Selection of Particle Size Standards for Verifying and Validating Dynamic Light Scattering Instruments Used in Life Sciences

Submicron particle size analysis using Nicomp® DLS system

INTRODUCTION

Dynamic light scattering (DLS) is the most accepted and used technique for submicron particle size analysis. Since DLS is a "first principles" technique, systems are not calibrated but accuracy performance should be verified during installation and annually thereafter. An installation and operational qualification (IQ/OQ) is often performed when an instrument is first installed in a life sciences laboratory to assure the system is operating to specification and/or for the intended application. One or more particle size standards are analyzed, and the results are compared to expected values. Not all particle size standards behave equally during the verification process and a nominal size near 100 nm works best while sizes near 400 nm are the least desirable.

INSIGHT FROM STANDARDS

Most DLS users rely on the manufacturer to write and execute the IQ/OQ. Almost all manufacturers use a 100 nm nominal polystyrene latex (PSL) standard, typically with a mean size ~92 nm, for accuracy testing. This size is chosen for several good reasons including:

- This size is suggested in ISO standard for DLS[1]
- This standard is narrow and easily sized by DLS
- This size is mentioned in USP 729[2]
- 100 nm PSL is referenced in the proposed USP 430 chapter[3]

The only current USP chapter written for size analysis by DLS is USP <729>, Globule Size Distribution in Lipid Injectable Emulsions. The sizes of 100, 250, 400 nm are mentioned in the Standard Preparation section, and the intensity weighted mean results "should coincide with the expected values within acceptable errors". USP <729> is unique in suggesting multiple size standards be tested. If the DLS system result is accurate at one size (100 nm), the system has proven to be operating properly. No additional insight is gained by testing at other sizes. The method validation process for other analytical techniques might test for linearity of results, but this is not appropriate for a light scattering technique like DLS.[4]

WHICH SIZE STANDARDS TO USE

While 100 and 250 nm PSL standards are easily handled by DLS, a 400 nm PSL presents a challenge to some systems due to the relationship between angle and scattering intensity. At sizes below ~10 nm the scattering from particles is isotropic – the same at every angle. As size increases, so does the angular scattering dependence and pattern complexity. The intensity form factor G^2 can be used to account for the net effect on scattered light from influences including intra-particle Mie scattering, refractive index (RI), angle of measurement[5] and difference between laser wavelength (λ) and particle size. Figures 1, 2, and 3 show G^2 overlayed on the particle size distribution results for a bimodal mixture of 10 % (wt) 100 and 1% (wt) 300 nm PSL standards.

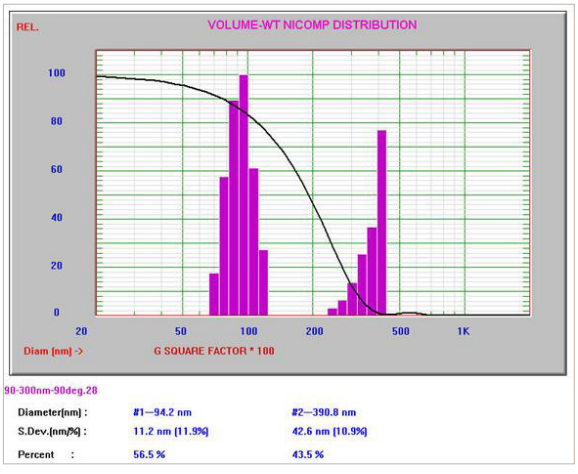

Figure 1. The bimodal sample measured at 90°.

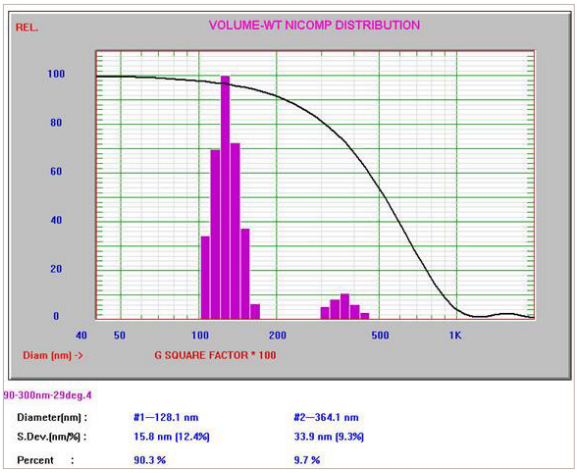

Figure 2. The bimodal sample measured at 29°.

