Lecture Notes Chem 524 -- (Part 19)- 2009

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XII. Light scattering for molecular size and shape — (Read Chap 16-3 and 16-4)

A. Classical methods

Turbidity—attenuation of light due to particulate matter in solution – high concentration

Measure like absorption spectrum, but simple filter setup, no spectrum (fig16-16)

$$\phi = \phi_0 \, e^{-\tau b}$$

here b- path, τ - turbidity coefficient, can relate to conc. (linear at low τ values)

$$-\log \phi/\phi_0 = kbc \qquad \text{where assume: } \tau = kc$$

Nephelometry refers to measurement of scattered intensity (at 90°) lower concentration

Measure like very simple full spectrum (filter) fluorescence, empirical relate conc.

$$\phi_{sc} = \phi_0 \, k_{sc}c$$

B. *Dynamic light scattering, DLS,* or quasi-elastic laser light scattering (sect Ch 16-4)

Based on fluctuations in interference between particles scattering a coherent monochromatic laser beam, due to Brownian motion of the particles. Detected by constructing an autocorrelation function to describe fluctuations in the scattered light

The intensity of the scattered light is proportional to the concentration of the macromolecules in solution; twice as many molecules scatter twice as much light.

← independent molecules
 – scatter phase random

aggregate — scatter →
 coherent (in phase)
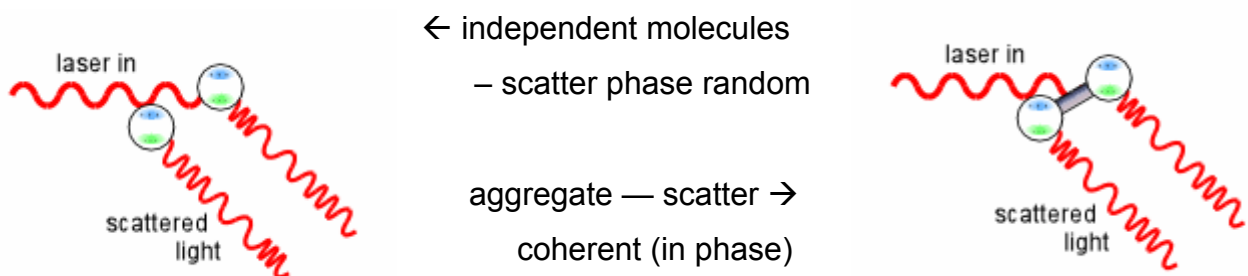
Independent molecules undergo random Brownian motion, giving a random phase to the scattered light, so the intensity from the molecules is incoherent and additive.

If molecules are strongly interacting with each other, their scatter is coherent, so the correlation of scatter will reflect molecular size (here shown as aggregation)

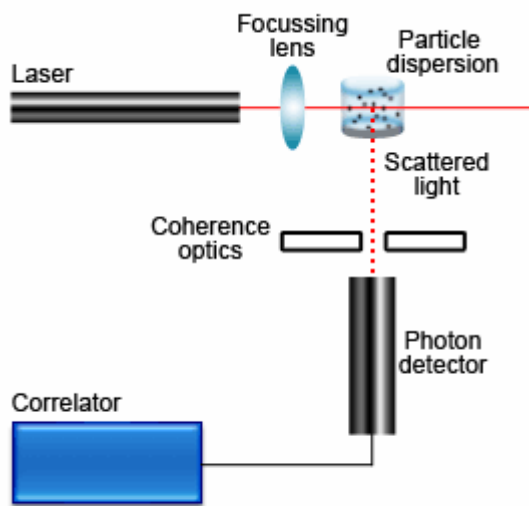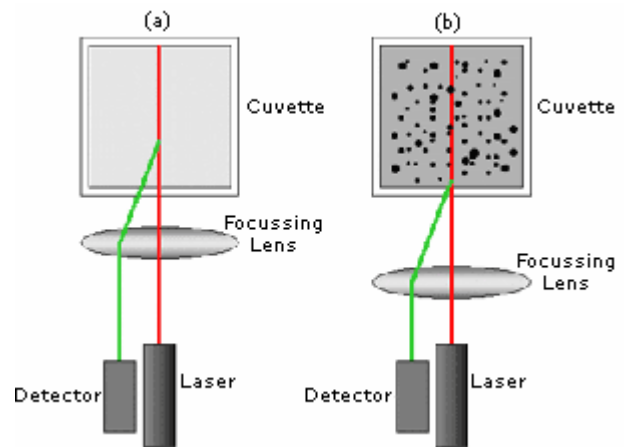Conventional design, use 90° scatter

