of view of the detector define a scattering volume. We can detect the scattered light (I_s) from this volume as a function of angle (θ) and polarization.
We can use light scattering to retrieve fundamental physical properties of the scattering medium.

- Elastic scattering
  - Photon retains energy
- Rayleigh
  - $r<\lambda/10$
  - Point form
- Mie
  - $r>\lambda/10$
  - Shape matters
- *Non-elastic*
  - *Absorption spectroscopy*
  - *Emission spectroscopy*

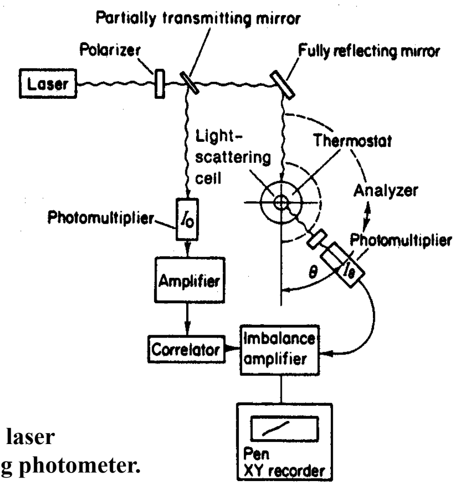


How much light radiates depends upon the polarizability.

- Typical size of a protein:
  r=1-3 nm
- Wave length of light source: 514nm (Ar+) 632nm (HeNe)
- Wave length of light is large with respect to molecule - Rayleigh scattering

Light Scattering

Light source : High pressure mercury lamp and laser light.
Limitation of molecular weight : $10^4 \sim 10^7$

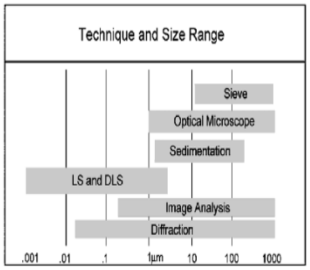


Schematic of a laser light-scattering photometer.

Applications

Light scattering is one of the most important ways to characterize macromolecules, particles and colloids.

1. Determine the molecular weight of macromolecules
2. Determine the particles size and distribution in a polymeric matrix.
3. Determine the shape and size of aggregates and colloids.
4. Determine the properties of dilute polymer solutions



Medical, pharmacy and biology: DNA colloids, toroid aggregates etc.
Chemistry, polymer and materials science : Macromolecules in solution
Fillers and polymer solutions
Climate research: Ice crystal, cloud radiation scattering

The intensity of scattered light or turbidity(τ) depends on the following factors

