# System Verification

*Nicomp® DLS*

All analytical instruments should be tested regularly to assure the system is performing properly. Dynamic light scattering (DLS) systems are typically very stable but should be tested using known standards to verify system performance and data integrity. Entegris recommends using polystyrene latex (PSL) standards to test the particle size results and one of several samples to test the zeta potential results. This technical note is written to aide customers who want to verify system performance of the Nicomp® 380 or 3000 series systems.

## CALIBRATION VS. VERIFICATION

DLS is a "first principles" technique meaning that the results are derived from first principles of physics, not from a calibration curve used to relate instrument response to known results. A particle size standard is introduced and the measured result is checked vs. the referenced result on the certificate of analysis (C of A). If significant differences are found the measurement is typically checked again one or more times to verify the cause of variance from the certified value and to investigate whether they occurred due to a real problem with the instrument, not sample integrity, dispersion or operator error. If there is a consistent, significant difference between the measured and reference result, it is not possible to alter a calibration curve or function. The variance is then deemed to be due to a mechanical and/or optical fault in the instrument and repairs are necessary. The repairs could include a change in laser, change in detector or optical re-alignment (the most common fix).

## FREQUENCY OF VERIFICATION

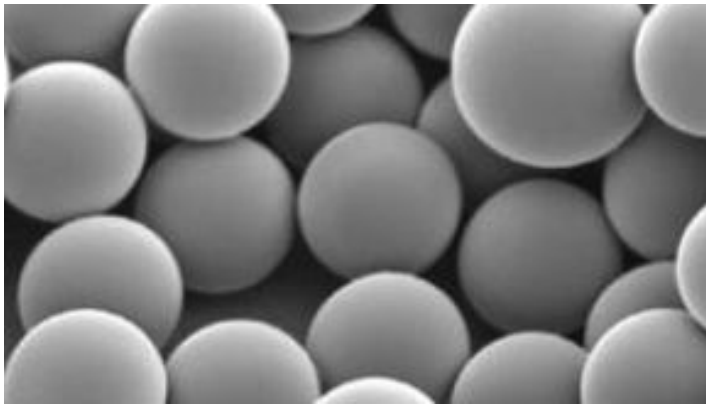
How often the system is verified should be a risk-based decision. If the product batch to be tested is worth millions of dollars we imagine the customer would test the system before testing the samples if the particle size result could fail the batch. If a researcher uses the system once a month for a quick size check of a sample, then annual verification should be sufficient. Most pharmaceutical companies require verification and a sticker on the system showing the last verification and next required date either every six - or twelve-months.

## HOW MANY SIZE STANDARDS TO TEST

Should one or more particle standards be tested during the system verification process? Unlike many other analytical techniques, DLS does not need to be tested for linearity of response. Therefore, a single particle size standard is typically sufficient to verify performance. The exception to this statement is for customers using the DLS system for USP <729> Method I testing. Then three standards should be used; approximately 100, 250, and 400 nm.

## WHICH STANDARD(S) TO USE

Many particle size standards are available from several vendors and the customer may choose the sample they wish. However, Entegris has experience with two PSL standards and these are the samples we typically suggest. The Thermo Fisher 3000 series NIST traceable 90 nm nominal PSL standard is often used to verify Nicomp performance. The sample is catalog number 3090A with a certified value of 92 ±3.0 nm. Another sample often used is Thermo Fisher catalogue number 5009 A with a value of 90 nm. This sample is not NIST traceable but we have sufficient experience to recommend the use of this sample, and it is less expensive than the 3000 series products.



## SAMPLE PREPARATION

The concentration in particle size standards discussed in this document are too high to use undiluted. Multiple scattering effects would create unacceptable errors in the reported vales. Therefore the user needs to dilute below the level where multiple scattering occurs, but high enough to obtain the ~300 kHz optimal count rate. Many users dilute with DI water, but a dilute salt solution will generate more accurate results by causing less change in the electric double layer surrounding the PSL particles.

Entegris recommends using a 10 millimolar (mM) NaCl solution when diluting the PSL standards. Measuring the PSL in DI water changes the electrical double layer surrounding the particles, effecting long distance interactions. This can change the measured mean size change by 2 – 10 nm.

To make a 10 mM NaCl solution, dissolve 0.5844 g NaCl in 1 L filtered DI water in a volumetric flask. Stir until all of the NaCl has dissolved.

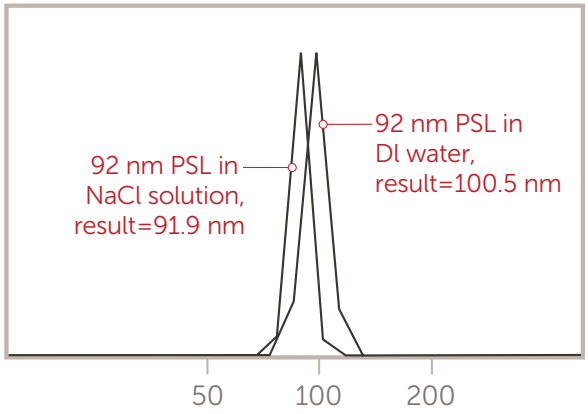



*Figure 1. 92 nm PSL results in DI water and 10 mM NaCl Solution*

Pipette 30 µL of NIST traceable standard into 5 mL of filtered 10 mM NaCl solution or 30 µL of the research standard (5009A) into 50 mL diluent. Even if the NaCl solution was originally made with filtered DI water we recommend still using a syringe filter when adding the 5 mL NaCl solution into the 5 mL container. Mix this sample vial and the standard is now ready for use.

