Static Light Scattering (SLS)

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INTRODUCTION

- **Static light scattering (SLS)** is a technique to measure absolute molecular weight using the relationship between the intensity of light scattered by a molecule and size, as described by the **Rayleigh theory**.
- **Rayleigh theory** states that larger molecules scatter more light than smaller molecules from a given light source and that the intensity of the scattered light is proportional to the molecule's molecular weight. There are two ways to measure absolute molecular weight by SLS:

1. Batch measurement using a cuvette
2. In combination with a chromatography instrument.

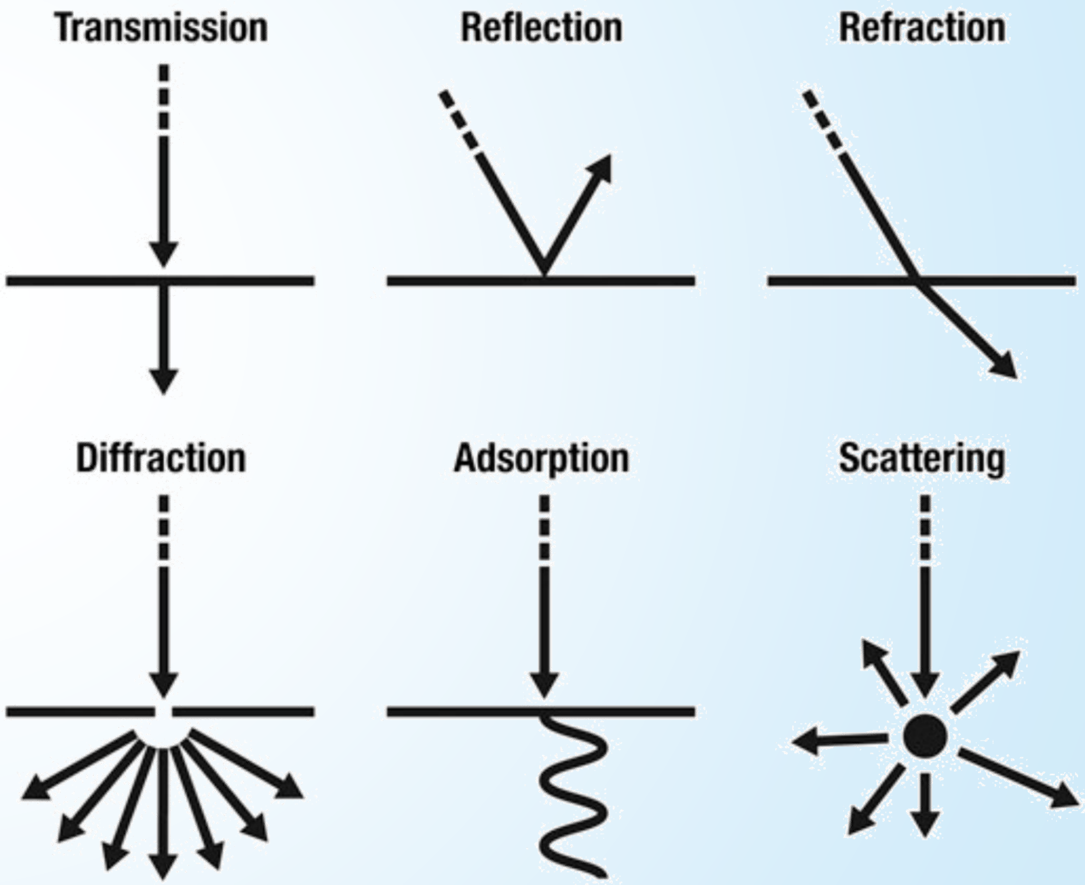
When light interacts with matter, it can....

- Reflection
- Refraction
- Diffraction

Scattering

- Absorption (light absorbed & converted to heat)

- When light is being scattered from ordered particles this can cause such phenomena as reflection, refraction or diffraction.



