DLS Sample Preparation

Nicomp® DLS System

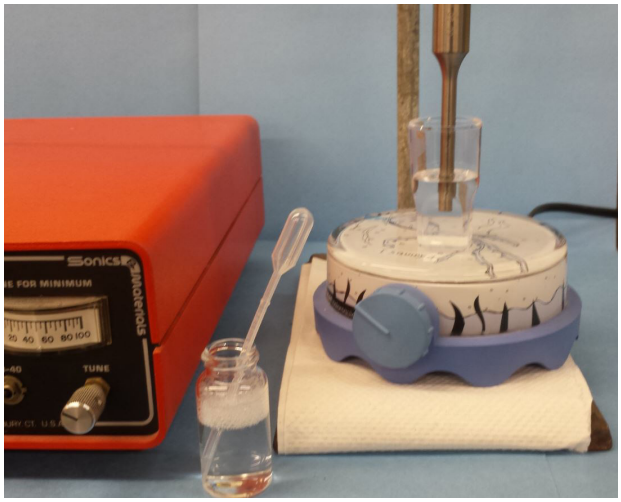
With many samples to be analyzed using dynamic light scattering (DLS), the chemist simply pours/pipettes the suspension into a cell, hits go, and a few minutes later has a perfect answer. This may be true for fairly dilute, monodisperse, and easy samples but certainly not for all samples. This technical note guides a Nicomp® DLS user through the various steps that may be required to generate accurate and repeatable results.

INTRODUCTION

A systematic approach to making any measurement is always a good idea. To begin with, every result must be repeatable in order to be believable. For a 90 nm PSL standard, the repeatability of the mean value should typically be within 5%. For a broad distribution, or bimodal sample ± 15% mean value may be acceptable. The absolute particle size matters as well. When measuring lysozyme you have an expected measurement size of 3.5 nm ± 5% (or 3.325 – 3.675 nm); the actual result is a measurement of 3.8 nm and should be considered acceptable. When results are not within an acceptable range of repeatability, or lie far outside the expected value parameters, to investigate include:

- The sample itself - Has it been contaminated? was it stored properly, etc.?
- Instrument performance - When was a standard last analyzed to verify system accuracy?
- Measurement parameters - How long was the measurement time (should be longer for wider distributions)? Was the count rate acceptable (ideally ~300 kcounts/sec)? Was the temperature and viscosity entered properly?
- Sample preparation - How was the sample dispersed? Is the result dependent on concentration?

All of these parameters can affect the result, and this document will focus on the sample preparation activities that can be important in generating good results.



A well organized particle size laboratory should include some of the basic supplies mentioned in this document, such as a range of chemicals, solvents, surfactants, and an ultrasonic probe when dispersing powder.

POWDER DISPERSION

Since DLS can only measure liquid suspensions; powder samples require dispersion into a suspension for measurement. This step may involve the following choices:

- Choice of diluent (water or solvent)
- How to wet the powder (choice of surfactant)
- Mixing and/or ultrasound to aid dispersion

Water is the easiest diluent choice, and is preferred whenever possible. If the sample dissolves in water, then an organic solvent may be required (note: make sure the proper viscosity is entered). Additional guidance for preparing the diluent is given in the next section of this document.

If the powder can be seen floating on top of the liquid as seen in Figure 1, then a surfactant is absolutely necessary to wet the sample for proper dispersion. Figure 2 shows the powder sample dispersing into the diluent after adding a drop of diluted surfactant to the sample.