advantages to back scatter, here ~170° – can vary focus and sample front side of cuvette, no need to dilute (Malvern)

**Figure 1:** Schematic diagram of a conventional, 90° dynamic light scattering instrument.

The second order autocorrelation curve is generated from the intensity trace as follows:

$$g^2(q;\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$

where $g^2(q;\tau)$ is the autocorrelation function at a particular wave vector, q, and delay time, τ, and I is the intensity.

As the time delays become longer, the correlation decays exponentially, since no correlation between the scattered intensity of the initial and final states. This exponential decay is related to the motion of the particles, specifically to the diffusion coefficient.
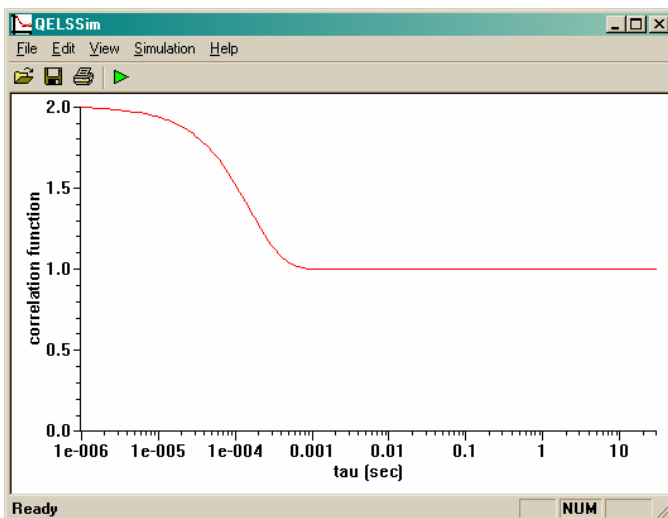
Exponential decay curve of correlation function   TurboCorr Digital Correlator (Brookhaven)

the correlation function for a monodisperse sample can be analyzed by the equation:

$$g^{(2)}(\tau) = B + \beta \exp(-2\Gamma\tau)$$ (2)

where B is the baseline of the correlation function at infinite delay, β is the correlation function amplitude at zero delay, τ is time and Γ is the decay rate.

The _software_ uses a nonlinear least squares fitting algorithm to fit the measured correlation function to eq. 2 to retrieve the correlation decay rate Γ.

Γ can then be converted to the diffusion constant D for the particle via:

$$D = \frac{\Gamma}{q^2}$$ (3)

where, q is the magnitude of the scattering vector, given by:

$$q = \frac{4\pi n_0}{\lambda_0}\sin(\theta/2)$$ (4)

where n_0 is the solvent index of refraction, λ_0 is the vacuum wavelength of the incident light, and θ is the scattering angle. [note in DLS, usually have fixed θ, but can vary]

Finally, the diffusion constant can be interpreted as the hydrodynamic radius r_h of a diffusing sphere via the Stokes-Einstein equation:

$$r_h = \frac{kT}{6\pi\eta D}$$ (5)

where k is Boltzmann's constant, T is temperature in K, and η is the solvent viscosity.

