REVIEW

# LASER LIGHT SCATTERING APPLICATIONS IN BIOTECHNOLOGY

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## ABSTRACT

*Recent advancement of laser light scattering applications in biotechnology are reviewed with emphasis on their use in the biopharmaceutical industry. Light scattering methods have been used to date to characterize biomolecules in solution. They can provide information about the size and conformation of proteins and their aggregation state as well as their ability to crystallise. In addition, modern light scattering instrumentation is becoming method of choice for studying macromolecular interactions. Interactions between macromolecules such as proteins and nucleic acids mediate fundamental processes and their modulation has led to new strategies for developing therapeutics. Light scattering approaches offer significant advantage to other approaches for studying molecular interactions. Compared with other techniques, light scattering is very quick, uses minimal sample quantities, allows recovery of the sample and does nor require derivatisation.*

## Introduction

Laser light scattering is a widely applied technique for studying biomolecules in solution. There are several types of light scattering studies. Measurements of the angular distribution of time-averaged scattered intensity is known as static light-scattering (SLS) while measurements of the time-dependent scattered intensity due to density or/and concentration fluctuations, is referred to as photon correlation spectroscopy (PCS) or dynamic light-scattering (DLS) (5, 10, 31). In this review, we summarize the principles of laser light-scattering techniques and their application in biotechnology. The most common applications include determination of size, shape, and structure of proteins, their aggregates, and complex formation with other molecules.

The theory of light scattering in conceptually simple and fundamentally the same as x-rays and neutrons scattering, even though light and x-rays are electromagnetic radiation with different wavelength, while neutrons have mass. The differences between the two main light scattering methodologies are as follows: Static light scattering deals with equilibrium measurements of the angular distribution of time-averaged scattered intensity. This technique can detect changes of the size, shape or structure of the analysed molecules or particles. Dynamic light scattering measures fluctuations in the intensity of the scattered light. It requires a coherent light source, such as laser, because the amount of temporal and spatial coherence in a conventional light source is quite small. Modern light scattering instruments are equipped with red-green wavelength diode lasers, which serve as light source that is stable and has low noise and good coherence.

Besides light scattering, many other techniques have also been used in characterizing macromolecules in solution. For

example quantitative or semiquantitative methods currently used for studying molecular interactions include 1. - affinity chromatography, of which the most advanced form is surface plasmon resonance; 2. - analytical ultracentrifugation; 3. - transport methods on columns or in the centrifuge; 4. - spectroscopic methods, including fluorescence energy transfer or depolarization, EPR, where a suitable probe can be introduced into one or both macromolecules under study; 4. - aqueous two-phase partition. All these approaches have their own distinct limitations: some are slow and expensive, other require large amounts (or rarely obtainable concentrations) of materials and many involve chemical derivatisation which are often uncertain in the consequences for activity. Compared with all these techniques, light scattering is quicker uses minimal sample quantities and is not destructive for the sample. In addition light scattering technique does nor require derivatisation of the sample.

Light scattering can be used to measure the second virial coefficient of a macromolecule, which is a measure of macromolecular self association, and is one of the few parameters that can be used to predict the crystallization properties of a sample.

## Basic theoretical background

According to the light scattering theory when light interacts with matter, the electric field of the light induces an oscillating polarization of electrons in the molecules. The molecules then serve as secondary source of light and subsequently scatter light. The frequency shifts, the intensity, and the angular distribution, of the scatter light are determined by the size, shape and molecular interactions in the scattering material.

The intensity of the scattered light is directly proportional to the weight-average molar mass and the concentration the macromolecule. For static light scattering measurements in dilute solution, the process can be described by the Rayleigh-Gans-Debye equation:

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

$R(\Theta)$ - is the excess intensity of scattered light at given angle ($\Theta$)
C - is the sample concentration
$M_w$- is the weight-average molecular weight (molar mass)
$A_2$ - is a second virial coefficient
K* is an optical parameter

The function $P(\Theta)$ describes the angular dependence of scattered light. At low angles the angular dependence of light scattering depends only on the mean square radius $\langle r_g^2 \rangle$ also called radius of gyration.

Two important parameters namely: weight-average molecular weight and radius of gyration can be determined from the Rayleigh-Gans-Debye equation. A typical way of data analysis is the Zimm plot. By extrapolating the total excess scattered intensity to zero scattering angle and zero particle concentration, the Mw can be calculated from the offset on the y-axis. Also, from the slope and the intercept at different concentrations, the value of radius of gyration and second virial coefficient can also be determined.

Dynamic light scattering, which is also known as "photon correlation spectroscopy" or "quasi-elastic light scattering" uses fluctuations in the intensities of the scattered light to measure the rate of diffusion of the protein particles. These fluctuations are inversely related to the size of the molecules or particles in solution since smaller particles move faster. Scientists have developed a method for quantifying how fast the correlation between the starting measurement and one a short time later takes to break down. The function used to calculate this correlation is the autocorrelation function. It describes how a given measurement relates to itself in a time dependent manner: The decay of the autocor-

relation is described by an exponential decay function G(t) which relates the autocorrelation to the diffusion coefficient D and the measurement vector K:

$$G(\tau) \infty e^{-2\,DK^2\tau}$$

$$K = \frac{4\pi\eta}{\lambda} \sin\left(\frac{\Theta}{2}\right)$$

n = refractive index of the solution (1.33 for water)
$\lambda$ = wavelength of the laser
$\theta$ = angle of scattering measurement