a. size

b. concentration

c. polarizability

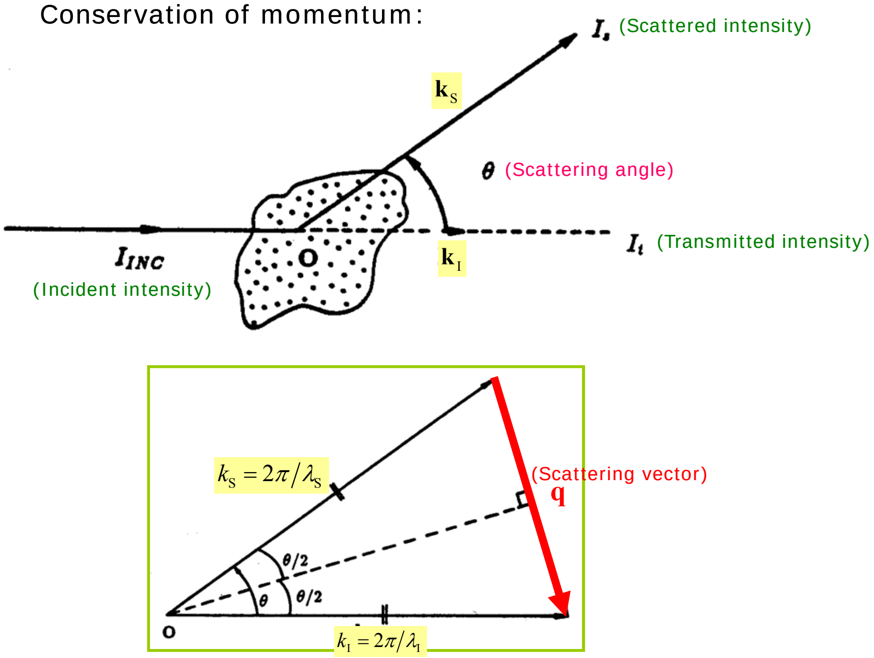
d. refractive index

e. angle

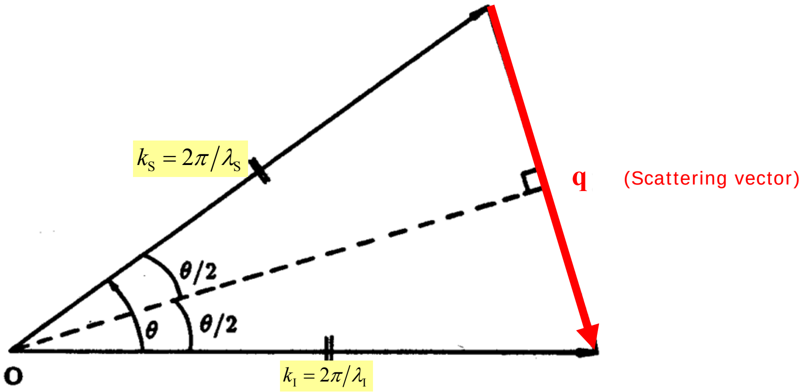
f. solvent and solute interaction

g. wavelength of the incident light

Conservation of momentum:



Magnitude of the Scattering Vector q

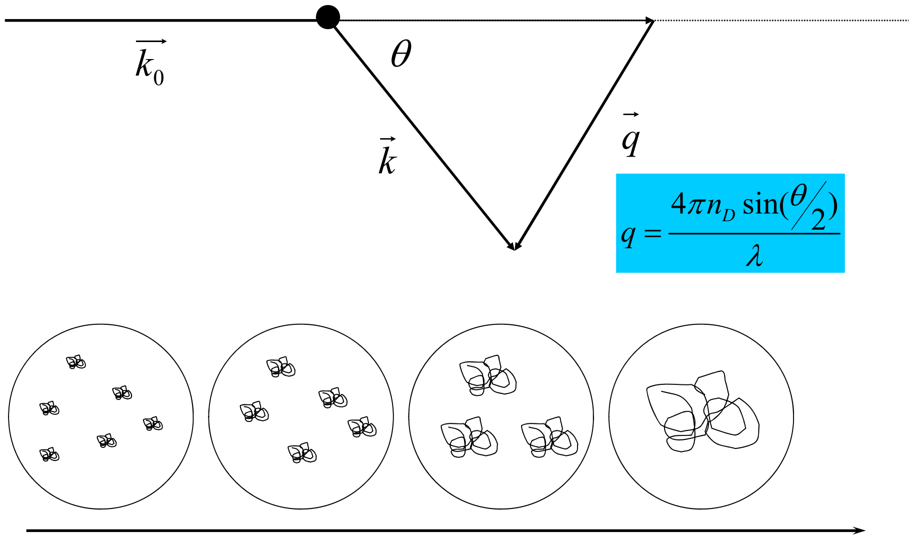


$$q^2 = |\mathbf{k}_I - \mathbf{k}_S|^2 = k_I^2 + k_S^2 - 2\mathbf{k}_I \cdot \mathbf{k}_S$$

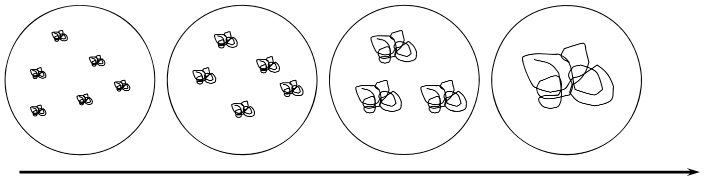
Assumption: $\lambda_I \approx \lambda_S$

$$= 2k_I^2 - 2k_I^2 \cos\theta = 4k_I^2 \sin^2(\theta/2)$$

$$\Rightarrow q = (4\pi n_D/\lambda_I)\sin(\theta/2)$$



$$q = \frac{4\pi n_D \sin(\theta/2)}{\lambda}$$

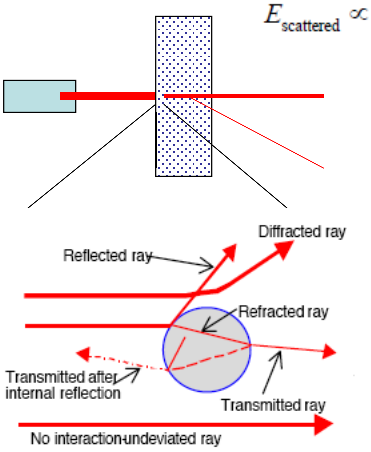


q-scale	resolution	information	comment
qR << 1	whole coil	mass, radius of gyration	e.g. Zimm plot
qR < 1	topology	cylinder, sphere, …	
qR ≈ 1	topology quantitative	size of cylinder, ...	
qR > 1	chain conformation	helical, stretched, ...	
qR >> 1	chain segments	chain segment density	

Static light scattering

- Particle size information is obtained from intensity of the scattering pattern at various angles.
- Intensity is dependent on
  - wavelength of the light
  - Scattering angle
  - particle size
  - relative index of refraction *n* of the particle and the medium.