So need to give several parameters to the instrument or software: temperature, index of refraction and viscosity of solvent, these generally stored in operating parameters

Applications:

determine average molecular size (diameter/radius)—0.6 nm -6 microns

12-20 microliter, protein concentrations 0.1 mg/ml

precision relatively good, but resolution poor

if two species should differ by more than factor of 3 in size

signals very non-linear, effectively depend on volume

so large particles dominate scatter

Can detect as low as nm size and up to many microns:

Chromatography mode, separate species and measure radii as come through the column, best since instrument does not resolve well, and if combined get very non-linear weighted average. – figures: red is chromatogram, blue is size
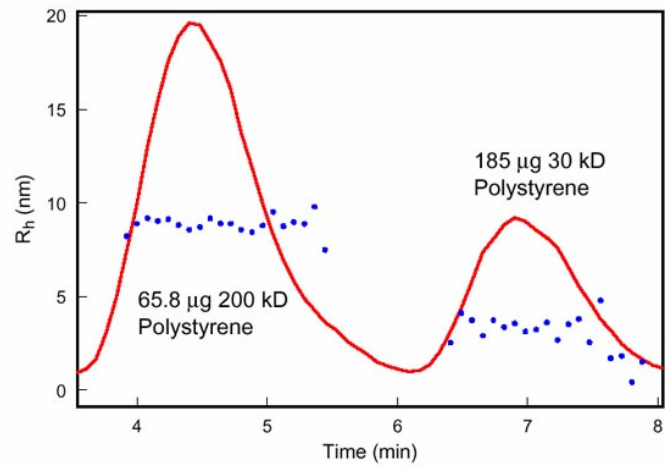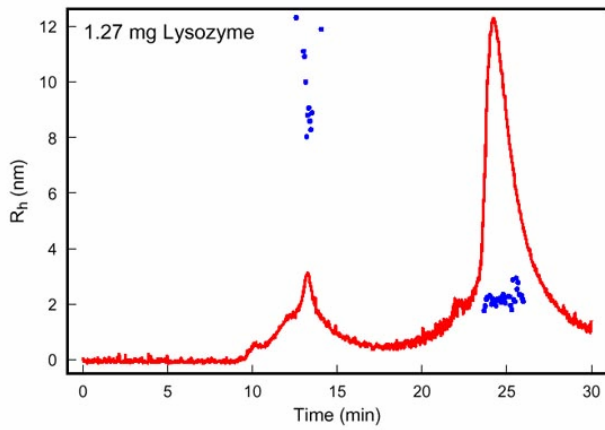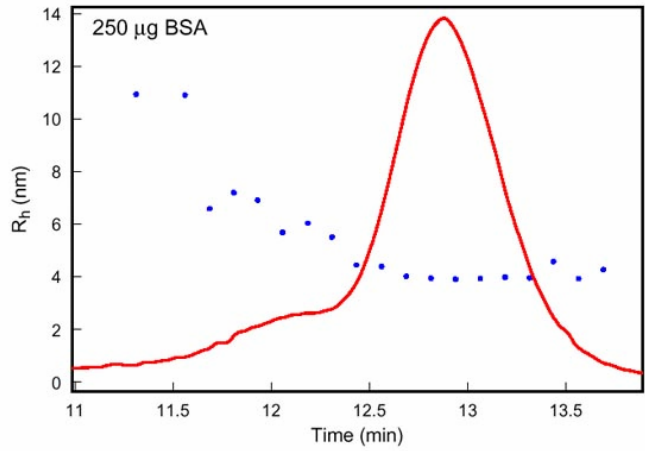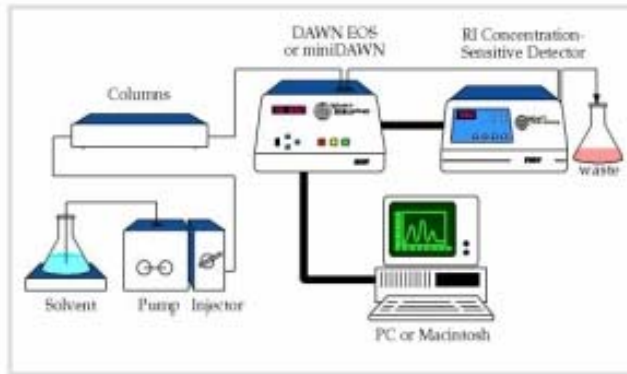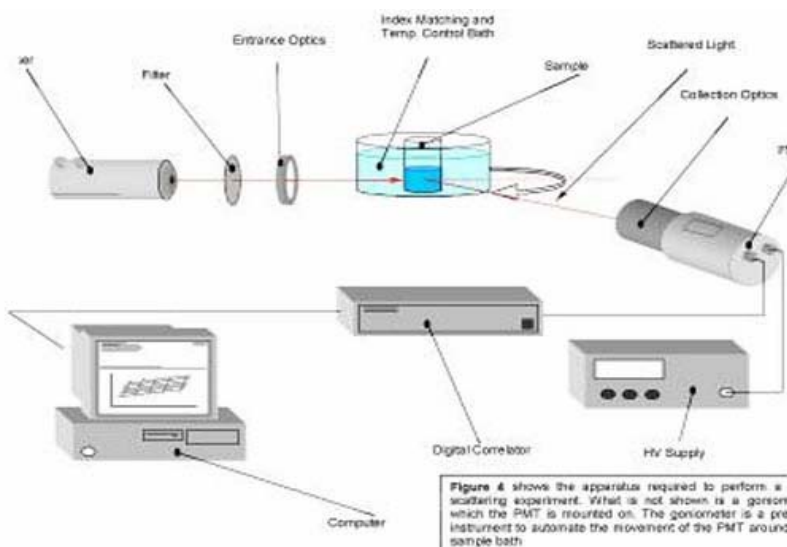