By fitting the points of autocorrelation to the function **G(t)**, the diffusion coefficient can be measured and related to the equivalent sphere of diameter **d** using the Stokes - Einstein equation

$$D = \frac{k_B T}{3\pi\eta d}$$

n = diluent viscosity (water = $8.94*10^{-4}$ kg/(ms)
T = temperature (K)
D = diffusion coefficient (in m2/s)
$k_B$ = Boltzmann constant ($1.3807*10^{-23}$ J/K)
d = sphere diameter (m)

For globular proteins the relationship between the diameter **d** and the molecular weight is given in the following equation:

$$M_W = (d*\alpha)^{\beta}$$

d = sphere diameter in nm
$\alpha$ = correction factor 1 = 1.68
$\beta$ = correction factor 2 = 2.3398
$M_W$ = molecular weight in kDa

## Light-scattering instrumentation

Several companies provide modern light-scattering instrumentation commercially. Brookhaven Instruments (Brookhaven, NY) and ALV GmbH (Langen, Germany) manufacture laser light scattering instruments capable of performing static light scattering experiments over a fairly wide range of angles, varying from 5 to $150^0$ as well as dynamic light scattering measurements. Wyatt (Santa Barbara, CA) offers laser light scattering instrument that has the same capabilities and in addition can be combined with HPLC. Protein Solutions (Charlottesville, VA) produces special dynamic light scattering equipment suitable for determining the sizes of biological particles in different size ranges while Pro2kem (Dundee, Scotland) has recently developed miniaturized light scattering instrument which uses only a few microliters of sample volume (**Figure**).

**Figure.** Miniaturized light-scattering instrument developed by Pro2kem (Dundee, Scotland). The instrument uses minimal amount of sample volume (less than 5 μl).

A light scattering instrument consists of several components: light source, optics, cell holder, and detectors. Traditionally Hg lamps were the light sources for the light scattering instruments. The development of powerful, single-wavelength lasers has led to a revolution in the light-scattering technique.

Conventionally, laser light scattering detectors have been equipped with standard photomultiplier tubes (PMT) as light detectors. Nowadays, modern laser light scattering instruments have a digital output (single photon counting) from a fast photomultiplier or photodiode. In order to perform DLS measurements, a digital correlator is fitted in some instruments, which allows calculation of the time autocorrelation function.

Dust is the major problem in conducting classical light-scattering studies. This is be-

cause the scattered intensity is proportional to the sixth power of the particle size and light is scattered much stronger by large dust particles that by the solute molecules. That is why special precautions should be taken to eliminate dust from the samples. This is easily achievable by disposable membrane filters.

## Application of light scattering for characterization of biomolecules in solution

The most common use of light scattering techniques is for the characterization of protein molecules and their aggregates, precrystallization processes and structures of protein-surfactant complexes. There are many examples where Weight-Average Molecular Weight and Radius of Gyration of proteins in solution have been characterized by measuring the angular dependence of total scattered intensity (1, 7, 8, 14, 15, 27, 28, 33, 39). In addition, Dynamic Light Scattering has been used to measure the Hydrodynamic Radius and the size distribution of proteins in solution (2, 3, 9, 11, 13, 16, 17, 20, 22, 34, 38, 40, 43).

As light scattering techniques are extremely useful for detecting changes in the size of molecules, they have been applied for monitoring protein aggregation and dissociation (4, 12, 18, 19, 21, 23, 24, 30, 32, 35). Aggregation behaviour of proteins is important not only because it affects their biological activity, but also it is directly related to the ability of proteins to form crystals (25, 26, 29), witch is of main importance for crystallographers.

The usefulness of light scattering as a screening tool for crystallography is linked to its ability to measure the second virial coefficient of a macromolecule. The second virial coefficient is a measure of macromolecular self-association, and is also a parameter that can be used to predict the crystallization properties of a sample. This was shown for the first time in 1994 by George and Wilson (14) who demonstrated that many proteins crystallize in conditions where the second osmotic virial coefficient becomes slightly negative, indicating net attractive interactions between protein molecules. The second virial coefficient is typically measured using static light scattering techniques. This technique has recently been successfully applied to predict ability of protein samples to crystallise (6, 36, 37).

In addition to protein aggregation light scattering techniques are proving very useful in studying interactions between proteins. We have used light scattering to study both protein-protein and protein-DNA interactions (42). This is of particular importance for the biopharmaceutical industry as developing new strategies for screening for molecules modulating such specific interactions can led to discovery of new therapeutic and research compounds.

## Conclusions and prospective for the future

Laser light scattering has proved as invaluable technique for characterisation of macromolecules in solution. With the development of modern instrumentation, the sensitivity of photon counting is so high that protein solutions in the concentration range 0.01 - 1 mg/ml (depending on molecular weight) suffice for accurate measurements. The sample volume required is no more than 5µl. The potential power and versatility of light scattering for the study of macromolecules is now therefore at last realized. This new developments, have motivated scientists to expand usability of this technology and adapt it for the drug-screening needs of biopharmaceutical companies. With the growing interest in protein-protein interactions (both for understanding living processes and perturbing these for therapeutic purposes) technologies, which offer new insights into these molecular associations, are of particular interest. For example, screening for specific inhibitors of protein association could

be used for testing compounds in the pharmaceutical industry. In the future, light scattering-based assays and instrumentation will offer an extremely rapid and inexpensive alternative to established screening methods.

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