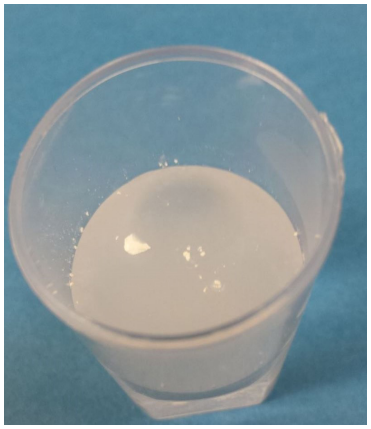


Figure 1. Poor sample powder wetting

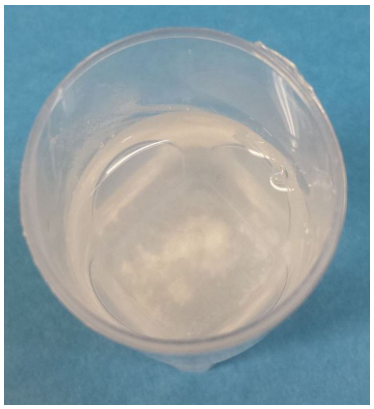


Figure 2. Powder now wetted and dispersed better after adding surfactant

Using surfactants may also be helpful to aid dispersion even when the sample isn't visibly floating on top of the liquid. The choice of optimum surfactant can sometimes be predetermined if the nature of the sample is well known. For example, use anionic (negatively charged head group) surfactants for positively charged particle surfaces.[1] Conversely, a cationic (positively charged) surfactant will adhere well to a negatively charged powder surface. But cationic surfactants are often pH dependent and less commonly found in typical labs, so nonionic surfactants are often used for negatively charged powder surfaces. Nonionic surfactants are the best options for many samples, especially if the surface properties of the powder are unknown. Some commonly used nonionic surfactants include:

- Triton X-100
- Igepal CA-630
- TWEEN® 20 and TWEEN® 80
- Span® 20 and Span® 80

All of these surfactants should be used at very low concentration in order to avoid bubble formation. A recommended practice is to dilute the surfactant to 1 – 10 volume % using filtered DI water. Place the powder in a small vial/beaker, place one drop of the diluted surfactant on top of the powder, and then add filtered DI water on top of the powder. The amount of water added is dependent on the desired concentration and total volume of the suspension. A larger total volume may be required if an ultrasonic probe is to be used to aid dispersion.

Powders naturally form agglomerates that must be broken apart if the goal is to measure the individual particle size. A common method to separate agglomerates is to apply ultrasonic energy, either by placing the suspension in an ultrasonic bath, or to place an ultrasonic probe into the sample. An ultrasonic probe is many times more powerful than an ultrasonic bath, and is used for more highly agglomerated samples. Care must be taken when using an ultrasonic probe in order to assure that agglomerates are separated, but individual particles are not broken.

During careful sample preparation and method development, it is suggested to perform a study of the amount of ultrasound energy added vs. measured particle size. An example of such a study is shown in Figures 3–6. Figure 3 shows the results before ultrasound is applied. Figures 4 – 5 show the results after applying different amounts of ultrasound. Figure 6 shows an overlay of no ultrasound vs. 60 sec ultrasound.