$$E_{\text{scattered}} \propto \frac{dn}{dc}$$



$$I_{\text{scattered}} \propto \left(\frac{dn}{dc}\right)^2$$

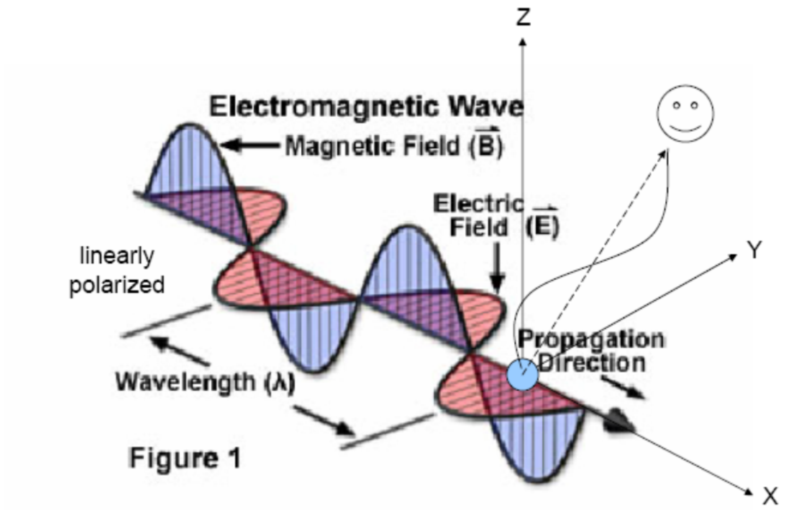


Figure 1

Classically, the oscillation of the external field produces a corresponding oscillation in the electrons within the molecule, dispersing some of the energy in directions other than the direction of the incident radiation

Scattering arises from the oscillating dipole moment, a vector determined by the polarizability $\mu = \alpha E = aE_o \cos 2\pi\nu t$, where $E(x,t) = E_o \cos 2\pi(\nu t - x/\lambda)$

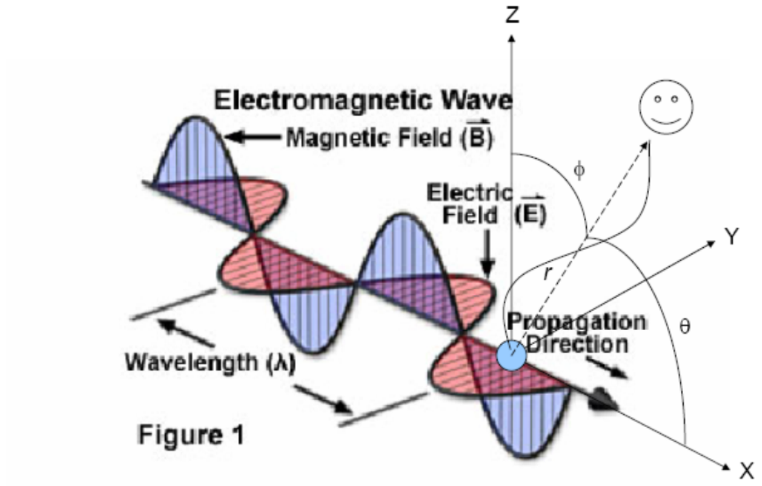


Figure 1

The radiated field may be described in terms of coordinates r, ϕ and θ , with respect to the direction of the polarization (Z):

$$E_r = [\alpha E_o 4\pi^2 \sin\phi \,/\, r\lambda^2] \cos 2\pi(\nu t - r/\lambda)$$

Amplitude is of interest, because square of amplitude is intensity.

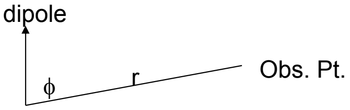
The incident electric field is

$$E = E_0 \cos 2\pi \left(\nu t - \frac{x}{\lambda}\right)$$

Interaction with molecules drives their electrons at the same frequency to induce an oscillating dipole

This dipole will radiate producing a scattered E field from the single molecule

$$E_r = \frac{\alpha E_0 4\pi^2 sin\phi}{r\lambda^2} \cos 2\pi(\nu t - \frac{r}{\lambda})$$



$$E_r = \frac{\alpha E_0 4\pi^2 sin\phi}{r\lambda^2} \cos 2\pi(\nu t - \frac{r}{\lambda})$$

$\cos 2\pi(\nu t - \frac{r}{\lambda})$ polarized oscillating field at particle and detector

$\frac{4\pi^2 sin\phi}{r\lambda^2}$ angular and distance dependence of scattering