Brownian Motion

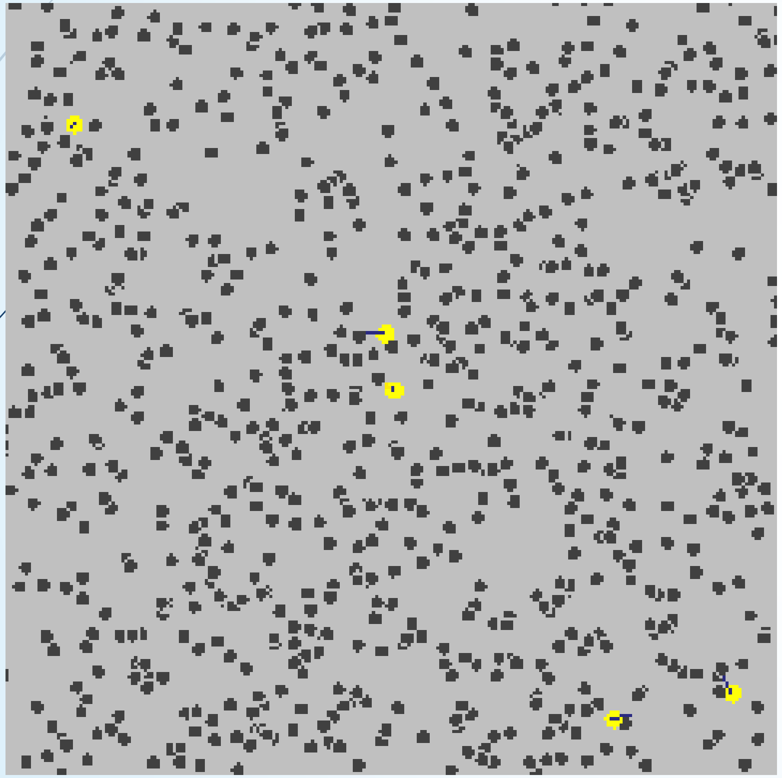
- Brownian motion is the random motion of particles suspended in a fluid (a liquid or a gas) resulting from their collision with the fast-moving molecules in the fluid.
- This motion is named after Robert Brown (botanist, born 1773). In 1827, while looking through a microscope at particles trapped in cavities inside pollen grains in water, he noted that the particles moved through the water; but he was not able to determine the mechanisms that caused this motion.

Brownian Motion and Diffusion

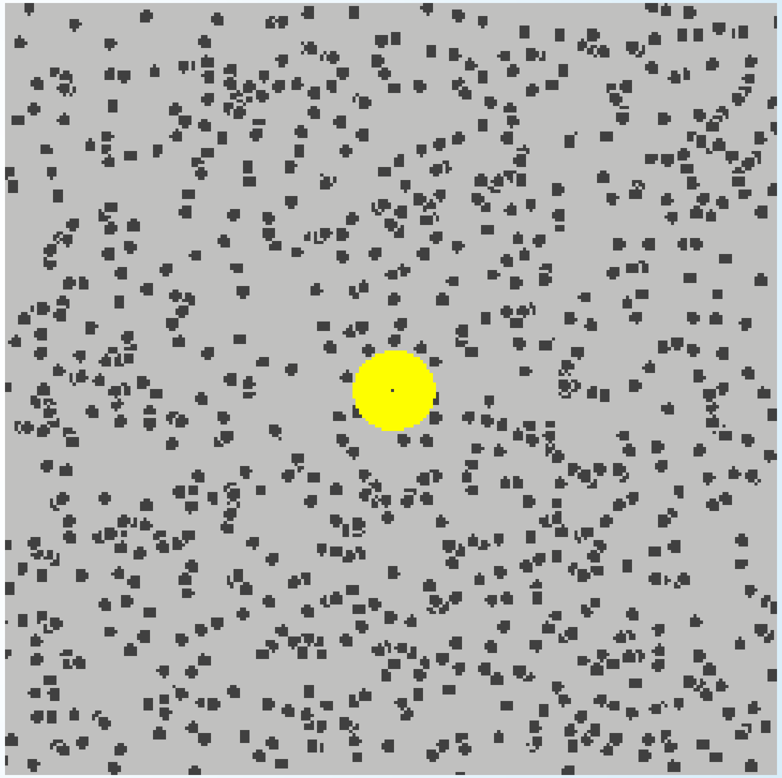
- If a number of particles subject to Brownian motion are present in a given medium and there is no preferred direction for the random oscillations.
- The physical process in which a substance tends to spread steadily from regions of high concentration to regions of lower concentration is called diffusion. Diffusion can therefore be considered a macroscopic manifestation of Brownian motion on the microscopic level.
- Thus, it is possible to study diffusion by simulating the motion of a Brownian particle and computing its average behavior.
- A few examples of the countless diffusion processes that are studied in terms of Brownian motion include the diffusion of pollutants through the atmosphere.
- The diffusion of "holes" (minute regions in which the electrical charge potential is positive) through a semiconductor.
- The diffusion of calcium through bone tissue in living organisms.