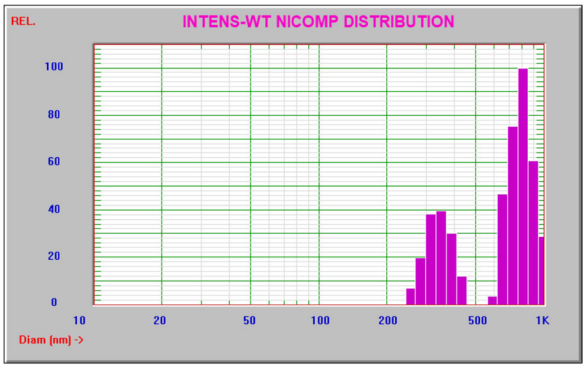


Figure 3. No ultrasound; two peaks at 804 nm and one at 348 nm

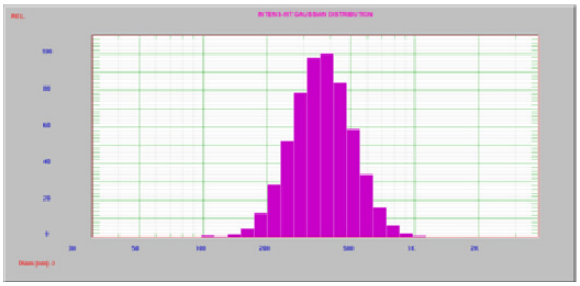


Figure 4. After 30 sec ultrasound, one peak at 385 nm

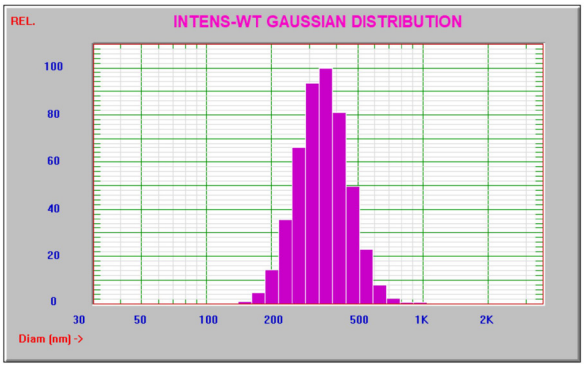


Figure 5. After 60 sec ultrasound, one peak at 355 nm

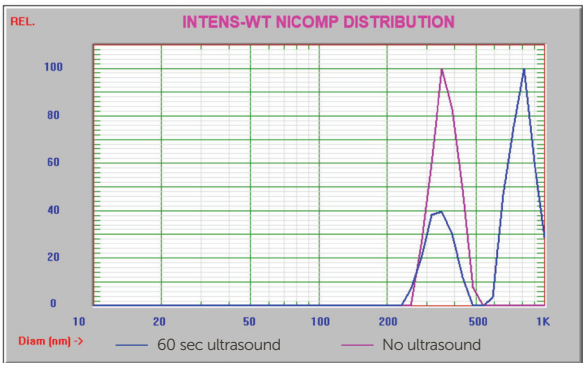


Figure 6. No ultrasound vs. 60 sec ultrasound

The energy level of the probe was kept constant during these procedures, while varying the time (in sec) of exposure of the sample to the probe. Pulsing the ultrasound (5 sec on, 5 sec off) helps reduce heat buildup in the sample.

DILUENT SELECTION

Whether the diluent will be aqueous or organic, the most important characteristic is that the diluent be (essentially) particle free. Since the scattering intensity is proportional to the diameter to the 6th power, a few large particles contribute greatly to the overall scattering from the sample of mostly small particles. The easiest way to remove contamination particles from the diluent is through filtration. For large diluent volumes required for high sample loads, a laboratory DI water filtration system may be appropriate just be sure to place a 0.2 µm (or better) final filter on the end. The megohm-cm rating is actually not important, only the particle concentration. Just building a homemade system with multiple filters in series is quite acceptable for most samples.

For individual measurements a syringe filter is the best way to prepare clean diluent. A common practice is to use a 10 mL syringe with a 25 mm diameter filter. The filter rating depends on the size of the particles to be analyzed and the seriousness of the measurement. The highest rated syringe filter to use is the Whatman™ Anotop 0.02 µm (Whatman 6809 – 1102) filters, but be forewarned they are expensive. For more typical DLS measurements >100 nm, a filter such as the Pall Acrodisc® 0.8/0.2 µm PN 4187 is often used in the Entegris applications labs.

As mentioned, the conductivity rating of the water is usually not critical. In fact, it is better to use a low salt concentration diluent than pure DI water for many samples, because DI water can strip ions off the surface of the particle, lower the zeta potential, and cause agglomeration. When Entegris prepared the 90 nm PSL standard used to verify system performance, we use 10 mM NaCl or KCl water to achieve the best results.

NOTE:
In order to make 10 mM of NaCl solution dissolve 0.5844 g NaCl into 1 L DI filtered water
In order to make 10 mM of KCl solution dissolve 0.7455 g in 1 L filtered DI water

Many biological samples are measured in phosphate buffered saline (PBS) to match the osmolarity and ion concentrations in the human body. In the Entegris applications labs we typically take the easy route and just dissolve PBS tablets (Sigma Aldrich P4417 – 50TAB) in filtered DI water when working with PBS. There are many different ways to prepare PBS. Some formulations do not contain potassium, while others contain calcium or magnesium. The most common composition of PBS is shown below in Table 1:

Salt	Concentration	Concentration
NaCl	137 (mmol/L)	8 (g/L)
KCl	2.7 (mmol/L)	0.2 (g/L)
Na_2HPO_4	10 (mmol/L)	1.42 (g/L)
KH_2PO_4	1.8 (mmol/L)	0.24 (g/L)

Table 1. PBS composition

Start with 800 mL of distilled water to dissolve all salts. Adjust the pH to 7.4 with HCl. Add distilled water to a total volume of 1 liter. The resultant PBS should have a final concentration of 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl.

