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

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

\[ I \propto |E|^2 \]

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 ($\lambda$), 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 ($\theta$) 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.](image)

**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 (\(\tau\)) 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:

\[ q = k_s = 2\pi/\lambda_s \]

\[ \theta \] (Scattering angle)

\[ k_i \] (Incident intensity)

\[ k_s \] (Scattering vector)

\[ I_s \] (Scattered intensity)

\[ I_t \] (Transmitted intensity)
Magnitude of the Scattering Vector \( q \)

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

\( \lambda \) is the wavelength of the incident light, \( n_D \) is the refractive index of the scatterer, and \( \theta \) is the scattering angle.

Assumption: \( \lambda_s \parallel \lambda \)

\[
q^2 = |k_1 - k_s|^2 = k_1^2 + k_s^2 - 2k_1 \cdot k_s
\]

\[
q = 2k_1^2 - 2k_1^2 \cos \theta = 4k_1^2 \sin^2(\theta/2)
\]

\( k_s = \frac{2\pi}{\lambda} \) and \( k_1 = \frac{2\pi}{\lambda} \)
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.

\[
I_{\text{scattered}} \propto \left( \frac{dn}{dc} \right)^2
\]
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 \( \mu = \alpha E = aE_0\cos2\pi vt \), where \( E(x,t) = E_0\cos2\pi(vt - x/\lambda) \).

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

\[
E_r = \left[ \frac{\alpha E_02\pi^2 \sin \phi}{r^2} \right] \cos 2\pi(vt - r/\lambda)
\]

Amplitude is of interest, because square of amplitude is intensity.
The incident electric field is
\[ E = E_0 \cos 2\pi \left( vt - \frac{x}{\lambda} \right) \]

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

\[ E_r = \frac{\alpha E_0 4\pi^2 \sin \phi}{r\lambda^2} \cos 2\pi \left( vt - \frac{r}{\lambda} \right) \]

\[ \cos 2\pi \left( vt - \frac{r}{\lambda} \right) \] polarized oscillating field at particle and detector

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

\[ \alpha E_0 \] oscillating dipole moment of particle in terms of oscillation field

\[ \frac{I_{\text{scatt}}}{I_o} \bigg|_{\text{particle}} = \frac{\left( \alpha E_0 4\pi^2 \sin \phi \right)^2}{r^2 \lambda^4} = \frac{16\pi^4 \alpha^2 \sin^2 \phi}{E_o^2} \]
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 \( \Phi \)

Express \( \alpha \) in measurable terms

Express \( \alpha \) in measurable terms

\[
\frac{I_{\text{scatt}}}{I_o} \propto \left( \frac{\alpha E_o 4\pi^2 \sin \phi}{r \lambda^2} \right)^2 = \frac{16\pi^4 \alpha^2 \sin^2 \phi}{r^2 \lambda^4}
\]

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

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 \((n - n_{solv})/C \approx dn/dc\)

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

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

\[\alpha = \frac{n_{solv}}{2\pi N_A} \frac{dn}{dc} M\]
Scattered Intensity

- Detect intensity, not $E$, where

$$\frac{I_{\text{scatt}}}{I_o} \bigg|_{\text{particle}} = \left( \frac{\alpha E_o 4\pi^2 \sin \phi}{r \lambda^2} \right)^2 \frac{E_o^2}{r^2 \lambda^4} = 16\pi^4 \alpha^2 \sin^2 \phi$$

- Substituting for $\alpha$, we have

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

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

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

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

- We define the Rayleigh ratio $R_\theta$:

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

\[ \frac{Kc}{R_\theta} = \frac{1}{M} \]

Expand in a power series around \( c \) for nonideal solutions

\[ \frac{Kc}{R_\theta} = \frac{1}{M} + 2Bc + \ldots \]

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

---

**Basic Measurement**

- If the intensity ratio \( I/I_0 \), \( n_{solv} \), \( dn/dc \), \( \lambda \), \( c \), \( \phi \), and \( r \) are all known, you can find \( M \).
- Usually write \( \frac{Kc}{R_\theta} = \frac{1}{M} \)
- Measurements are usually made as a function of concentration \( c \) and scattering angle \( \theta \)
- 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 $\neq N \times$ single scatter
- Power series expansion
  $$\frac{Kc}{R_\theta} = \frac{1}{M} + 2Bc$$
- $B = 2^{nd}$ 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 $R_h$. 