αE_0 oscillating dipole moment of particle in terms of oscillation field

$$\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{\left(\frac{\alpha E_o 4\pi^2 \sin\phi}{r\lambda^2}\right)^2}{E_o^2} = \frac{16\pi^4\alpha^2 \sin^2\phi}{r^2\lambda^4}$$

$$\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{\left(\frac{\alpha E_o 4\pi^2 \sin\phi}{r\lambda^2}\right)^2}{E_o^2} = \frac{16\pi^4\alpha^2\sin^2\phi}{r^2\lambda^4}$$

This equation tells us:

- Intensity of scattering falls off with r^2
- Intensity of scattering increases rapidly with decreasing wavelength
- Intensity of scattering depends on ϕ

Express α in measurable terms

Express α in measurable terms

$n^2 - n_{solv}^2 = 4\pi N\alpha$

where n = index of refraction of solution; n_{solv} of solvent

$(n-n_{solv})(n+n_{solv}) = 4\pi N\alpha$

At low conc. $(n + n_{solv}) \approx 2\, n_{solv}$ and $(n - n_{solv})/C \approx dn/dc$

$\alpha \approx (n_{solv} / 2\pi)\,(dn/dc)\,(c/N)$

$$\alpha = (n+n_{solv})(n-n_{solv})/4\pi N \sim \frac{2n_{solv}}{4\pi}\frac{dn}{dc}\frac{c}{N} = \alpha = \frac{n_{solv}}{2\pi N_A}\frac{dn}{dc}M$$

$$\alpha = \frac{n_{solv}}{2\pi N_A}\frac{dn}{dc}M$$

Scattered Intensity

- Detect intensity, not E, where

$$\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{\left(\frac{\alpha E_o 4\pi^2 \sin\phi}{r\lambda^2}\right)^2}{E_o^2} = \frac{16\pi^4\alpha^2\sin^2\phi}{r^2\lambda^4}$$

- Substituting for α, we have

$$\boxed{\alpha = \frac{n_{solv}}{2\pi N_A}\frac{dn}{dc}M}$$

$$\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{4\pi^2 M^2 n_{solv}^2\left(\frac{dn}{dc}\right)^2 \sin^2\phi}{r^2\lambda^4 N_A^2}$$

- If there are N scatterers/unit volume and all are independent with N = N_Ac/M, then

$$\left.\frac{I_{scatt}}{I_o}\right]_{per\ unit\ volume} = N\left.\frac{I_{scatt}}{I_o}\right]_{1\,particle} = \frac{4\pi^2\sin^2\phi\, n_{solv}^2\left(\frac{dn}{dc}\right)^2 Mc}{N_A r^2\lambda^4}$$

- We define the Rayleigh ratio R_θ:

$$R_\theta = \frac{I_{scatt,\theta}r^2}{I_o\sin^2\phi} = \frac{4\pi^2 n_{solv}^2\left(\frac{dn}{dc}\right)^2 Mc}{N_A\lambda^4} = KMc$$

Rayleigh ratio and molecular weight determination

With real solutions, equations must be modified to account for non-ideality (particles are not truly non-interacting) but

$Kc/R_\theta = 1/M$

Expand in a power series around c for nonideal solutions

$Kc/R_\theta = 1/M + 2Bc + \ldots$

where B is a measure of the nonideality, the second virial coefficient

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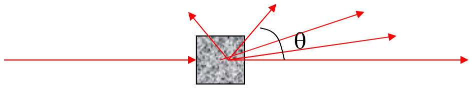
Basic Measurement

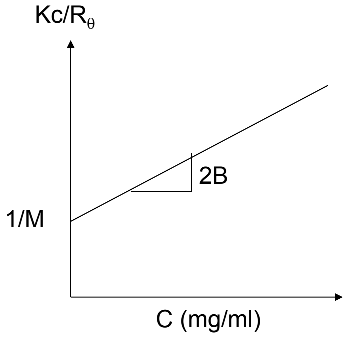
- If the intensity ratio I_θ/I_o, n_{solv}, dn/dc, λ, c, ϕ, and r are all known, you can find M.
- Usually write $Kc/R_\theta = 1/M$
- Measurements are usually made as a function of concentration *c* and scattering angle θ
- The concentration dependence is given by

$$\frac{Kc}{R_\theta} = \frac{1}{M} + 2Bc$$

where B is called second virial coefficient



Concentration effect

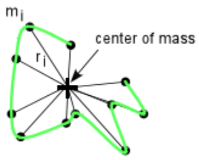


- Non-ideal solution
  - Particles not independent
  - Scatter ≠ N x single scatter
- Power series expansion

$$\frac{Kc}{R_\theta} = \frac{1}{M} + 2Bc$$

- B = 2nd virial coefficient
  - Measure of solution non-ideality
  - General thermodynamic property

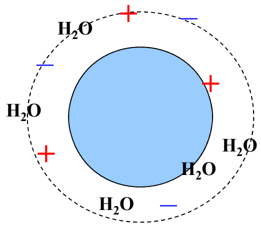
- Radius of gyration (rms): a measure of the size of molecule by the mass distribution about its center of mass.