Other buffers can be used for proteins or when working with organic solvents. The most frequently used salts to screen charge interactions in aqueous solution are KCl, KBr, NaBr, or NaCl. But for organic solvents either THF, DMF, or DMA are recommended. Proteins can be measured in a variety of buffers that include both a salt and pH adjustment. One basic goal is to avoid the isoelectric point (IEP)[2] of any sample. Lysozyme has the highest zeta potential at pH <4, so particle size measurements should be most stable below this pH. The buffer recipe for measuring lysozyme at 0.1 mg/mL[3] near pH 2.4 is shown below.

1. 0.2 M KCl solution was prepared by dissolving 14.91 g of KCl in 100 mL filtered DI water
2. 0.2 M HCl solution was created by adding 8.58 mL concentrated HCl into 491.4 mL water
3. 50 mL of the 0.2 M KCl solution was added into a 200 mL clean bottle
4. 7.8 mL of the 0.2 M HCL was slowly added to the KCL solution
5. Filtered DI water was added to fill up to 200 mL total volume

NOTE: The pH can be altered to higher values by adding less HCl in step 4. Any pH below 5 should create fairly stable lysozyme.

CELL SELECTION

Every Nicomp comes delivered with a standard 10 mm path length glass cell, plus a box of 100 round glass cells that fit into a metal cell holder. These round glass cells have a sample volume of 800 µL, but only 300 µL is required for a measurement. Disposable 10 mm plastic cells are acceptable for many easy samples, but the glass cells are better for smaller size, lower concentration measurements. Make certain not to leave finger prints on the cells in the measurement zone (~1.5 cm from the cell bottom). For best results clean the 10 mm glass cell with filtered water three times prior to use. A photo of typical cells used with the Nicomp is shown in Figure 7.

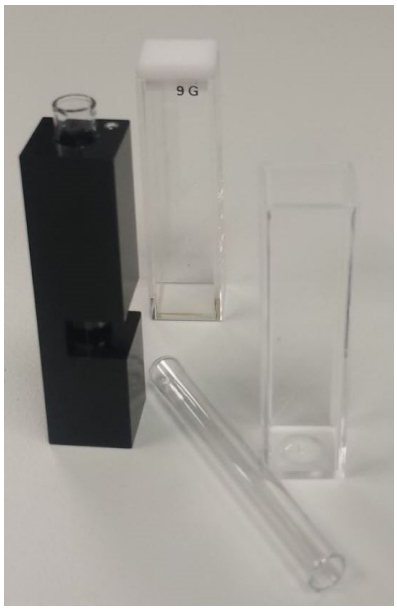


Figure 7. Clockwise starting at the top; 10 mm glass cell, 10 mm disposable plastic cell, round glass cell, round glass cell inside metal cell holder

Another approach to help with smaller size, lower concentration samples is to place a small volume cell into a refractive index (RI) matching fluid (Sigma Aldrich P/N M3516 or Acros Organics P/N 12402) inside a 10 mm cell as shown in Figure 8. This was the cell used to collect the lysozyme data at 0.1 mg/mL documented in Entegris Technical Note - 0.1 mg/mL lysozyme[3].

