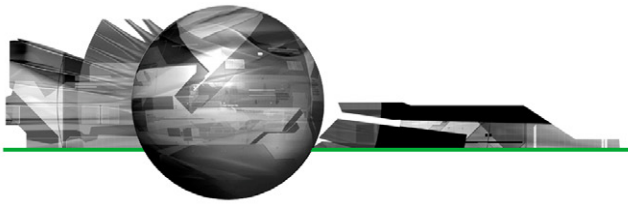


application note

Measuring Latex Standards by Dynamic Light Scattering

Introduction

Dynamic light scattering (DLS) is a non-invasive technique suitable for the size characterization of nanoparticles and low-molecular weight molecules such as proteins and polymers [1-3]. The technique measures the time-dependent fluctuations in the intensity of scattered light that occur due to the random movement of the particles or molecules undergoing Brownian motion. The velocity of this Brownian motion is measured and is called the translational diffusion coefficient (D) which can be converted into a hydrodynamic diameter (D_H) using the Stokes-Einstein equation [1-3].

DLS is an absolute technique using first principles and therefore calibration is not required. However, verification of the instrument should be regularly performed to check correct operation.

Polymer latex spheres are very commonly used to verify correct instrument performance. This is because they are available as monosize dispersions of near perfect spheres. The sphere is the only three-dimensional shape whose size can be unambiguously described by a single figure and being monodisperse removes any uncertainty regarding the calculation of a mean size.

Polymer latex samples have other benefits. They have a similar density to water, so particles less than 1micron will remain in suspension during measurement. Dispersions can be stored at room temperature and have storage lifetimes of months or years.

A wide range of monodisperse polystyrene lattices are available from a variety of manufacturers. However, not all are supplied with an individual calibration certificate. Thermo Scientific Nanosphere 3000 series size standards [3] are each supplied with a calibration certificate, measured by transmission electron microscope, (TEM) traceable to NIST [4]. The specification for the standards also includes a hydrodynamic diameter measured by dynamic light scattering (DLS).

Thermo Scientific Nanosphere 3000 series size standards are available from 20nm to 900 nm. The easiest sizes to measure are in the range 20nm to 300nm. Particles larger than 60nm are large enough to give very reproducible results at suitable dilutions for DLS. Particles larger than 300nm start to show a marked variation in scattering intensity with angle and measuring standards smaller than this removes the requirement to consider the angle.

The Thermo Scientific Duke Standards 2000 and 4000 Series are NIST traceable standards of sizes which can also be used for DLS verification applications and contain sizes greater than 1 micron.

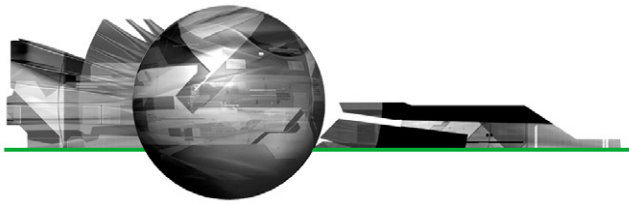
Certified and Hydrodynamic Sizes

The result quoted on the Thermo Scientific Nanosphere bottle is the certified TEM result. The DLS result (i.e. the hydrodynamic size) is quoted in the specification sheet provided and is not a certified value. For all Thermo Scientific Nanosphere 3000 standards, the size accuracy by DLS should be within the specified hydrodynamic size range ±2% for samples prepared in a 10mM NaCl [2,3]. Sodium chloride is used to suppress the electrical double layer. Dilution of the standard in deionised water will give an extended double layer and result in an artificially increased size which may be out of specification.

Comparison of sizes measured by TEM and DLS

Different measurement techniques will measure different properties of a particle and so can give different results in terms of the size interpreted from the measured property. Therefore, the question which often arises, is which one is the correct result? For many people ‘seeing is believing’ so the electron microscope result is ‘correct’. In fact, samples prepared for electron microscope examination are often harshly treated and this treatment can distort soft materials such as polymer lattices and change, or mask, surface structures. It can make the size measurement of some types of materials like surfactant micelles impossible. In contrast, DLS measures the hydrodynamic diameter of dispersed particles in their native environment.

Any surface structure such as an adsorbed polymer layer, or a change in the electrical double layer that affects the Brownian motion of the particle, will change the effective particle size [5]. Increasing the surface structure or extending the electrical double layer by using a very low salt dispersant, will reduce Brownian motion and increase the measured size. For these reasons, the hydrodynamic size or DLS size of particles that are not smooth, hard



spheres is usually larger than the TEM size.

Sample Preparation of Latex Standards for DLS Measurement

For instruments with a 90° detection angle (for example, Zetasizer Nano S90, μV or APS), all latex standards are supplied at a concentration that is too high for DLS measurement, typically 1%w/v. Too concentrated a sample will produce multiple scattering resulting in the apparent size being too small. Therefore, the latex dispersion should be diluted with 10mM NaCl as previously discussed which has been filtered at 0.2 microns.

For backscatter detection instruments which have variable measurement positions (for example, Zetasizer Nano S or ZS), measurements are possible on neat concentrations of latex. The reduction in path length arising from the variable measurement position results in a reduction in multiple scattering.

However, the final concentration of any latex used should be such that the result is independent of the actual concentration [2,3] with the optimum concentration being dependent upon size of the latex.

Various Thermo Scientific Nanosphere 3000, 2000 and 4000 series size standards were prepared accordingly and measured on a range of Zetasizer instruments for this application note. Some of these standards were measured at neat concentration, others were diluted in 10mM NaCl and others were prepared in 13% w/v sucrose solution. The density of polystyrene latex spheres is close to that of a 13% w/v sucrose solution and reduces problems of sedimentation of the latex. The concentration and diluent used for the various latex standards used in this application note are shown in Table 1.