- Hydrodynamic radius: depends on the mass and the shape of the molecule (conformation).
- Second virial coefficient (B): measure of solute-solvent interaction.

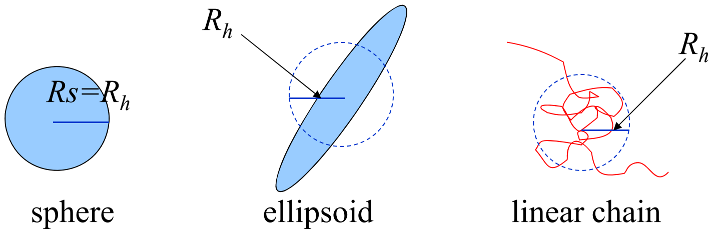
Hydrodynamic radius, R_h

- R_h is the radius of a hypothetical hard sphere that diffuses with the same speed as the particle under examination
- The radius calculated from the diffusional properties of the particle is indicative of the apparent size of the dynamic hydrated/solvated particle



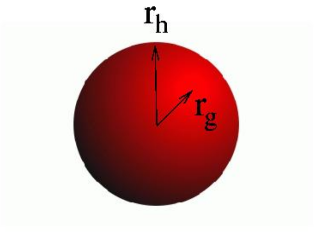
R_h and shape of particles

- For the center-of-mass motion, an ellipsoid with a hydrodynamic radius R_h receives the same friction as sphere of radius R_h does
- A linear chain with a hydrodynamic radius R_h diffuses with the same diffusion coefficient as the sphere of radius R_h



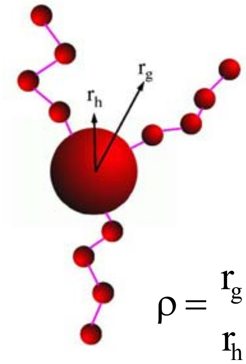
Conformation: r_h vs. r_g

solid sphere



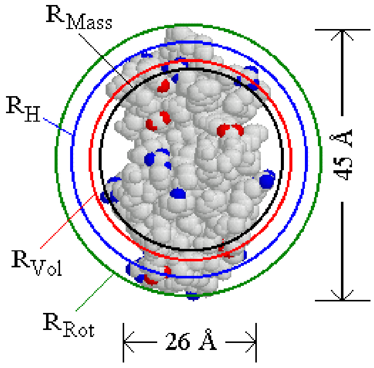
$$\rho = \frac{r_g}{r_h} = 0.77$$

3-arm star polymer



$$\rho = \frac{r_g}{r_h} \approx 1.4$$

By comparing r_g to r_h we may learn about the compactness of a molecule and so gain information concerning the molecular conformation.



Lysozyme
MW = 14,700 Da
V_p = 0.73 mL/g
a/b = 22.5/13 = 1.731
F = 1.022

R_{Mass}: RMS radius
R_H: hydrodynamic radius
R_{Vol}: Radius of a hypothetical sphere that occupies the same volume as the macromolecule
R_{Rot}: Radius subtended by rotating the macromolecule

Hydrodynamic radius from diffusion coefficient

$$D = \frac{k_b T}{f}$$

Einstein equation

and

$$f = 6\pi\eta R_h$$

Stokes equation

$$D = \frac{k_b T}{6\pi\eta R_h}$$

Stokes-Einstein equation

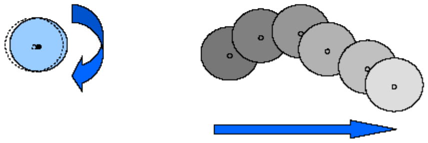
$$R_h = \frac{k_b T}{6\pi\eta D_T}$$

translational diffusion coefficient, D_T

Diffusion of molecules ---- Brownian Motion

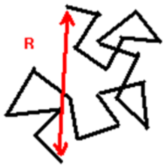
Rotational diffusion: no signal change

Translational diffusion: signal change



Radius of Gyration of a Polymer Coil

The radius of gyration R_g is defined as the RMS distance of the collection of atoms from their common centre of gravity.

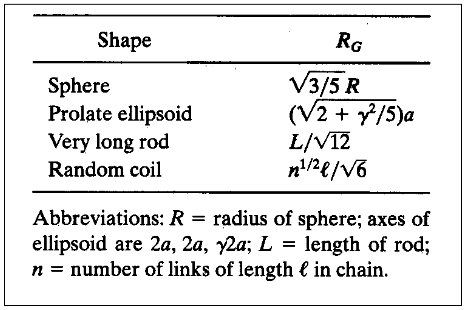


For a polymer coil with rms end-to-end distance R ;

$$R_G = \frac{1}{6}\left\langle \overline{R}^2 \right\rangle^{1/2} = \frac{l}{6} N^{1/2}$$

$$R_G^2 = \frac{1}{2N^2} \sum_{i=1}^{N} \sum_{j=1}^{N} r_{ij}^2$$

Distance between particles *i* and *j*



Shape	R_G
Sphere	$\sqrt{3/5}\,R$
Prolate ellipsoid	$(\sqrt{2 + \gamma^2/5})a$
Very long rod	$L/\sqrt{12}$
Random coil	$n^{1/2}\ell/\sqrt{6}$

Abbreviations: R = radius of sphere; axes of ellipsoid are $2a$, $2a$, $\gamma 2a$; L = length of rod; n = number of links of length ℓ in chain.