Academic Enthusiasm- NAAC-UGC team appreciated Innovative Practices

Brownian Motion



This is a simulation of the Brownian motion of 5 particles (yellow) that collide with a large set of 800 particles.



This is a simulation of the Brownian motion of a big particle (dust particle) that collides with a large set of smaller particles (molecules of a gas

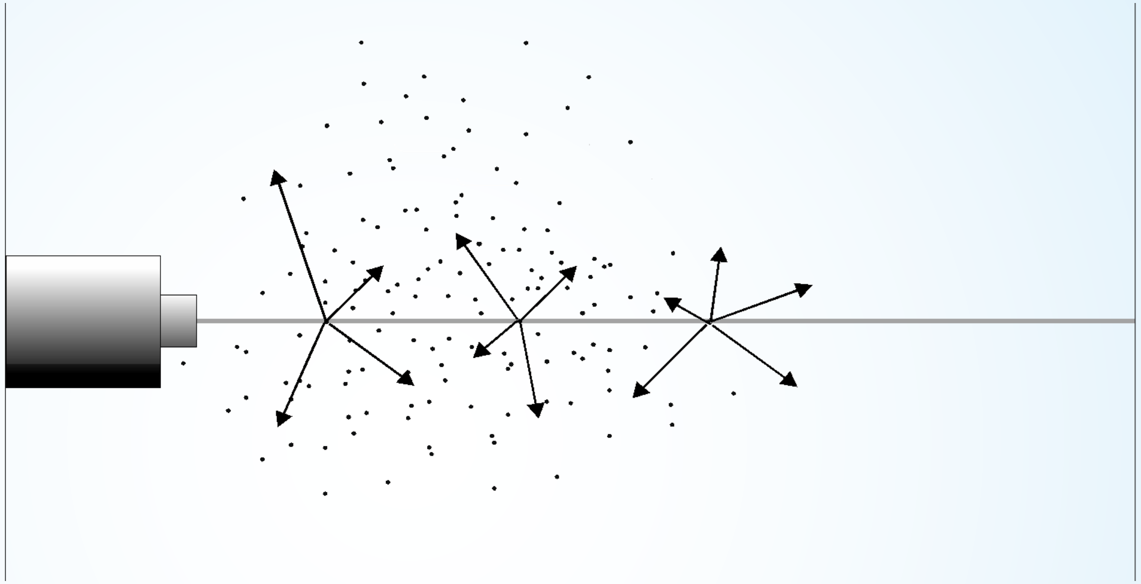
Scattering of Light

- Scattering is a general physical process where some forms of radiation, such as light, sound, or moving particles, are forced to deviate from a straight trajectory by one or more paths due to localized non-uniformities in the medium through which they pass. In conventional use, this also includes deviation of reflected radiation from the angle predicted by the law of reflection.
- *Reflections that undergo scattering are often called diffuse reflections and un-scattered reflections are called specular (mirror-like) reflections.*
- *Scattering may also refer to particle-particle collisions between molecules, atoms, electrons, photons and other particles. Examples include: cosmic ray scattering in the Earth's upper atmosphere; particle collisions inside particle accelerators; electron scattering by gas atoms in fluorescent lamps; and neutron scattering inside nuclear reactors.*

Single & Multiple Scattering

- When radiation is only scattered by one localized scattering center, this is called single scattering.
- It is very common that scattering centers are grouped together; in such cases, radiation may scatter many times, in what is known as multiple scattering.
- The main difference between the effects of single and multiple scattering is that single scattering can usually be treated as a random phenomenon, whereas multiple scattering, somewhat counterintuitively, can be modeled as a more deterministic process because the combined results of a large number of scattering events tend to average out.
- Multiple scattering can thus often be modeled well with diffusion theory.