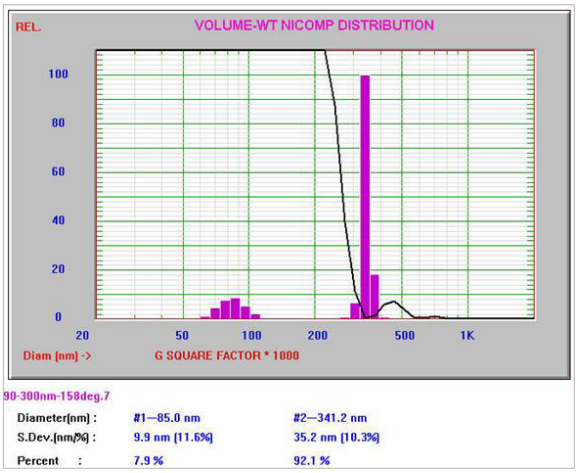

Figure 3. The bimodal sample measured at 158°.

Notice in Figures 1 and 3 that the G^2 plot hits the baseline near 400 nm, indicating a Mie scattering minimum. This scattering minimum has a deleterious effect on sizing accuracy at 90° and 158°. For this reason, instruments collecting scattered light at 90°, such as the Entegris Nicomp® DLS system, may perform less well near 400 nm than other sizes. In addition, the polydispersity (width of the distribution) increases with size, adding another reason why 400 nm less ideal to test at than 100 nm.

ACCEPTABLE ERRORS

The instrument manufacturer sets the accuracy pass/fail criterion in the OQ. USP <729> leaves the acceptable error open with the "acceptable errors" clause. The only other guidance at this time on this topic comes from ISO 22412 and the proposed USP <430> chapter (which is based on ISO 22412), both of which define a ±2% error limit. The ISO committee that wrote this standard aspires to be an influence to promote best practices to help generate the best possible data when using the DLS technique. This is a different mindset than a USP chapter that more likely wishes to promote a standardized approach to sample preparation, instrument calibration/verification, test procedures, and result reporting. These different goals may create conflicts and concerns when taking a copy/paste approach from ISO standard to USP chapter. This is clearly the case with USP <430>, and the 2% error limit is inappropriate for regular use of the DLS technique. A DLS result within 10% of the expected result proves a DLS system is functioning properly. A result greater than 10% of the expected value is most likely due to the sample dispersion/preparation technique and/or shelf life of the standard.

SAMPLE PREPARATION

Sample preparation can be one of the more challenging steps in generating quality DLS results depending on the suspension being analyzed.[6,7] Sample preparation influences results even with a relatively easy sample like the 92 nm PSL as shown in Figure 4.

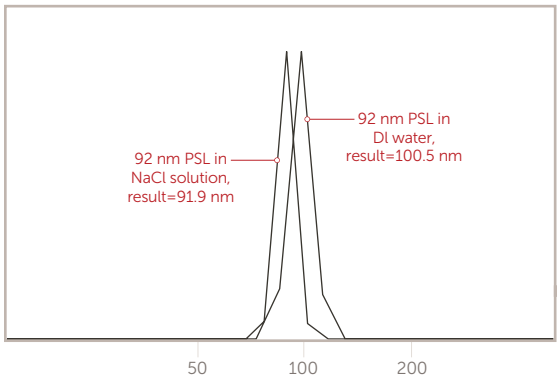

Figure 4. 92 nm PSL results dispersed in DI water and 10 mM NaCl solution.

Dispersing PSL standards in 10 mM salt solution hinders aggregation and typically generates more accurate results – in this case by 8.5 nm or 9% better. If the result had been 1 nm larger, the system might not pass a ±10% acceptance criteria, even though the system is operating perfectly as proven by the salt solution result. This example highlights the problem with the perhaps well intentioned, but improper acceptable error range of 2%.

This result dependence on sample preparation becomes more pronounced with testing a 400 nm PSL standard due to the Mie scattering minimum described earlier in this document. Careless sample preparation of a 400 nm PSL standard when measured on a 90° DLS system can generate results with errors up to 20% greater than the expected value.

EXPERIMENTAL

A rigorous sample preparation method was created to generate accurate DLS results at 90° for a 400 nm PSL standard.