Wyatt, Dynamic light scatter

Static, multiangle light scatter
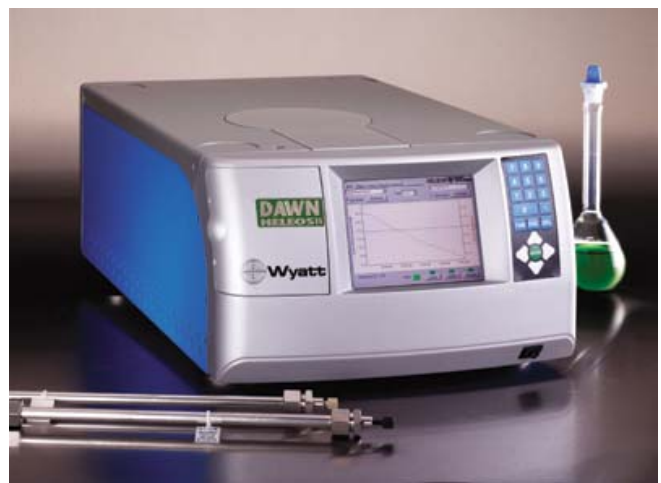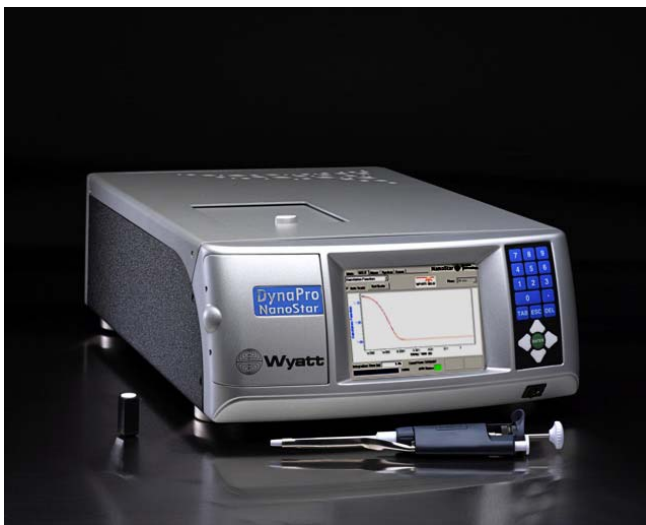
4. **Static/angular light scattering** uses the intensity scattered at a number of angles to derive information about the radius of gyration R_g, molecular mass M_w of the molecule or complex, and the second virial coefficient A_2, for example, following micellar formation [1-5].