Why LASER Beam Visible as a Line



- A light beam is not a physical object.
- Objects can be seen when they emit light into our eyes, either because the objects emit light themselves or because they reflect or scatter light that falls upon them.
- The reason why the LASER beam is visible from the side is that some of the photons hit dust particles in the air and are thereby deflected into our eyes.

Basic Principle

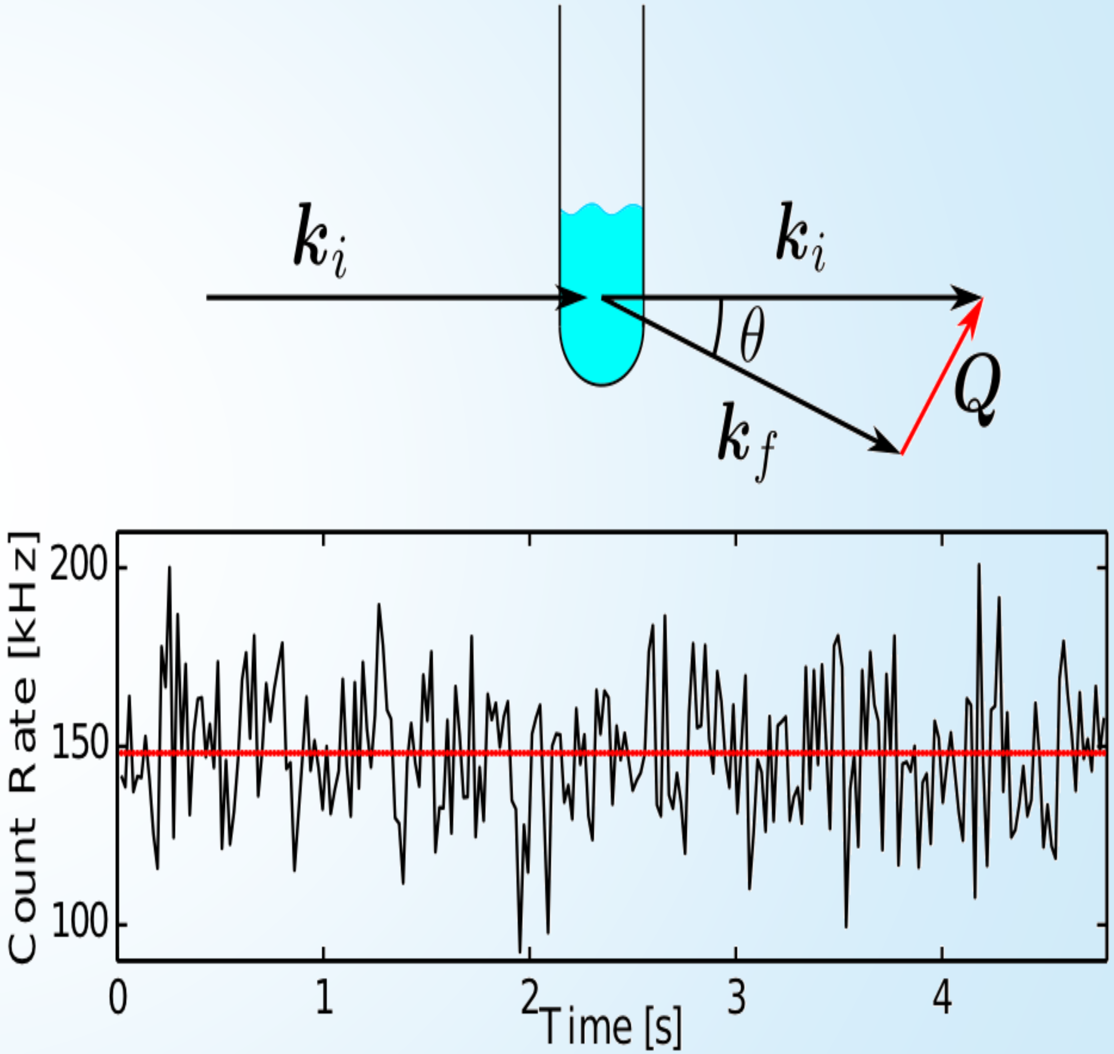
From the intensity fluctuations in time (see Figure 1), information on the dynamics within the solution are obtained.