Materials

- 400 nm PSL standard, Thermo Fisher cat no. 3400A, batch no. 3400-003
- Entegris Nicomp 3000ZLS system, 35 mW 639 nm wavelength laser, APD detector
- 10 mM NaCl solution
- Square glass cuvette
- Whatman Anotop 0.02 µm syringe filters (WHA 68091102)

Procedure

1. Prepare a 10 mM NaCl solution, dissolve 0.584 g NaCl into 1 liter filtered DI water.
2. Stir until all NaCl is dissolved.
3. Rinse a beaker that will hold at least 15 mL volume with filtered DI water. Entegris typically uses 20 mL scintillation vials. Pull 15 mL of the 10 mM NaCl solution into a 20 mL syringe. Attach an Whatman Anotop 0.02 µm filter. Push the syringe plunger to drive 15 mL of the NaCl solution into the vial/beaker.
4. Remove the cap and dropper tip from the PSL standard bottle. Pipette 10 µL of PSL standard into the 15 mL NaCl solution. Double the volumes if you do not have an accurate pipette at 10 µL.
5. Mix the vial and place in an ultrasonic bath for 30 seconds.
6. Clean the square glass cell three times with either DI water or salt solution using a syringe and 20 nm filter.
7. Pipette ~3 mL PSL standard into the square glass cell.
8. Place the cell into the Nicomp 3000ZLS system.

Instrument Settings

Create a measurement protocol using these settings:

- Autoset channel width
- Autoset sensitivity
- Autoset baseline adjustment
- Temperature = 23°C (73°F)
- Viscosity = 0.933 cP
- Run time = 5 minutes
- Scattering angle = 90°

Make three measurements and report the intensity mean diameter.

Results

Two samples were analyzed on two Nicomp systems following the sample preparation and measurement settings described in this document. Both Nicomp systems had the same 35 mW laser and APD detector, but one system was operating the Entegris ZPW software platform and the second was operating the Nicomp.Net software platform. The selection of software platforms does not affect calculated results since the exact same DLS algorithm is used for both. The Nicomp.Net software platform conforms to the requirements described in 21 CFR part 11 for electronic records and signatures required in the pharmaceutical industry. The result from the Nicomp instrument with ZPW software is shown in Figure 5, and the result with Nicomp.Net software is shown in Figure 6.

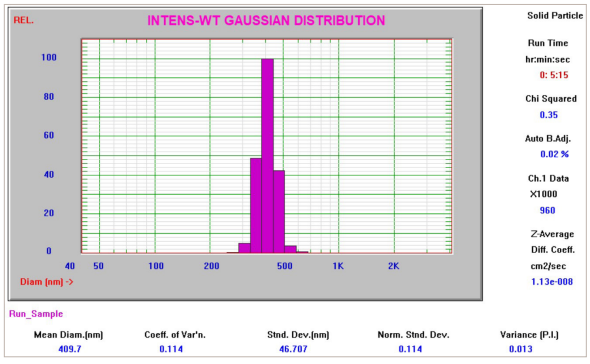

Figure 5. 400 nm result, ZPW software.

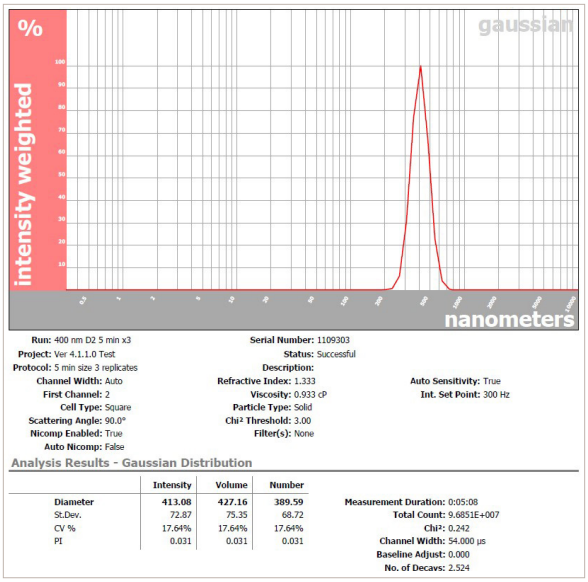

Figure 6. 400 nm result, Nicomp.Net software.

Repeatability was also tested and an overlay of three results are shown in Figures 7 and 8. The results in Figure 7 were performed using a square glass cell while the results in Figure 8 were performed using disposable a round glass cell.

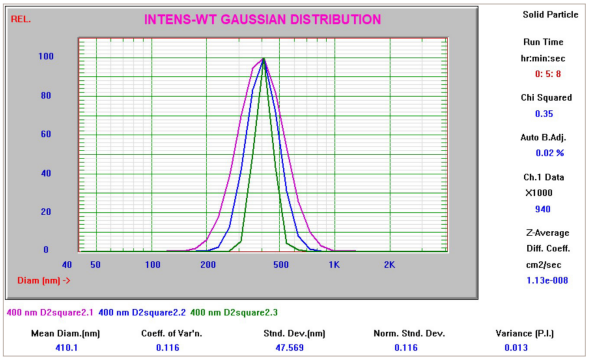

Figure 7. Overlay of three 400 nm results, square glass cell.