Scattering from particles with dimensions comparable to the wavelength of the incident radiation: size and shape information.

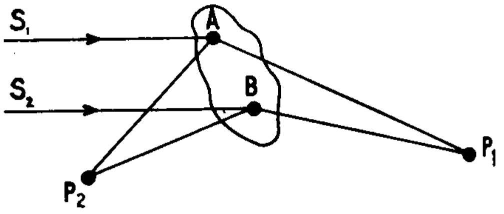
If λ is of the same order as the particle size, then radiation scattered from different points on the same particle can interfere. The bigger the phase difference between scattered beams, the stronger the effects of interference.

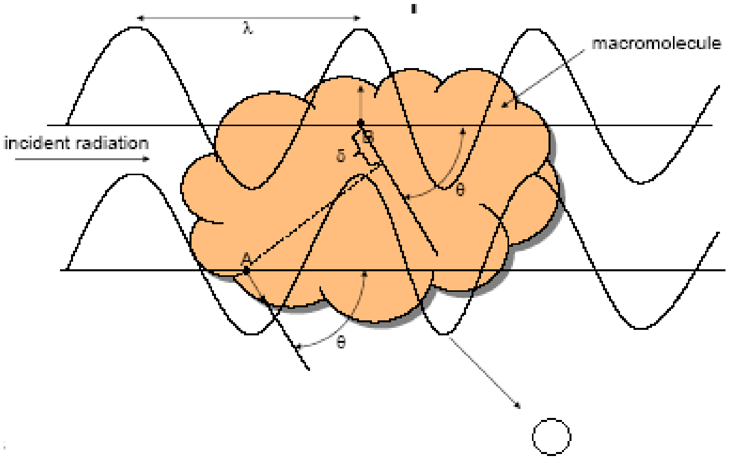
Phase differences will be magnified at large scattering angles.

In the figure below, AP_1 is approximately equal to BP_1, so the phase difference will be small.

However, AP_2 is larger than BP_2, resulting in a larger phase difference.

S_1 and S_2 are sources





Two points from which scattering occurs are labeled A and B. The phase of the radiation (and thus the two induced dipoles) is different at the two points. The points are also different distances from the observer.

For larger particles, we define a function $P(\theta)$, sometimes called the *particle form factor*, which is the ratio of scattering from the finite-sized particle to that from an infinitely small particle of the same mass.

P(θ) = scattering by real particle at θ / scattering by hypothetical particle at θ

$P(\theta)$ is sensitive to the shape of the particle. Typically, calculations are carried out for a variety of shapes and compared to the experimental data.

$$P(\theta) \approx 1 - \frac{q^2 R_G^2}{3} \text{ where } R_G^2 \text{ is the}$$

$$\text{radius of gyration and } q = (4\pi / \lambda)\sin(\theta / 2)$$

$$\ln(1 - x) \approx -x$$

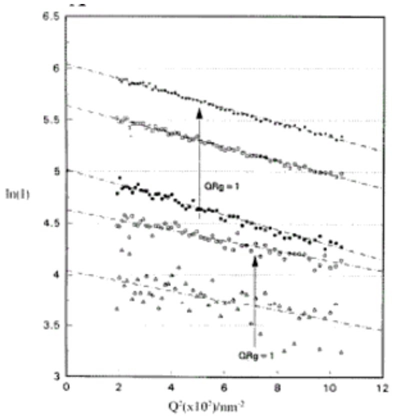
$$\text{Thus, } \ln P(\theta) \approx -\frac{q^2 R_G^2}{3}$$

There are two commonly used plots for determining R_G and M_w

The Guinier Plot and the Zimm Plot.

The **Guinier Plot** is a plot of $\ln(I_\theta)$ vs q^2, which should have a slope of $-R_\mathrm{G}^2/3$.

