

Guide for Making Zeta Potential Measurements

Using the Nicomp® DLS system

INTRODUCTION

Zeta potential is a measure of the surface charge on particles in suspension and emulsions. This is the electrical potential measured in millivolts at the slipping plane – the interface separating fluid attached to the surface from the bulk continuous phase as shown in Figure 1.

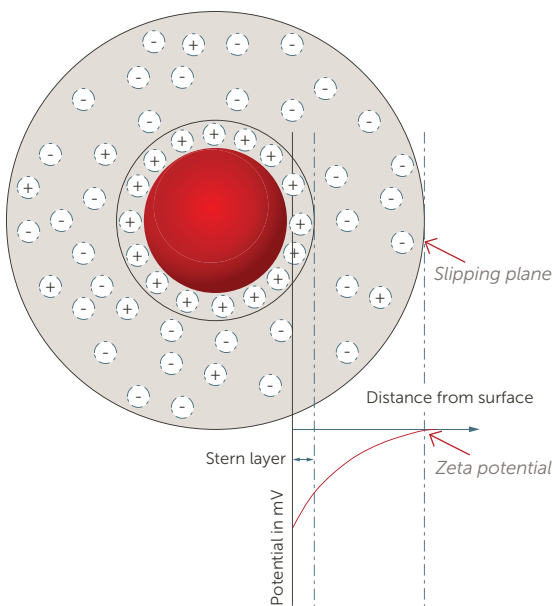


Figure 1. Zeta potential on the surface of a negatively charged particle.

The zeta potential is an indicator of dispersion stability. The magnitude (not the sign of the charge) of the zeta potential provides information on the electrostatic repulsion between the particles or emulsion droplets in a suspension. A higher zeta potential value indicates the suspension should be more stable than a value near zero. When the zeta potential is near zero the suspension is likely to destabilize, leading to particles agglomerating and settling or emulsions phase separating.

The Nicomp® dynamic light scattering (DLS) system measures the electrophoretic mobility of a suspension and then calculates the zeta potential. The electrophoretic mobility is measured by applying a known electric field into the sample cell and determining the velocity of the particles in the applied field, Figure 2.

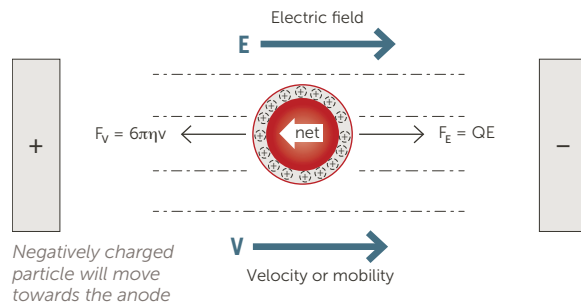


Figure 2. Zeta potential measurement.

The particle velocity is determined either by measuring the frequency (Doppler) or phase shift, known as phase analysis light scattering (PALS). The particle motion is used to calculate the electrophoretic mobility (μ) using the equation below.

$$\mu = \frac{\Delta\omega\lambda_0}{4\pi n E \sin(\frac{\theta'}{2})}$$

Where:

μ = electrophoretic mobility

ω = frequency shift

λ_0 = wavelength of the laser

n = refractive index of the medium

E = electric field strength

θ = angle between the incident beam and scattered light

After determining the electrophoretic mobility, the zeta potential is then calculated using the Henry equation below.

$$\mu = \frac{2\zeta\epsilon}{3\eta_0} f(\kappa a)$$

Where:

- μ = electrophoretic mobility
- ζ = zeta potential
- ϵ = dielectric permittivity
- η_0 = viscosity
- $f(\kappa r)$ = function describing the relationship between particle radius a and the Debye length $1/\kappa$, see Appendix I

SOFTWARE SETUP PROCEDURES

There are two software platforms used to control measurements – ZPW and Nicomp.NET. ZPW is the older software platform that includes both frequency and phase shift algorithms. Nicomp.NET only provides PALS and has a 21 CFR part 11 compliant option for electronic records/electronic signatures used in the pharmaceutical industry. This technical note will cover both software platforms.

ZPW Software Setup

Open the ZPW software. If the system was last used for making particle size measurements, switch to zeta potential by clicking on “Particle Sizing”, then “To Zeta Potential”, or click “Alt + Z”, Figure 3.

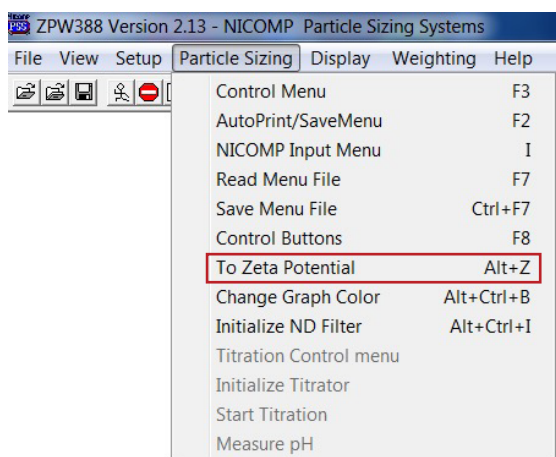


Figure 3. Switch to zeta potential mode.

This opens the Zeta Control Menu, Figure 4.

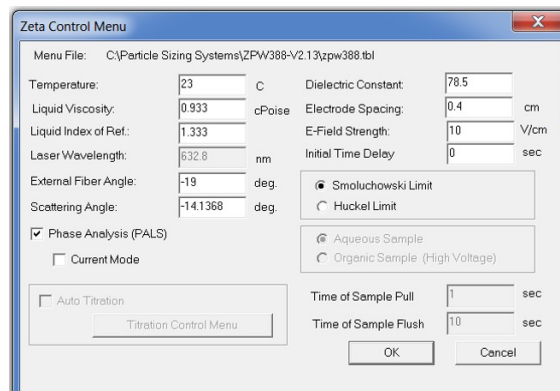


Figure 4. Zeta Control Menu.