The evaluation of the fluctuations is commonly named as dynamic light scattering (DLS) while the analysis of the absolute mean intensity is known as static light scattering (SLS).

The intensity is very sensitive to variations in size of the solutes, so that it is advantageous to investigate aggregation in solution.

The scattering vector Q is defined as difference between outgoing and incoming wave vector and its magnitude is

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

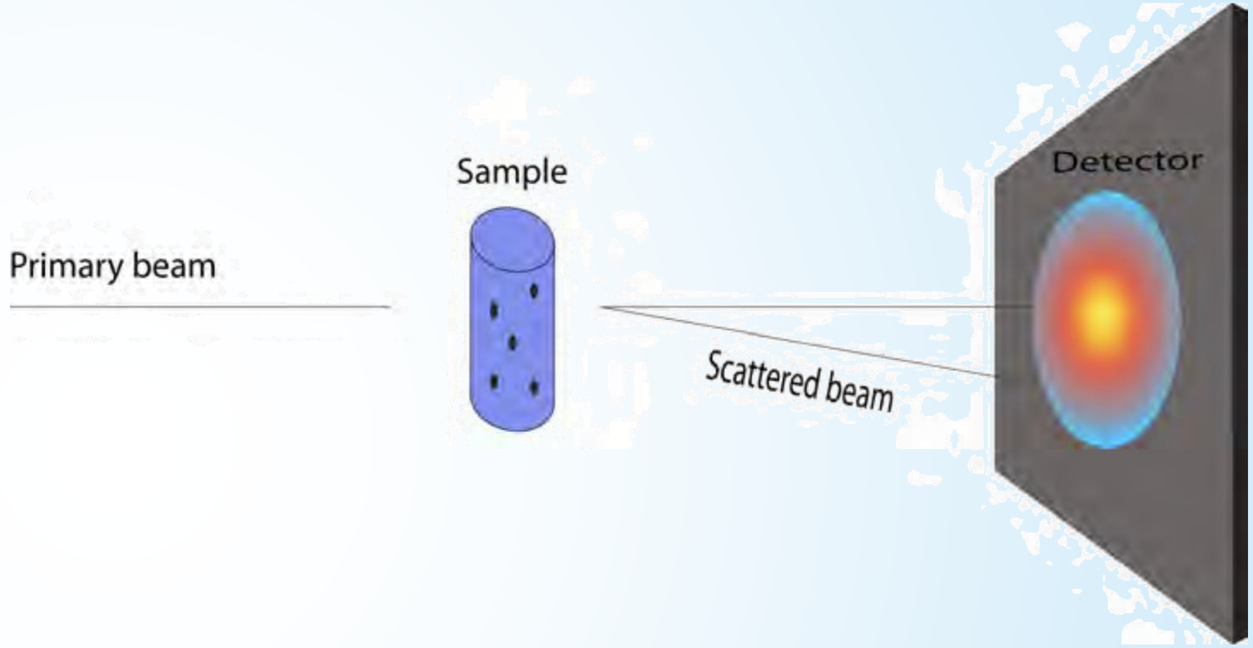


Scattering geometry and intensity fluctuations at detector

Basic Principle

Intensity depends on:

