2 Main Types

(1) Classical or "STATIC" Light Scattering

- Measure scattered intensity $i_\theta$ as a function of angle $\theta$ $\rightarrow M_w$ (weight average molecular weight)
- Measure $R_g$ (radius of gyration)

(2) Dynamic or "QUASI-ELASTIC" Light Scattering

- Measure rapid fluctuations of $i_\theta$ as a function of time $t$ $\rightarrow D$ (diffusion coefficient)
CLASSICAL LIGHT SCATTERING

... for characterisation of biomolecular molecular weights and conformations (via "radius of gyration," $R_g$)
The blue colour of the sky and the polarisation of skylight ... constitute, in the opinion of our most eminent authorities, the two great standing enigmas of meteorology. Indeed it was the interest manifested in them by Sir John Herschel in a letter of singular speculative power that caused me to enter upon the consideration of these questions so soon.

J. Tyndall, 1969
Historical

17th C
SNELL
NEWTON
HUYGENS
FERMAT

19th C
YOUNG
FRESNEL

1865: MAXWELL - ELECTROMAGNETIC THEORY

1869: TYNDALL - LIGHT SCATTERING;
"THE 2 GREAT STANDING ENIGMAS"
JOHN WILLIAM STRUTT (LORD RAYLEIGH)

In 1874, aged 28, photographed by himself with wet collodion plate
1881: Rayleigh - Single Particle Theory (Small Scatterers)

1908: Mie
1909: Debye

1914: Rayleigh
1915: Debye

1925: Gans

1908: Smoluchowski
1911: Einstein

1947-50: Debye
1947-50: Zimm

General Theory
Approximate Theory
Thermodynamic Theory of Solution Scattering
Scattering by Solutions of Macromolecules
Classification of Light Scattering

(by particle type)

(1) Rayleigh \( d \leq \frac{\lambda}{20} \)  
\( M \leq 40,000 \)  
- Lysozyme, Myoglobin etc.

(2) Rayleigh - Gans - Debye (RGD)  
\( d \sim \frac{\lambda}{20} \Rightarrow \lambda \)  
\( \frac{n_f}{n_o} - 1 \leq 0.1 \)  
\( \left\{ \right\} \text{OF MOST INTEREST!} \)

(3) Mie \( d \geq \lambda \)  
- LARGE VIRUSES  
- BACTERIA etc.
RAYLEIGH - GANS - DEBYE (RGo) SCATTERING.

- For biomolecules of \( M = 40,000 \rightarrow 20 \times 10^6 \)

Measure a parameter 'Rayleigh Ratio'

\[
R_0 = \frac{i_0}{I_0} \left[ \frac{C^2}{1 + \cos^2 \theta} \right]
\]

dir. of particle from detector

Light scattering by a solution of macromolecules can be summarized by the equation:

\[
\frac{K_C}{R_0} \sim \left\{ 1 + \frac{16\pi^2 R_g^2 \sin^2 \theta}{3 \lambda^2} \right\} \left( \frac{M}{1 + 2BC} \right)
\]

\( K \) is a collection of constants: \( \frac{2\pi^2 n_0^2 (dn/dC)^2}{N_A \lambda^4} \)

\( B \): 2nd virial coeff. ; \( C \): concentration

\( R_g \): "radius of gyration".

If \( B \) is known, or \( C \) is small enough (20C << 1)

a plot of \( \frac{KC}{R_0} \) vs \( \sin^2 \frac{\theta}{2} \) \( \rightarrow \) Molec. wt = \( R_g \).
$\frac{kC}{R^2_{\theta}} \mid_{c=0} = \frac{1}{M} \left\{ 1 + \frac{16\pi^2}{3} \frac{R^2_{g}}{\lambda^2} \sin^2(\theta/2) \right\}$

$3 \frac{1}{n}$

$10^5 \times Kc/R^2_{\theta}$

$\sin^2 (\theta/2) + kc$
Besides classical light scattering, $R_g$ can also be obtained from solution x-ray scattering or neutron scattering. Why might these alternative techniques be more suitable for smaller biomolecules ($M \leq 40000$)?
Radius of Gyration \( R_g \)

- Root mean square distance of mass elements in a particle from centre of mass.

**Sphere** (radius \( R \))

\[ R_g = \sqrt{\frac{3}{5}} R \]

**Rod** (length \( L \))

\[ R_g = \frac{L}{\sqrt{12}} \]

**Ellipsoid** (semi-axes \( a, b, c \))

\[ R_g = \sqrt{\frac{a^2 + b^2 + c^2}{5}} \]

(nb. prolate \( c = b \) oblate \( c = a \))

**Random coil** (mean square end-to-end distance \( R^2 \))

\[ R_g = \left< R^2 \right>^{\frac{1}{2}} / \sqrt{6} \]

**Bead models**

\[ R_g = \text{some complicated function!} \]
<table>
<thead>
<tr>
<th>Material</th>
<th>( M )</th>
<th>( R_g ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>14,100</td>
<td>1.52</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>70,000</td>
<td>2.98</td>
</tr>
<tr>
<td>Tumip yellow mosaic virus</td>
<td>( 5 \times 10^6 )</td>
<td>30.0</td>
</tr>
<tr>
<td>Myosin</td>
<td>493,000</td>
<td>46.8</td>
</tr>
<tr>
<td>DNA sample</td>
<td>( 4 \times 10^6 )</td>
<td>117.0</td>
</tr>
</tbody>
</table>
OVALBUMIN SEC-MALLS

10 mg/ml

Volume (mL)

Elution Volume
Molecular Weight: SEC–MALLS

MALLs detector

SEC - columns

Concentration detector
Molecular Weight: SEC–MALLS

Molecular Weight: SEC–MALLS

3

Combined Differential Light Scattering with Various Liquid Chromatography Separation Techniques

By Philip J. Wyatt

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1. INTRODUCTION

The combination of light scattering measurements with various particle/molecular separation techniques often permits an unparalleled characterization of the separated particles. In a sense, this is but an application of the so-called “inverse scattering” problem, i.e. from measurements of the light scattering...
Follow up bibliography:

1. On-line tutorials from: Wyatt Technology and Viscotek corporation (see their web sites)