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Mini Review

Dynamic Light Scattering: Advantages and Applications

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Abstract

Dynamic light scattering (DLS), also known as Quasi Elastic Light Scattering (QELS) or Photon Correlation Spectroscopy (PCS) is a non-invasive, fast, precise, reliable, promising technology which is well established for size measurement and study of size distribution of molecules in submicron region. DLS works on the principle of measurement of Brownian motion of particles and correlates motion with the size of the particles. Particle size measurements using DLS can be achieved within one or two minutes and the measurements were found to be accurate, reliable and provided unbiased information on shape (flexible coil, rods, cylinders, spheres) of macromolecules (DNA, RNA, polysaccharides, proteins, and viruses). This technology is also suitable for molecular weight determination and size measurements of molecules in the range of 10μm to less than 1 nm and having molecular weight less than 1000 Da can be determined. DLS has been used as a promising technology for conducting studies on homogeneity of RNA, proteins and their complexes. Major application of this technology is in milk and other oil in water emulsions since milk contains large number of colloidal particles which has the ability to scatter light well and also the structure of milk is least affected due to dilution of milk. Although DLS possess some limitations such as variations in results due to changes in temperature, viscosity and inability to differentiate molecules that are closely related, it is gaining popularity in academic and industrial laboratories as it provides reliable results in a short period of time.

Keywords: DLS; Light Scattering; Brownian Movement

Dynamic light scattering (DLS), sometimes referred to as Quasi Elastic Light Scattering (QELS) or Photon Correlation Spectroscopy (PCS) is a non-invasive, well-established technique for measuring the size and size distribution of molecules and particles typically in the submicron region, and with the latest technology, lower than 1nm.

DLS is a non-invasive method that provides fast, precise, and reproducible quality check to study aggregation in bio molecular preparation requiring very little amounts of sample. DLS works on the principle of measurement of Brownian motion of particles and correlates motion with the size of the particles. Brownian motion is defined as the random movement of particles due to the successive collision by the solvent molecules that surround them. DLS is widely used for measurement of particles which are suspended within liquid.

Brownian motion will be slowed down with increase in size of the particle. Smaller particles move more rapidly as they are thrusted further by the solvent molecules. During DLS measurement, the temperature should be accurately known to determine the viscosity as viscosity has direct relationship with temperature. Stable temperature during measurement is essential as variations in temperature may result in occurrence of convection currents in the sample will induce non-random movements that will alter the accurate interpretation of size. Translational diffusion coefficient (D) is used to express the velocity of the Brownian motion and is dependant not only on the size of the particle, but also on superficial structure, concentration and type of ions in the medium.

Applications of DLS includes categorization of particles,

emulsions or molecules which have been distributed or dispersed in liquid. Laser light is scattered at different intensities due to random movement of particles or molecules in suspension and these intensity fluctuations are analysed to obtain the velocity of the Brownian motion and using Stokes-Einstein relationship, the particle size was determined. Stokes-Einstein relationship is given by

$$d(H) = \frac{kT}{3\pi\eta D}$$

where d(H) = hydrodynamic diameter, K = Boltzmann's constant, D = translational diffusion coefficient, T = absolute temperature and η = viscosity

Most DLS systems uses laser technology and laser of known wavelength is allowed to pass through dilute sample and the intensity of scattered light is collected by detector is measured. The particle size distribution of the sample is obtained by analysis of measured value by use of algorithms. The quantum of light scattered by particle depends upon the shape, size and molecular weight of a particle as well as the refractive indices of both particle and solvent. The scattered light from individual particles is interrupted by the scattered light from other particles before reaching the detector since the molecules are in random movement due to Brownian motion which will result in random fluctuations in time.

Theories based on light scattering

1. Rayleigh Scattering- If the size of particles in comparison to the wavelength of the laser used is small, then the light scattering by the particle which is illuminated by a vertically polarised laser will be equal in all directions, i.e. isotropic. The Rayleigh also stated that I α 1/λ^4 ,where I= intensity of light scattered, d = particle diameter and λ= laser wavelength.
2. Mie Theory- Mie stated that if the particle size approximates to that of the wave length of illuminating light, then a complex function of maxima and minima in terms of angle is observed.

Advantages

Particle size measurements using DLS can be achieved within one or two minutes and the measurements were found to be accurate, reliable and provided unbiased information on shape (flexible coil, rods, cylinders, spheres) of macromolecules (DNA, RNA, polysaccharides, proteins, and viruses). Measurement of particle concentration can be achieved calibration free and the mean size measurement only requires the knowledge of viscosity of the liquid. Samples having different concentrations such as simple or no sample preparation, high concentration and turbid samples can be analysed directly using DLS. This technology is also suitable for molecular weight determination and size measurements of molecules in the range of 10μm to less than 1 nm and having molecular weight less than 1000 Da can be determined. DLS' s major advantage is that very less amount of sample (< 3μL) is essential for analysis. DLS is found to be suitable for protein- RNA interaction studies and modified DLS in-line process systems are found to be suitable. It is possible to conduct experiments on wide range of sample buffer and wide temperature range using DLS technique.

Limitations

Measurements obtained by DLS are altered by variations in temperature and solvent viscosity as it is highly sensitive to it. Therefore, both temperature and solvent viscosity must be kept constant for reliable results. DLS cannot differentiate molecules that are closely related (e.g., monomer and dimer) since it is a low-resolution method. DLS must be used on highly dilute solutions of food particles. It is difficult to dilute many food particles as it may result in variation of structural properties, for instance complex particles may dissociate. So, it is essential to ensure that the particles under study in a DLS experiment must retain their structures which are native to the food. Presence of large aggregates even in a small amount will adversely affect the measurements since scattering intensity is proportional to 6^{th} power of the size of macromolecules (I α d^6, where d is a diameter of macromolecule). Therefore, proper cleaning of the sample-holding cuvette prior to measurement is essential. The signals from DLS vary with changes in size and concentration of macromolecules and so the concentration range is to be optimized prior to measurement for attaining reliable results.

Applications

Major application of this technology is in milk and other oil in water emulsions since milk contains large number of colloidal particles which has the ability to scatter light well and also the structure of milk is least affected due to dilution of milk. A modified version of DLS, DWS (Diffusion Wave Spectroscopy) can be used for concentrated suspensions which is having similar apparatus as that of DLS except that back scattered light and incident light are conducted through fibre optics. DLS has been used as a promising technology for conducting studies on homogeneity of RNA, proteins and their complexes. Multi-Angle Dynamic Light Scattering (MADLS®), a modified DLS technique, has the ability to improve

the resolution of DLS and the size results obtained were found to be angular independent so that accuracy of DLS technique is improved. DLS has been widely used to determine the size distribution of casein micelles in bovine milk and also for molecular weight determination of molecules [1-4].

Conclusion

DLS is a non-destructive technique that provides quick, precise, accurate and reproducible quality check for interaction studies during bio molecular preparation and the analysis requires very less amount of sample. Although DLS possess some minor limitations, it is gaining popularity in academic and industrial laboratories as it provides reliable results in a short period of time.

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