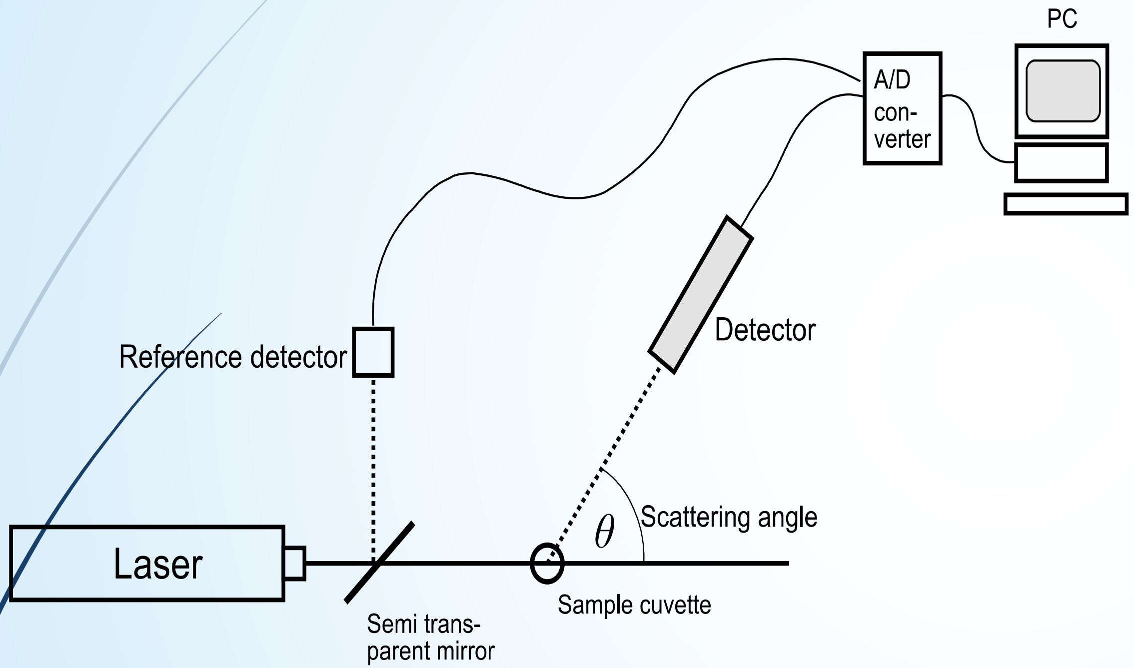
- The molecular weight of the particles
- The concentration of the particles
- The size of the particles
- The refractive index of the pure solvent
- The refractive index of the suspended molecules
- Interaction forces between particles