Data fit to Zimm plot, which is described by the equation

$$\frac{Hc}{R(\theta,c)} = \frac{1}{M_w}\left[1 + \frac{r_g^2 K^2}{3}\right] + 2A_2C$$

Figure 4 shows the apparatus required to perform a scattering experiment. What is not shown is a goniometer which the PMT is mounted on. The goniometer is a precision instrument to automate the movement of the PMT around sample bath.

Angular Dependence of Scattered Light and Size

For small molecules, the scattered light in the plane perpendicular to the polarization of the incident light is independent of scattering angle.
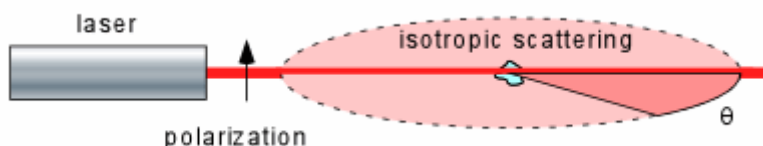
For larger macromolecules, there are variations in the phase of the scattered light from different parts in the macromolecule. This can lead to the destructive or constructive
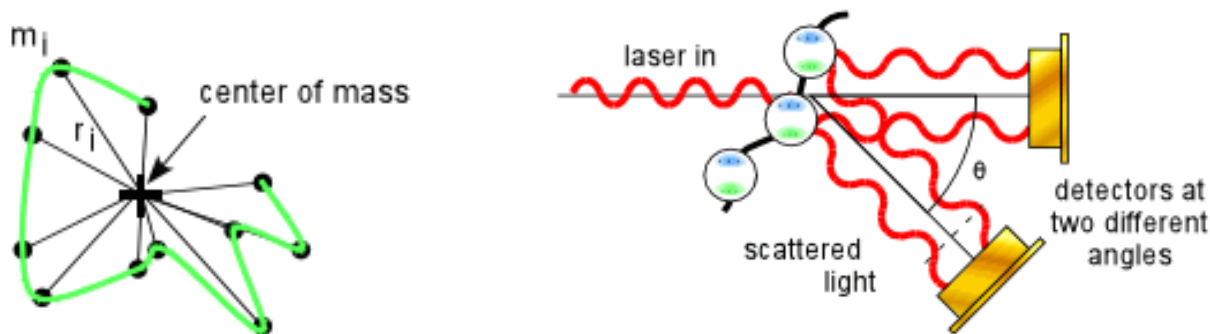
interference of the scattered light. The net result is that the intensity of scattered light away from the direction of the laser beam is changed and, for molecules small compared to the wavelength of the incident light, is reduced. The scattered waves will add destructively or constructively producing

constructive or destructive interference in certain directions. If the angular dependence of the scattered light is measured, it is possible to determine the size of the molecule.

A simple static light scattering experiment entails the average intensity of the sample that is corrected for the scattering of the solvent will yield the [Rayleigh ratio](), R as a function of the angle or the wave vector q as follows:

$$R(\theta_{sample}) = R(\theta_{solvent}) I_{sample} / I_{solvent}$$

yielding the difference in the Rayleigh ratio, $\Delta R(\theta)$ between the sample and solvent:

$$\Delta R(\theta) = R(\theta_{sample}) - R(\theta_{solvent})$$

As described in the review article by ***Philip Wyatt*** [P.J. Wyatt, Anal. Chim. Acta 272, 1-40 (1993)] Zimm's development leads to the expression:

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

where:

- $R(\theta,c)$ is the excess Rayleigh ratio of the solution as a function of scattering angle θ and concentration c. It is directly proportional to the intensity of the scattered light in excess of the light scattered by the pure solvent.
- c is the solute concentration.
- M_w is the weight-averaged solute molar mass.
- A_2 is the second virial coefficient in the virial expansion of the osmotic pressure.
- K^* is the constant $4\pi^2 (dn/dc)^2 n_0^2 / N_a \lambda_0^4$.
- N_a is Avogadro's number. This number always appears when concentration is measured in g/ml and molar mass in g/mol.
- $P(\theta)$ describes the angular dependence of the scattered light, and can be related to the rms radius.