![Diagram of hydrodynamic radius and particle shapes](image-url)
Conformation: $r_h$ vs. $r_g$

solid sphere

3-arm star polymer

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

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

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
$a/b = 22.5/13 = 1.731$
$F = 1.022$

$R_{\text{Mass}}$: RMS radius
$R_h$: hydrodynamic radius
$R_{\text{Vol}}$: Radius of a hypothetical sphere that occupies the same volume as the macromolecule
$R_{\text{Rot}}$: 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

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

translational diffusion coefficient, \(D_T\)

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 \(R\);

\[
R_G = \frac{1}{6} \left( \overline{R^2} \right)^{1/2} = \frac{I}{6} N^{1/2}
\]

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

Distance between particles \(i\) and \(j\)

<table>
<thead>
<tr>
<th>Shape</th>
<th>(R_G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sphere</td>
<td>(\sqrt{3/5} R)</td>
</tr>
<tr>
<td>Prolate ellipsoid</td>
<td>((\sqrt{2} + \gamma^2/5)a)</td>
</tr>
<tr>
<td>Very long rod</td>
<td>(L/\sqrt{12})</td>
</tr>
<tr>
<td>Random coil</td>
<td>(n^{1/2}\ell/\sqrt{6})</td>
</tr>
</tbody>
</table>

Abbreviations: \(R\) = radius of sphere; axes of ellipsoid are \(2a, 2a, \gamma 2a\); \(L\) = length of rod; \(n\) = number of links of length \(\ell\) in chain.
Scattering from particles with dimensions comparable to the wavelength of the incident radiation: size and shape information.

If $\lambda$ 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.

$$P(\theta) = \frac{\text{scattering by real particle at } \theta}{\text{scattering by hypothetical particle at } \theta}$$

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

$$P(\theta) \approx 1 - \frac{q^2 R_G^2}{3} \text{ where } R_G^2 \text{ is the radius of gyration and } q = (4\pi / \lambda) \sin(\theta / 2)$$

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

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 $\ln(I_0)$ vs $q^2$, which should have a slope of $-R_G^2/3$.

The plot shows scattering data for $\gamma$-gliadin at different concentrations.
Analysis of LS Data

- Measure $I(\theta, c)$ and plot $Kc/R_0$ vs $\sin^2(\theta/2) + (\text{const})c$

\[
\frac{Kc_2}{R_0} = \left( \frac{1}{M} + 2Bc_2 \right) \left[ 1 + \frac{16\pi^2 r_0^2}{3\lambda^2} \sin^2 \left( \frac{\theta}{2} \right) \right]
\]

Extrapolations:
- Extrapolation to zero scattering angle
- Extrapolation to zero concentration
The **Zimm Plot** utilizes this relationship for finite particles:

\[
\frac{Kc_2}{R_\theta} = \frac{1}{M} \left( \frac{1}{M_w} + 2BC \right)
\]

\(KCR_0\) 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 \((1/M)(16\pi^2R_G^2/3\lambda^2)\).

-- The slopes of the horizontal lines yield the second virial coefficient, \(B\).
Sample Construction of a Zimm Plot

Measure $R_0$ as a function of scattering angle and sample concentration. Correct the $R_0$ values for solvent scattering.

Plot $Kc/R_0$ vs $\sin^2(\theta/2)$ for each concentration; extrapolate to $\sin^2(\theta/2) = 0$.

Plot $Kc/R_0$ vs $c$ for each angle; extrapolate to zero concentration.

Plot $Kc/R_0$ 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.

The limiting slope of the $c = 0$ line is equal to $(1/M)(16\pi^2R_G^2/3\lambda^2)$, 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).
**Figure 7.** Zimm plot for PMMA solved in acetone at room temperature (24°C).

---

**Figure 6.7** 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⁻¹·g⁻² (from D. Jolly and H. Eisenberg, *Biopolymers*, 15(1976):61).
For nonideal solutions, scattering measured at several concentrations and KC/Rθ vs C plotted – extrapolation to C=0 gives the correct molecular weight

For mixture of macromolecular substances: \( R_0 = \Sigma R_{i0} = \Sigma K_i C_i M_i = KCM_w \)

\[
B (\text{slope}) = \text{second virial coefficient}; B(+) means good solute solvent interaction or bad solute-solute interactions; B(-) means bad solute solvent interactions or good solute-solute interactions
\]

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