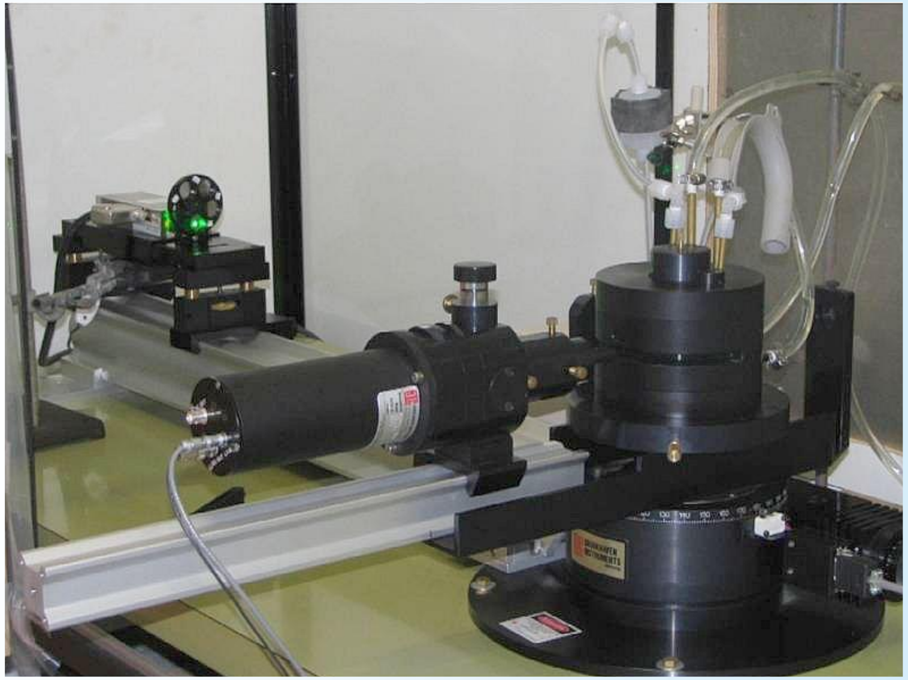


Static Light Scattering

- The main quantities influencing the static light scattering intensity, are the molecular weight M, concentration and size of the particles in solution.
- Due to the long wavelength, particles of size of nanometers (typical for proteins) can be interpreted as (independent) scattering centers whose intensity interferes constructively.
- If the particles are big enough ($>\lambda/20$), an angle dependent change in intensity can be observed.
- The Rayleigh ratio R $\propto$ is introduced as quantity independent of the experimental setup.

Equipment Set-up





The scattered light intensity is measured as a function of the scattering angle, θ. The measured intensity is divided by the incident laser beam intensity, which is measured by a reference detector. This can be much less sensitive than the detector measuring the scattered light.

Working Principle

- The laser beam passes through a beam splitter in order to detect a reference intensity at the monitor diode.
- In transmission it impinges on a glass cuvette immerged in a toluene bath.
- This bath avoids reflexes and its temperature can be controlled accurately.
- The beam scattered by the sample is detected at an angle θ by an avalanche photo diode (APD), or photomultiplier tube, a PMT transferring the signal to a PC.
- The purpose of the attenuator is to protect the APD from a too high photon flux.
- Detector used for measuring the scattered light is mounted on a so-called goniometer which makes it possible to control from what angle (usually θ) the scattered light is recorded.

Precautions Taken

- The sample solution must be completely transparent.
- The sample concentration should be high enough. The lower the molecular weight the higher the necessary concentration.
- Experiment can be performed only if the sample solution should not absorb light of the wavelength used.
- Refractive Index of the solvent should have different from the molecules in solution.
- Before Conduct the experiment must know The weight concentration (i.e. g/L) of the solute molecules.
- Should know the refractive index increment dn/dc of the solute/solvent system.

Similar Technique(s)

- Coupling with Size Exclusion Chromatography.
- Dumas method of molecular weight determination
- Ebullioscopy
- Dumas method of molecular weight
- Mass spectrometry (MS)

Some Examples (1/5)

Trends Trends in Analytical Chemistry, Vol. 30, No. 1, 2011

Measurement of nanoparticles by light-scattering techniques

Satinder K. Brar, M. Verma

Nanoparticles (NPs), due to their unique physical and chemical properties, especially their minute particle size ($\leqslant$100 nm), find applications in numerous industrial, commercial and consumer products. After their end-user applications, these NPs find their way into the environment and food products. The NPs so discharged need to be quantified accurately to determine their toxicity and exposure levels.

At this time, there is a need to develop a unified method for their determination. There are plenty of techniques available in the market that were initially used for colloidal particles (e.g., microscopy, spectroscopy and the recent addition of magnetic resonance), but each of these techniques has a certain degree of uncertainty.

Further, sample homogeneity, sample preparation, instrument-operating procedures, and statistical practices are likely to add to the complexity of the problem. In this context, this review attempts to understand the widely-used light-scattering techniques, including their theory, practice and real-world use in determination of NPs in environmental and food applications.

Some Examples (2/5)

Characteristics	Advantages	Disadvantages	Remarks
Size range (nm)		2–3000	Other methods (e.g., tracking analysis) can have a larger size-range analysis
Size resolution	1:3 in theory, 1:4 in practice*	-	Relatively higher than other known methods
Measurement of NPs in polydispersions		Average particle size, which is intensity biased towards the larger or contaminant particles within a sample	Other approaches can have particle-by-particle approach enhancing resolution
Measurement of NPs in monodispersions	Slightly more reproducible than other methods due to average particle size from many more particles		Other methods look at fewer NPs, hence decreasing reproducibility
Refractive index		Requires solvent refractive index. In samples with NP mixture, analysis is weighted towards the more refractile particles	Other methods may not need this information, causing no interference
Size distribution	Intensity distribution which can be converted into a volume distribution. No accurate information about particle concentration can be calculated.		It is equivalent to other methods where particle distribution is possible and volume distribution is not. Often in liquid environmental and food samples, volume is more important
Dilution	Lower dilution		Less compromise on particle aggregation, a major factor in NP measurement

