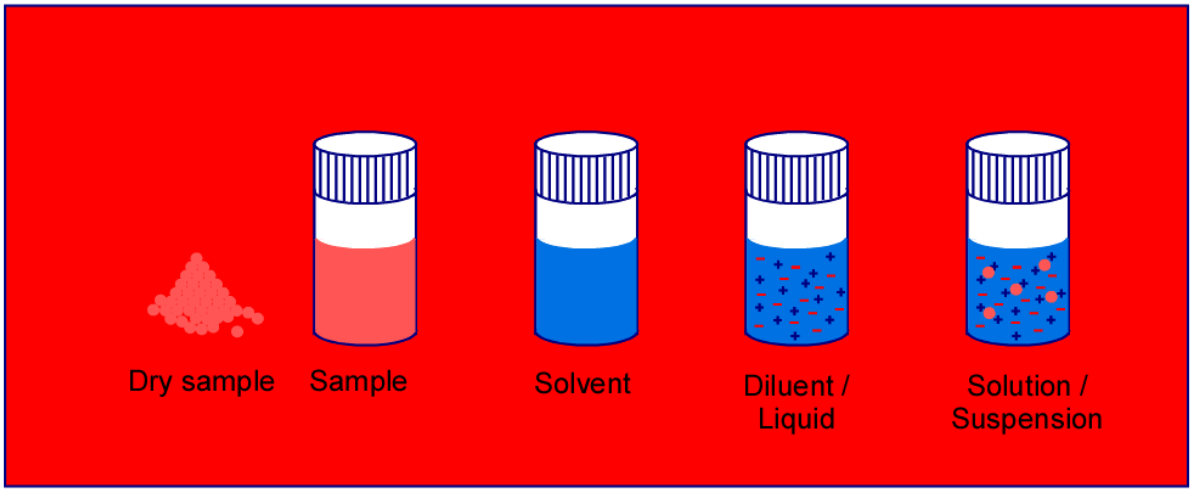
Guide for DLS sample preparation

Eric Farrell & Jean-Luc Brousseau Ph.D.

This document is intended to help the user determine the best way to prepare samples for dynamic light scattering (DLS) measurements. If the user wants to know how to prepare the 92nm latex standard for best measurements, please refer to the document entitled "A Guide to Proper Sample Preparation: Electrostatically-Stabilized Nanoparticles in Water."

Nomenclature

The term solvent refers the pure solvent used to prepare the diluent. Examples of solvents are toluene or water. The diluent may also be referred to as the liquid in DLS textbooks. Diluents are solvent with additives, for example a 10% by weight methanol in water or a 10 mM KNO_3 salt in DI water solution. The samples to be analyzed by DLS will be prepared in the liquid. The solution or suspension to be measured is the sample in the liquid. Although there are differences, the terms suspended and dissolved will be used interchangeably in this document.



Aqueous measurements

Particle size measurements by DLS should not be conducted in pure de-ionized (DI) water, as the electrical double layer surrounding the particles will have long distance interaction. The size measured in DI water will usually be too big by 2 to 10 nm due to the electrostatic interaction between the particles. To screen any charge on the particles, it is a good idea to measure in water with a trace amount of salt. The ions with opposite charge will condense around the particle, screening long distance electrostatic interactions. A general salt like NaCl can be used but often the chloride ions are too aggressive and may react with the particles or adsorb to their surface. We recommend the use of KNO_3 for aqueous diluents. A concentration of 10 mM KNO_3 is ideal for all concentrations of particles.

Diluent / Liquid

If the liquid is a pure solvent like toluene, the purest solvent possible should be sourced. Non-polar solvents do not usually dissolve or carry dust. These can

Brookhaven Instruments
750 Blue Point Road
Holtsville, NY 11742
Phone: +1 (631) 758-3200
Fax: +1 (631) 758-3255
E-mail: info@bic.com
Web: bic.com

be used as is, as any filtering and manipulation will only risk adding dust. If the solvent is polar, then chances are that filtering will help remove dust in the solvent. In the case of aqueous diluents like KNO_3 in water, filtering is needed as salts are notoriously full of dust. It is always a good practice to filter the aqueous diluent using a 0.1 or 0.2 micrometer filter that has been previously rinsed according to the manufacturer's practice. Rinsing the filter is an important step that should not be omitted.

Dry sample dissolution

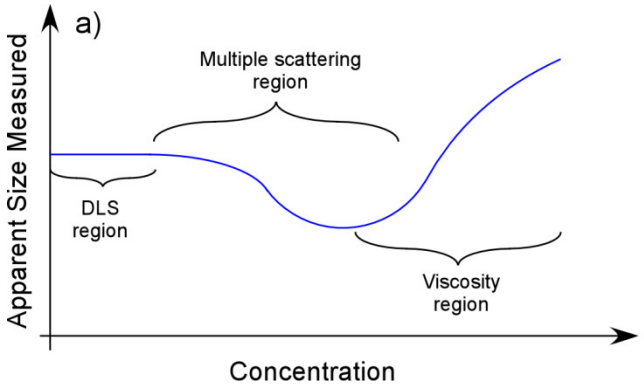
If your sample is a dry powder, it will need to be dissolved or suspended before it can be measured. If the sample is a protein, the solution should not be stirred too aggressively and should never be sonicated. If the sample is sturdy, then sonication, vortex, spinning and other methods can be used to dissolve/suspend the sample. It is for the user to figure out the dissolution time and method. Experiment with different dissolution methods and time. In the case of a sample that disperses quickly, the time to disperse with agitation may be only a few minutes. Large polymers may require more than 24 hours to completely dissolve into solution. When preparing your sample, take a good look at how the sample is dispersing. For further information on preparing stable suspensions from dry powders, refer to ISO standard 14887 entitled "Sample preparation – Dispersing procedures for powders in liquids."