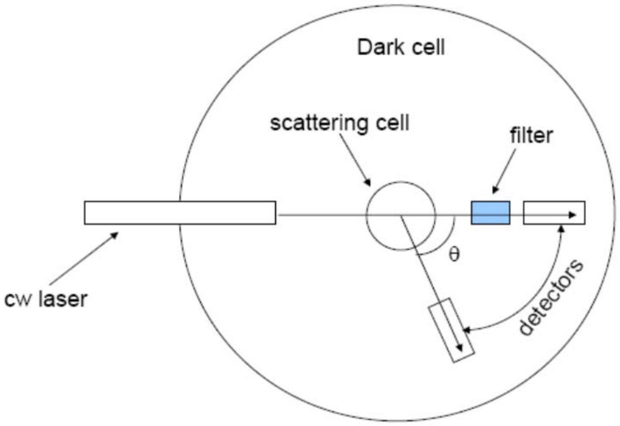
The plot shows scattering data for γ-gliadin at different concentrations



For mixture of macromolecular substances:

$R_\theta = \Sigma R_{\theta i} = \Sigma K_i C_i M_i = KCM_w$

schematic of modern light scattering instrument

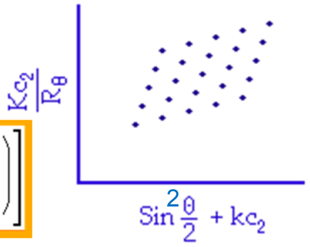


Analysis of LS Data

- Measure $I(\theta, c)$ and plot

  Kc/R_θ vs $\sin^2(\theta/2)$ + (const)c

$$\boxed{\frac{Kc_2}{R_\theta} = \left(\frac{1}{M} + 2Bc_2\right)\left[1 + \frac{16\pi^2 \overline{r_g^2}}{3\lambda^2}\sin^2\left(\frac{\theta}{2}\right)\right]}$$

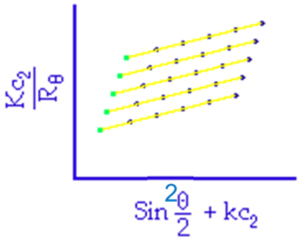


Extrapolations:

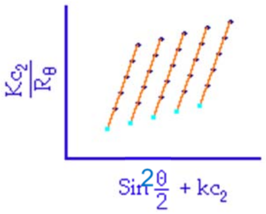
$c \longrightarrow 0$

$\theta \longrightarrow 0$

Extrapolation to zero scattering angle

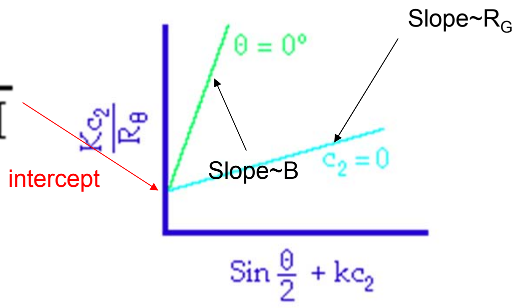


Extrapolation to zero concentration



Final result

$$\frac{Kc_2}{R_\theta} = \frac{1}{M}$$



The **Zimm Plot** utilizes this relationship for finite particles:

$$\frac{KC}{R_\theta} = \frac{1}{P(\theta)}\left(\frac{1}{\overline{M}_w} + 2BC\right)$$

KCR_θ is plotted vs. $\sin^2(\theta/2)$+ KC.

Data is gathered at 4 or 5 concentrations over a range of scattering angles.

Data for C=0 and $\theta/2$=0 are obtained by extrapolation.

The final plot contains both experimental and extrapolated values.

- The intercept on the vertical axis is $1/M_w$.
- The limiting slope at $C = 0$ equals $(1/M)(16\pi^2 R_G^2/3\lambda^2)$.
- The slopes of the horizontal lines yield the second virial coefficient, B.

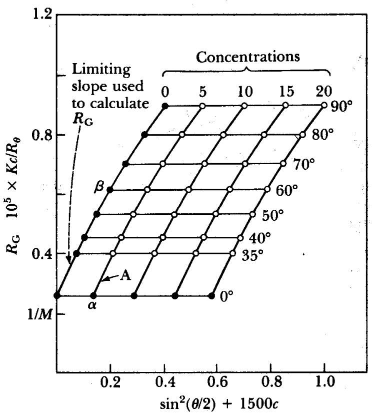
Sample Construction of a Zimm Plot

Measure R_θ as a function of scattering angle and sample concentration. Correct the R_θ values for solvent scattering.

Plot Kc/R_θ vs $\sin^2(\theta/2)$ for each concentration; extrapolate to $\sin^2(\theta/2) = 0$.

Plot Kc/R_θ vs c for each each angle; extrapolate to zero concentration.

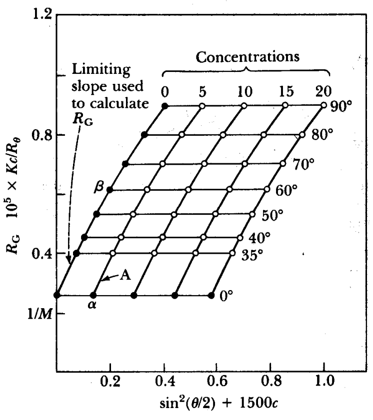
Plot Kc/R_θ vs $\sin^2(\theta/2) + kc$ using both experimental and extrapolated points. The $c = 0$ and $\sin^2(\theta/2) = 0$ lines should intersect at $1/M$ on the y-axis.