Results and Discussion

Table 1 summarises the results obtained for various latex standards measured by DLS. The table includes details of the latex used (with the Thermo part number displayed in brackets), the certified (#) or hydrodynamic (*) size range, the concentration at which the latex was measured, the diluent used for preparation, the instrument on which the measurements were taken and the z-average diameters obtained for each latex. All of the standards available from Thermo have a certified size range but some also have hydrodynamic size ranges quoted. The certified size range (#) is obtained using transmission electron microscopy and is traceable to NIST. The hydrodynamic size range (*) is determined by DLS.

The results show that there is a wide concentration range over which latex standards can be measured. The backscatter detection used in the Zetasizer Nano allows some latex samples to be measured at neat concentration i.e. 1% w/v.

As the size of the latex particles increase, the issue of number fluctuations and sedimentation become important. During a DLS measurement, the intensity of scattered light fluctuates due to the Brownian motion of the particles. The scattering intensity is proportional to the sample concentration and so the number of particles within the scattering volume should remain constant during the course of the measurement. However, as the particle size increases, the number of particles in the scattering volume decreases until severe fluctuations of the momentary number of particles in the scattering volume will occur. Number fluctuations are defined as variations in the number of particles within the scattering volume during the course of a DLS measurement.

In order to avoid number fluctuations, the concentration of the sample must be increased. However, this will increase multiple scattering effects which in turn will influence the result obtained. Back scatter detection, in combination with a variable measurement position, allows for higher sample concentrations to be measured thereby avoiding number fluctuation problems. Number fluctuations normally manifest themselves by elevated and fluctuating baselines in the correlation functions.

A second problem when measuring large sized particles by DLS is that of sedimentation. All particles will sediment and the rate will depend upon the particle size and relative densities of the particles and suspending medium. For DLS, the rate of sedimentation should be much slower than the rate of diffusion. Large particles diffuse slowly so sedimentation is a more important issue.

The presence of sedimentation can be determined by checking the stability of the count rate from repeat measurements of the same sample. Count rates which are decreasing with successive measurements indicate that sedimentation is present and the Expert Advice system in the Zetasizer software will highlight this to the user.

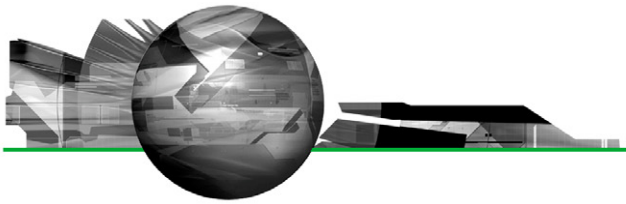
It may be advantageous to suspend the particles in a medium of similar density if the viscosity is not significantly increased. For the measurements of 3, 6 and 8.9μm latex in this application note, the samples were prepared in 13% w/v sucrose which has the same density as the latex.

The results obtained for the 3, 6 and 8.9μm latex samples are in the expected size range using the DLS technique. They were measured at concentrations between 0.15 and 0.24% w/v. These results confirm that multiple scattering effects were

minimised using back scatter detection and that number fluctuations and sedimentation did not influence the results obtained.

Table 1: Summary of DLS measurements performed on a series of Thermo latex standards. The table includes details of the latex used (with the Thermo part number displayed in brackets), the certified (#) or hydrodynamic (*) size range, the concentration at which the latex was measured, the diluent used for preparation, the instrument on which the measurements were taken and the z-average diameters obtained for each latex.

Thermo Scientific Nanosphere 3000 Series Latex Nominal Size (d.nm)	Certified Size Range#/ Hydrodynamic Size Range* (d.nm)	Concentration (%w/v)	Diluent	Instrument	z-Average Diameter (nm)
20 (3020A)	19.5 to 22.5nm#	1% Neat	-	Zetasizer Nano S	21.5nm (± 0.09)
30 (3030A)	31.6 to 34.4#	0.1%	10mM NaCl	Zetasizer Nano S	32.7nm (± 0.11)
60 (3060A)	59 to 65*	1% Neat	-	Zetasizer Nano S	62.8nm (± 0.16)
150 (3150A)	147 to 157*	1% Neat	-	Zetasizer Nano S	152.9nm (± 0.72)
200 (3200A)	195 to 205*	0.001%	10mM NaCl	Zetasizer Nano S	201.7nm (± 0.76)
220 (3220A)	217 to 227*	0.001%	10mM NaCl	Zetasizer Nano S	223.0nm (± 0.93)
300 (3300A)	298 to 310*	0.001%	10mM NaCl	Zetasizer Nano S	301.1nm (± 0.48)
500 (3500A)	495 to 530*	0.05%	10mM NaCl	Zetasizer Nano S	507.9nm (± 1.95)
700 (3700A)	697 to 740*	0.1%	10mM NaCl	Zetasizer Nano S	711.4nm (± 6.46)
1000 (4010A)	1000 to 1042#	0.02%	10mM NaCl	Zetasizer Nano S	1057nm (± 26.2)
3000 (4203A)	3036 to 3090#	0.24%	10mM NaCl	Zetasizer Nano S90	2902nm (± 95.4)
6000 (4206A)	5844 to 5945#	0.15%	13% w/v sucrose	Zetasizer Nano S	6254nm (± 40.9)
8900 (2009A)	8500 to 9200#	0.165%	13% w/v sucrose	Zetasizer Nano S	9108nm (± 598)



application note

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