## INSTRUMENT SETTINGS

Depending on the instrument vintage and software being used there may be minor differences in the settings used for the verification process. But the basic settings used are shown below.

| | |
|---|---|
| **Refractive index** | 1.333 |
| **Viscosity** | 0.933 CP |
| **Particle type** | Solid |
| **Auto sensitivity** | Checked |
| **Channel width** | 10 µsec |
| **Temperature** | 23°C |
| **Intensity set point** | 300 kHz |
| **First channel used** | 2 |
| **Scattering angle** | 90° |
| **Cell type** | Square or round |
| **Run time** | 10 minutes |

Do not worry about Nicomp settings, the Gaussian results will be used. Make at least three measurements and print each run. Record the mean diameter values of the Gaussian Intensity distribution and calculate average of at least three runs.

## PASS/FAIL CRITERIA

The determination if the Nicomp is operating within specification is determined by two criteria.

### 1. Accuracy

The average of three mean values is +/15% from the certified value.

Lower limit: certified value * .85

Upper limit: certified value *1.15

### 2. Repeatability

The deviation from all three runs is ±5% from the mean value.

Each result must lie between this range:
.95* average – average * 1.05

If the measurements pass these two criteria the Nicomp has been properly certified for use. A good size result is shown in Figure 2.

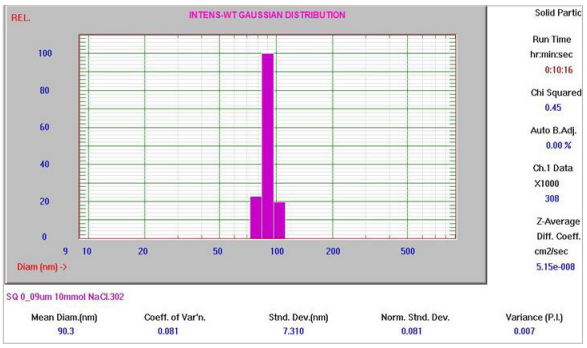



*Figure 2. A good size result*

## ZETA POTENTIAL

Many Nicomp systems also measure zeta potential. Zeta potential is typically regarded as more of a relative then absolute measurement, but system verification against a known sample is still advised.

## WHICH STANDARD TO USE

The only NIST certified zeta potential material is SRM 1980, Positive Electrophoretic Mobility Standard. This material is a goethite powder that must be suspended in liquid and carries a positive charge. The dispersion process, positive charge, and requirement to manually calculated zeta potential from the electrophoretic mobility all contribute to poor acceptance of this sample. This sample and procedure will not be discussed in this document. We will instead explain the procedure for a less expensive, easy to use sample that customers can use to verify the zeta potential measurements on a Nicomp system. This sample is a powdered non dairy creamer that we have named the zeta reference standard (ZRS).

## SAMPLE PREPARATION

Place 0.1 g of the ZRS sample in 200 mL DI water and stir for 3 minutes. Measure the pH of the prepared sample to verify that the pH is between about 6 – 8.

Inject about 3 mL of sample into a standard disposable cell. Insert the zeta potential electrode and wipe any liquid that drips onto the external cell windows. Assure there are no bubbles between the electrodes. Connect the standard cable (not HV) to the electrode and place the cell in the instrument so that the cable is on the back side of the instrument.

## INSTRUMENT SETTINGS

Configure the software for zeta potential measurements. Set up the zeta potential control menu as shown below.

| | |
|---|---|
| **Temperature** | 23°C |
| **Viscosity** | 0.933 CP |
| **Refractive index** | 1.333 |
| **External fiber angle** | -19° |
| **Scattering angle** | -14.1368° |
| **Phase analysis (PALS)** | Checked on |
| **Dielectric constant** | 78.5 |
| **Electrode spacing** | 0.4 cm |
| **E-Field strength** | 4 V/cm |
| **Smoluchowski limit** | Checked on |

Make at least three, five minute measurements and print each run. Calculate the average of the three runs.

## PASS/FAIL CRITERIA

The system passes the verification procedure if the average result lies between the upper and lower values provided on the certificate of analysis provided with the standard.

An example of a good zeta potential result is shown in Figure 3.

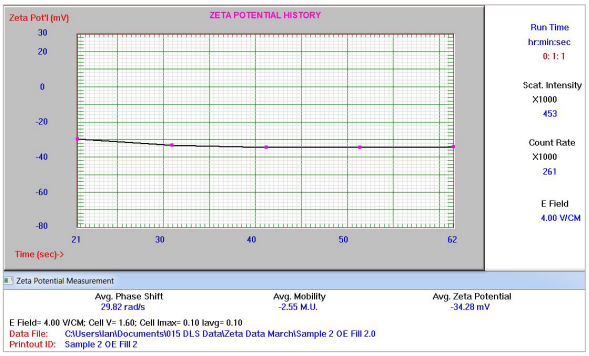



*Figure 3. Good zeta potential result*

*Note: If one result varies significantly from the others Entegris recommends discarding that result as an outlier and either performing another measurement or calculating the average without the outlier value. Zeta potential is inherently less stable than particle size measurements.*

## CONCLUSIONS

Entegris encourages customers to regularly perform verification tests on their Nicomp system. Please feel free to contact your local Entegris representative to schedule a visit by an authorized specialist to verify your system. Otherwise, follow the instructions described in this document to verify performance yourself.

### FOR MORE INFORMATION

Please call your Regional Customer Service Center today to learn what Entegris can do for you. Visit entegris.com and select the Contact Us link to find the customer service center nearest you.

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