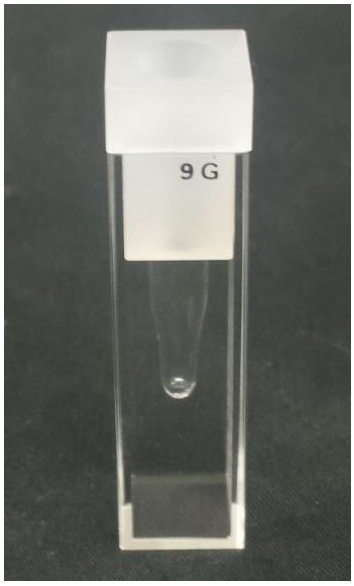


Figure 8. RI matching cell

REMOVING LARGE PARTICLES

Many users want to measure the size of the smallest single particles in the sample, not the agglomerates. Remember that DLS measures the hydrodynamic diameter; a sphere of the reported diameter will diffuse at the rate that was derived from the correlation function. Three agglomerated spheres will be measured at ~3 times the single particle size. If the goal is to measure only the single particle size in the presence of agglomerates, then the agglomerates must be removed from the sample. The easiest way to do this is to filter out the larger particles. Use a syringe filter with a pore size at least 2x larger than the expected single particle size. For example, if trying to measure particles expected to be ~20 nm, use a 50 nm pore size filter to remove agglomerates. Be warned, the filtration step may reduce the concentration (count rate) significantly, and concentration is an important factor, as discussed below.

At the end of a measurement it is a good idea to look at the bottom of the cell to see if any large particles have settled to the bottom. If large particles are seen at the bottom of the cell the result is suspect and better sample dispersion is required.

CONCENTRATION EFFECTS

Every time you make a DLS measurement you will get a result, but not always the best result. Despite the wide concentration ranges specified for all DLS systems, it is impossible to measure every sample at any desired concentration and generate an accurate result. When measuring an unknown sample, and it is typically worth the time to conduct a short experiment to investigate the effect of concentration on generated results. For many typical samples this can be as simple as measuring at concentration A, record result, dilute 2:1 with a proper diluent, measure, compare result B to A. If the results are the same, you have proven both repeatability and a lack of concentration effect on the measurement – both worth the time investment. If result A is not the same as B, then dilute the sample again, and record the differences. In general, the lower concentration result is likely to be more accurate. The ISO standard for DLS[4] suggests performing measurements at multiple concentrations, and then extrapolating to the apparent size at zero concentration. While this may qualify as a best practice, it is rarely done or reported.

There are several reasons that DLS measurements may be concentration dependent. Multiple scattering occurs when light interacts with one particle and then another before arriving at the detector. Multiple scattering effects can be reduced when using the multi-angle Nicomp system by measuring at a backscatter angle (~173 degrees) rather than 90 degrees. The other alternative is to dilute the sample as explained above. Other reasons DLS measurements are concentration dependent include hindered diffusion and other particle-particle interactions. These effects become important as a function of both size and concentration with the negative effects occurring at lower concentrations for smaller particle sizes. The lower concentration limit for making measurements on the Nicomp depend on particle size, concentration, relative refractive index particle/diluent, and also instrument configuration (light source and detector). The count rate generally provides a good indication if there is enough signal for good measurements, ideally near 300 kcounts/sec. If a sample is generating a lower count rate, this can be improved sometimes by placing the sample in a centrifuge to increase the concentration at the end of the cell.

A note on protein concentration: measuring the size of proteins on the Nicomp DLS system is a common application. If the user has control of the sample concentration, it becomes easier at concentrations >1 mg/mL but certainly lower concentrations are feasible[3].

CONCLUSIONS

The Nicomp DLS system can measure a wide range of sample types easily and accurately. Sample preparation is sometimes the key to generating repeatable results as described in this document. Other factors can also influence results including experimental setup, including:

- Measurement duration (longer for broader distributions)
- Measurement angle
- Channel width
- Neutral density filter setting (sensitivity)
- Baseline adjustment

Please see the Nicomp user manual for a discussion of these and other instrument settings.

References:

[1] ISO 14887 Sample preparation — Dispersing procedures for powders in liquids

[2] Entegris Application Note – Isoelectric Point

[3] Entegris Technical Note – 0.1 mg/mL Lysozyme

[4] ISO 22412 - 2008, Particle Size Analysis— Dynamic Light Scattering (DLS)

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