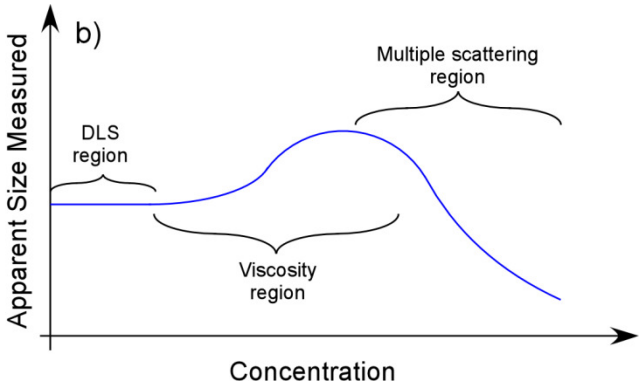
Liquid sample preparation

For liquid samples, the sample may need to be diluted. It is ideal to dilute the sample in the same exact liquid it was originally prepared in, using the same concentrations of additives (i.e. salts, surfactants, dispersing agents) if any were present. In highly concentrated liquid samples, the samples may appear opaque or milky-white. If the sample is highly concentrated, the sample should be diluted in the liquid of choice. Usually putting a drop of the neat sample in 20 mL of liquid or doing a 1:1000 dilution should be sufficient.

Concentrations

Solutions prepared for DLS will need to be clear to very slightly hazy. Although the instrument can measure solutions at concentration up to 40% and possibly more, the size measured in these cases will be wrong. It is not a DLS measurement if the sample is not clear. The Stokes-Einstein equation applies to infinitely dilute solutions. Highly concentrated measurements can be made for diffusing wave spectroscopy (DWS) and other applications. For DLS particle sizing, the sample needs to be water clear to very slightly hazy. If the solution is white or too hazy, it should be diluted further before attempting a DLS size measurement. If the sample is too concentrated, the measured size of your particles will be inaccurate due to multiple scattering or viscosity effects. Multiple scattering occurs when the particle concentration is high enough where the light scattered from a single particle is re-scattered by others in the suspension. This will cause your measured particle size to be artificially low. Viscosity effects occur when the volume of sample added to the liquid is enough such that it will alter the viscosity of the liquid. If the liquid viscosity is wrong due to viscosity effects, your measured particle size will also be wrong. A simple schematic is shown to describe the effects of measuring at high concentration. Depending on the sample and the liquid, a size reduction or a size increase can be seen when the





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concentration is too high for DLS. At extremely high concentrations, this phenomenom usually reverses.

When the solution is ready for analysis, it should be inspected for particles at the bottom of the cuvette. If there are particles at the bottom of the cuvette, the sample distribution will not be accurate, as the large settled particles will not be measured, resulting in an inaccurate size distribution. Samples with large particles settling are either not dispersed correctly (wrong pH, not enough sonication, not enough dilution time, etc.) or are not suitable for DLS. For low density particles, samples may exhibit the opposite behavior. Creaming is the term used for large particles of lower density than the solvent reaching the top of the sample. If the sample is creaming, there is either a dispersion problem or the particles are too big to be measured by DLS.

When the solution is ready for analysis and placed in the cuvette, care should be taken to avoid bubbles that may form on the walls of the cuvette. Slowly tilting or tapping the cuvette on a hard surface may help also.

Colored solutions

In the case of colored solutions, as long as the laser light is not absorbed completely by the solution, the sample can be measured. Colored samples and fluorescing samples may be harder to measure. Often the sample is available without the fluorescent dye or the absorbing chromophore. In this case it will be easier to measure the sample without the dye/chromophore present. To distinguish the scattering from the inherent color of a sample, try reading text through the sample. If the text can be read, then the scattering is not creating most of the opacity.

Filtering the solution

If the solution is to be filtered, keep in mind that the size distribution may be changed if the particles are removed by the filter. A good rule of thumb is to use a pore size (filter size) 3 times larger than the largest size to be measured. For DLS a 5 micrometer filter can be used in most cases. Always verify that the largest size measured is smaller than the filter pore size by a factor of 3. Always rinse the filter prior to use. These recommendations are valid for all filters except the Whatman Anotop series filters. For Whatman Anotop series filters, pass the first drop of sample to waste.

Measurements

Once the solution is homogenous and ready for DLS measurement, the solution can be placed in the instrument. For the novice, there are two ways of checking that the concentration is not too high and that the DLS measurement will be valid.

1) Count rate check: For an instrument equipped with an APD, the count rate for the scattering intensity of the dilute solution should be less than 2 Mcps (2,000,000 count per seconds) with the intensity maximized. Note that measurements SHOULD NOT be made at this high count rate. The maximum count rate for the measurement should be 500-600 kcps. The attenuator will need to be adjusted after measuring the scattering intensity on maximum intensity in most cases, especially if the count rate is greater than 600 kcps.

2) Dilution check: The second way to verify that the concentration is suitable for DLS measurements is to dilute your sample by 50% after the first measurement. If the size of the dilution is the same as the size of the more concentrated measurement and the count rate is reduced by a factor of 2, then the first measurement concentration was low enough.

After many measurements it will be easy to assess the concentration of the solution to be measured.

For best practices for measuring samples size, please refer to the document "Guide for DLS measurements".

Brookhaven Instruments
Nanoparticle, Protein, & Polymer Characterization

Brookhaven Instruments
750 Blue Point Road
Holtsville, NY 11742
Phone: +1 (631) 758-3200
Fax: +1 (631) 758-3255
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