(k is an arbitrary constant, 1500 in this example.)

The limiting slope of the $c = 0$ line is equal to $(1/M)(16\pi^2 R_G^2/3\lambda^2)$, from which R_G can be calculated.

The slopes of the horizontal lines provide information on the second virial coefficient (shown equal to zero in this example).



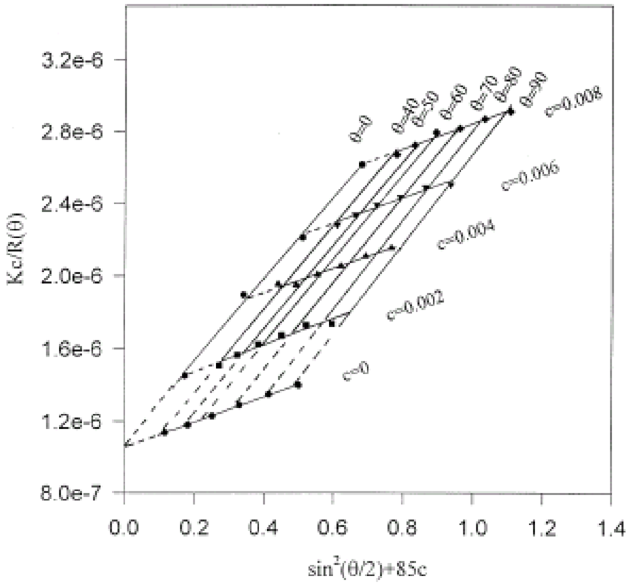


Fig. 7. Zimm plot for PMMA solved in acetone at room temperature (24°C).

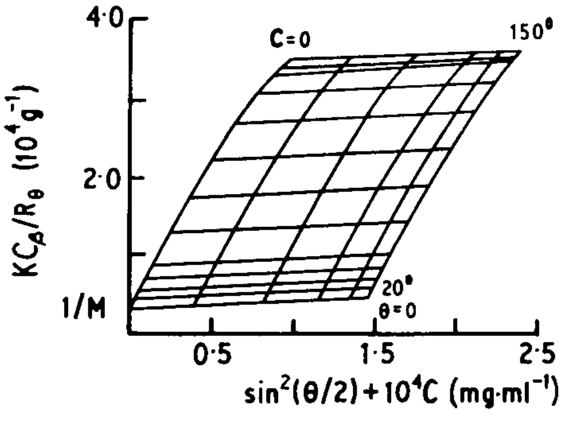
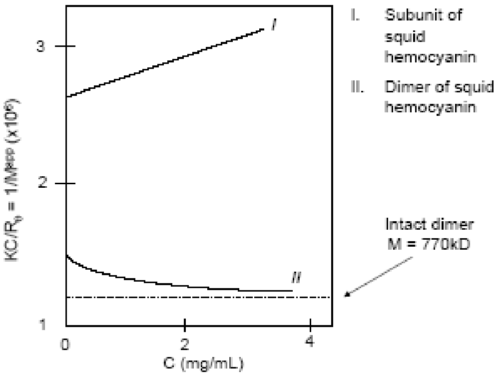


Figure 8.7 A Zimm plot of a DNA sample. The slope at zero angle gives B and the slope at zero concentration gives R_G. The results of this experiment gave $M_w = 3.7 \times 10^6$ daltons, $R_G = 213$ nm, and $B = 5.3 \times 10^4$ mL • mol • g^2 (from D. Jolly and H. Eisenberg, *Biopolymers,* 15(1976):61).

For nonideal solutions,

Scattering measured at several concentrations and KC/R_θ vs C plotted – extrapolation to C=0 gives the correct molecular weight

For mixture of macromolecular substances: $R_\theta = \Sigma R_{\theta i} = \Sigma K_i C_i M_i = KCM_w$



$$M_w = \frac{\sum_{i=1}^{n} C_i M_i}{\sum_{i=1}^{n} C_i}$$

$KC/R_\theta = 1/M + 2BC$

B (slope) is the *second virial coefficient; B(+) means good solute solvent interaction or bad solute-solute interactions; B(-) means bad solute solvent interactions or good solute-solute interactions*

Static light scattering vs. Dynamic light scattering

- Static light scattering measures time-average intensities (mean square fluctuations)

  molecular weight

  radius of gyration

  second virial coefficient

- Dynamic light scattering measures real-time intensities i(t), and thus dynamic properties

  diffusion coefficient

  (hydrodynamic radius)

  size distributions