The expansion of $P(\theta)$ to first order gives:

$$P(\theta) \approx 1 - \frac{16\pi^2 n_0^2}{3\lambda_0^2} \langle r_g^2 \rangle \sin^2 \frac{\theta}{2} + O\left(\sin^4 \frac{\theta}{2}\right) - \dots \tag{2}$$

where n_0 is the index of refraction of the solvent, λ_0 is the vacuum wavelength of the laser, and r_g is the rms radius. Here, the relation between the size and angular dependence of the scattered light is clear. For larger sizes (r_g greater than approximately 50 nm) it is necessary to include higher moments in the expansion of $P(\theta)$.

The mean square radius, $\langle r_g^2 \rangle$, may be calculated immediately from the slope at $\theta = 0$ of the measured ratios $1/R(\theta,c)$ with respect to $\sin^2(\theta/2)$.

Brookhaven Autocorrelator:

For photon counting applications including static and dynamic light scattering

Features at a glance

- USB communications
- Compact size, low power requirements, less than 300 mW
- Auto- & cross-correlation modes
- Up to 522 hardware channels
- Sampling time 25 ns to 40 ms
- Delay range 25 ns to 1,310 s
- 100% efficient, real-time operation over the full delay time range

Supplied with control software for use with Windows™

Wiki stuff:

The Siegert equation relates the second order autocorrelation function with the first order autocorrelation function $g^1(q;\tau)$ as follows:

$$g^2(q;\tau) = 1 + \beta \left[g^1(q;\tau)\right]^2$$

where the parameter β is a correction factor that depends on the geometry and alignment of the laser beam in the light scattering setup.

The simplest approach is to treat the first order autocorrelation function as the sum of single exponential decays with fractions $G(\Gamma)$, where Γ is the decay rate.

$$g^1(q;\tau) = \sum_{i=1}^{n} G_i(\Gamma_i) \exp(-\Gamma_i \tau) = \int G_i(\Gamma) \exp(-\Gamma \tau)\, d\Gamma.$$

One of the most common methods is the cumulant method [1] [2], from which in addition to the sum of the exponentials above, more information can be derived about the variance of the system as follows:

$$g^1(q,\tau) = \exp\left(-\bar{\Gamma}\tau\right)\left(1 + \frac{\mu_2}{2!}\tau^2 - \frac{\mu_3}{3!}\tau^3 + \cdots\right)$$

where $\bar{\Gamma}$ is the average decay rate and $\mu_2/\bar{\Gamma}^2$ is the second order polydispersity index (or an indication of the variance). A third order polydispersity index may also be derived but this is only necessary if the particles of the system are highly polydisperse. The z-averaged translational diffusion coefficient D_z may be derived at a single angle or at a range of angles depending on the wave vector q.

$$\bar{\Gamma} = q^2 D_z$$

with

$$q = \frac{4\pi n_0}{\lambda} \sin\left(\frac{\theta}{2}\right)$$

where λ is the incident laser wavelength, n_0 is the refractive index of the sample and θ is angle at which the detector is located with respect to the sample cell.

Static:

Here, the size measurement is known as the root mean square (rms) radius, or sometimes the "radius of gyration". The latter term is a misnomer since it describes a kinematic measure of a molecule rotating about a particular axis in space. The rms radius, on the other hand is a measure of its size weighted by the mass distribution about its center of mass. Once the molecule's conformation is determined, (e.g., random coil, sphere, or rod), the rms radius can be related to its geometrical dimensions. For a sample containing a broad distribution of molecular masses, following separation by ***chromatographic means***, the measured rms radius may be plotted against the correspondingly measured molar mass to determine the sample's conformation.

In addition, the setup of the laser light scattering is corrected with a liquid of a known refractive index and Rayleigh ratio e.g. toluene, benzene or decalin. This is applied at all angles to correct for the distance of the scattering volume to the detector.