Some Examples

3048 *Macromolecules* **2006,** *39,* 3048−3055

Temperature-Dependent Properties of Telechelic Hydrophobically Modified Poly(*N*-isopropylacrylamides) in Water: Evidence from Light Scattering and Fluorescence Spectroscopy for the Formation of Stable Mesoglobules at Elevated Temperatures

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ABSTRACT: The self-assembling properties of hydrophobically modified (HM) telechelic poly(*N*-isopropylacrylamides) (PNIPAM) were studied in aqueous solutions of concentration ranging from 0.1 to 11 g L^{-1} by fluorescence spectroscopy, using *N*-phenyl-1-naphthylamine as a probe, and by static (SLS) and dynamic (DLS) light scattering over a temperature domain encompassing their cloud point (T_{cp}) and coil-to-globule transition temperature (T_M). The telechelic HM−PNIPAM samples bear *n*-octadecyl termini, and their molar mass (M_n) ranges from 12 000 to 49 000 g mol^{-1} with a polydispersity index lower than 1.20. In cold aqueous solution, the HM−PNIPAM samples associate in the form of flower micelles ($10.8 < R_H < 17.5$ nm, $R_G/R_H \cong 1.3-1.5$) consisting of ≅16−27 polymer chains, depending on their molecular weight. In solutions heated under equilibrium conditions above T_M, individual flower micelles with collapsed loops associate to form stable mesoglobules ($R_G/R_H \sim 0.80$) comprising a few hundreds chains with a more rigid and more polar interior than the hydrophobic core of hydrated flower micelles. The size of the mesoglobules increases with increasing polymer concentration ($19 < R_H < 115$ nm), but in all cases the mesoglobule size distributions are narrower than those of the corresponding polymer micelles in cold solutions.

Case Study

1478 *Langmuir* **2006,** *22,* 1478−1484

Self-Assembly of *β*-Cyclodextrin in Water. Part 1: Cryo-TEM and Dynamic and Static Light Scattering

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In this article, we report evidence of β-cyclodextrin (β-CD) self-aggregation in water. A critical aggregation concentration (cac) between 2 and 3 mM was determined by using dynamic (DLS) and static (SLS) light scattering to investigate the presence of β-cyclodextrin aggregates. Transmission electron microscopy at cryogenic temperature (Cryo-TEM) was used to detect the structural features of cyclodextrin self-aggregates. The results show the occurrence of polymorphism depending on the β-CD concentration: polydisperse nearly spherical objects with diameters of about 100 nm are present at lower concentrations, whereas micrometer planar aggregates are predominant at higher concentrations.

REFERENCES

- https://en.wikipedia.org/wiki/Brownian_motion (Dt. 17-May-2018; 01:13 IST)
- https://en.wikipedia.org/wiki/Scattering (Dt. 17-May-2018; 01:13 IST)
- Bente Vestergaard - BioSAXS group - University of Copenhagen, 24/06/16, University of Copenhagen
- https://www.britannica.com/science/Brownian-motion (Dt. 17-May-2018; 01:13 IST)
- R. Peters. ALV - Technical Documentation, 2009.
- https://www.lsinstruments.ch/technology/static_light_scattering_sls/ (Dt. 17-May-2018; 01:13 IST)
- https://www.retsch-technology.com/applications/technical-basics/static-laser-light-scattering/ (Dt. 18-May-2018; 23:13 IST)

Academic Enthusiasm- Interaction with Prof. A. K. Chaudhary, IIT Roorkee (Ph.D. examiner) and engaging Teachers workshop as Resource person in a Programme of DST-Govt. of India

THE END