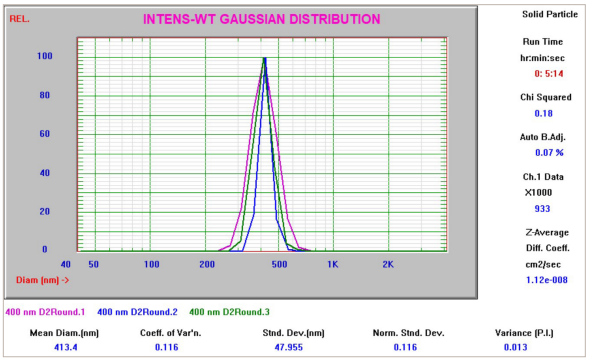

Figure 8. Overlay of three 400 nm results, disposable round glass cell.

DISCUSSION

The results from both systems are within 2-3% of the reported mean from the certificate of analysis of the PSL standard used, indicating an acceptable measurement procedure was developed.

Note that there are multiple justifiable options for choosing how to calculate the reported deviation from expected results. The certificate of analysis for this standard provides the following information:

- Certified mean diameter = 400 nm ±9 nm, k = 2
- Hydrodynamic diameter = 398 – 430 nm (PCS/DLS)
- Standard deviation = 7.3 nm
- Coefficient of variation = 1.8%

One simple way to calculate allowable error is mean x allowable error %.

Example for 10% allowable error:

Lower limit = 400 x 0.9 = 360 nm

Upper limit = 400 x 1.1 = 440 nm

Another option including the expanded uncertainty is:

Lower limit = (400 – 9) x 0.9 = 351.9 nm

Upper limit = (400 + 9) x 1.1 = 449.9 nm

Using the 2% error mentioned in ISO 22412 and USP 430:

Lower limit = 400 x 0.98 = 392 nm

Upper limit = 400 x 1.02 = 408 nm

Or

Lower limit = (400 – 9) x 0.98 = 383.18 nm

Upper limit = (400 + 9) x 1.02 = 417.18 nm

The stated range for the hyrodynamic dynamic diameter by DLS of 398 – 430 nm highlights the inappropriateness of specifying a 2% acceptable error limit. The official DLS results by the standard manufacturer fails the 2% error limit even when including the stated expanded uncertainty of 9 nm. It appears the proposed USP chapter is expecting every lab in the U.S./world that uses DLS to have significantly higher accuracy than the standard manufacturer's lab with over 30 years of DLS experience measuring this sample.

The reported intensity mean of 413 nm shown in Figure 8 is still quite accurate but notice the differences in distribution width, the variance or PI (polydispersity index). The PI values from these results ranged from 0.006 – 0.025. This sample remains more difficult than 100 nm PSL with respect to consistent PI values, a characteristic also attributed to the Mie scattering minimum.

The slightly better results shown in Figure 7 vs. Figure 8 suggest that the square glass cell provides better results than the disposable round glass cell. These differences are minor but may possibly be the difference between passing and failing depending on how the acceptable error is calculated.

CONCLUSIONS

The results shown in this document imply that acceptable results for 400 nm PSL measured by DLS at 90° are possible for an expert user carefully following the procedures described in this document. This does not imply that Entegris endorses using 400 nm PSL standard for verifying DLS system during the OQ. Testing the system with a single 100 nm nominal PSL is sufficient to verify system performance. Of the other sizes to be considered for testing accuracy, the worst option is 400 nm PSL.

References

[1] ISO standard 22412:2017 Particle size analysis – Dynamic light scattering, https://www.iso.org/standard/65410.html

[2] USP <729> Globule Size Distribution in Lipid Injectable Emulsions

[3] USP <430> Particle Size Analysis by Dynamic Light Scattering, PF 46(3), Notice: Documents in PF Online are not official and may never become official

[4] Entegris technical note DLS Method Development and Validation, https://www.entegris.com/content/dam/product-assets/nicompnanodlszlssystems/technote-dls-method-development-validation-10581.pdf

[5] Entegris technical note Multiangle DLS Measurements, https://www.entegris.com/content/dam/product-assets/nicompnanodlszlssystems/technote-multiangle-dls-measurements-10569.pdf

[6] Entegris technical note DLS Sample Preparation, https://www.entegris.com/content/dam/product-assets/nicompnanodlszlssystems/technote-dls-sample-preparation-10568.pdf

[7] Entegris technical note DLS System Verification, https://www.entegris.com/content/dam/product-assets/nicompnanodlszlssystems/technote-system-verification-10564.pdf

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