Description of options in the Zeta Control Menu:

Temperature: Input sample temperature, default is 23°C (73°F).

Liquid Viscosity: Input liquid viscosity, typically water since most samples are dilute or have been diluted. Note that viscosity changes with temperature.

Laser wavelength is set once upon instrument installation.

External Fiber Angle: Always set to -19°.

Scattering Angle: Always set to -14.1368°.

Note: The system will not operate properly if these angles are set to positive angles.

Phase Analysis: Two measurement modes are available; frequency and phase analysis light scattering (PALS). The PALS method is more sensitive and is the suggested mode of operation used by most operators.

Current Mode: Keeps current constant during measurement if checked. If unchecked, then measurements are made in constant voltage. Constant current is recommended for samples with a high number of counter ions such as CMP slurries.

Dielectric Constant: 78.5 for water, or see Appendix II for other values.

Electrode Space: 0.4 cm for standard cell.

E-Field Strength: Varies with sample, see Table 1 for suggestions.

Sample	Electrode spacing	Applied field (V)
Aqueous low salt conc.	0.4 cm	5 – 10 V
Aqueous high salt conc.	0.4 cm	3 – 5 V
Protein, biomolecule	0.4 cm	1 – 5 V
Nonaqueous	0.4 cm	50 – 250 V

Note: always use the HV connector for nonaqueous samples

Table 1. Suggested Electric Field Strengths.

Initial Time Delay: Delay between applying electric field and initiation of data collection.

Smoluchowski Limit: Default choice for κa for most samples, Appendix I.

Huckel Limit: Alternative κa value for unique samples, Appendix I.

Aqueous Sample: Standard measurements.

Organic Sample: Check when organic solvent is used as continuous phase.

Titration controls are not available currently.

Next, open the Auto Print/Save Menu by clicking "Zeta Auto Print/Save Menu" or click "F2".

This opens the dialog box shown in Figure 5.

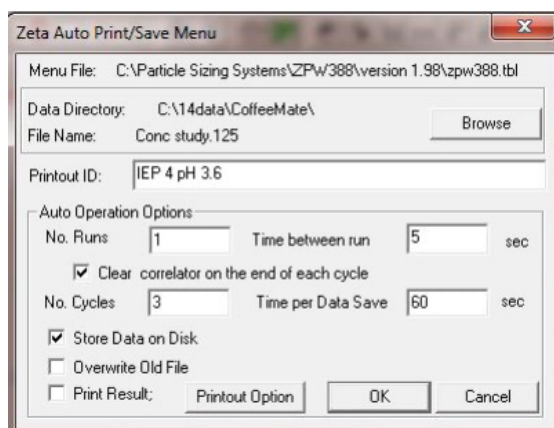


Figure 5. Zeta auto print/save menu.

Specify a place to store the data collected. Data should never be stored to the root (C:) directory. From within the browse function, you can create a new folder to place data in. The file name must include a decimal (point), for example, Zeta Sample 1.0.

Printout ID allows a maximum of 80 characters to be entered as a description of the sample that will appear on all printouts.

No. Runs: by choosing more than 1 run the user can use the same sample for multiple measurements.

Time between run: the user may choose to let the sample rest between each run for a chosen amount of time. This can be useful in combating Joule heating that may begin to cause convection currents within the sample cell thereby moving particulate around and out of the inspection zone.

Clear correlator: the user may choose to clear all past information from the correlator if performing multiple measurements. Or allow the information to accumulate.

No. Cycles: the number of files saved to complete a measurement.

For example, if # of cycles = 2 and time per = 30 sec. or # of cycles = 1 and time per = 60 sec. the total measurement time for both conditions will be 60 seconds. In the first set of conditions there will be two files saved with increasing extension numbers each 30 seconds worth of data, in the second set only a single file containing all data for the 60 seconds.

Nicomp.NET Software Setup

Click "Protocol", select "Manual – Zeta" in the box at the bottom left, and the Protocol window shown in Figure 6 will appear.

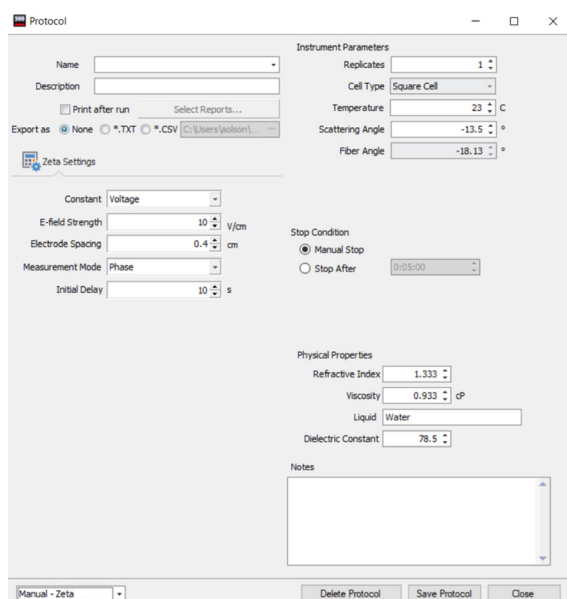


Figure 6. Zeta potential protocol.

Enter a unique name for the protocol.

Enter additional description details if desired.

Select "Print after run" and the desired report.

And/or click "Export as" and select the desired file format.

Select the number of Replicates (number of measurements).

Cell type is always "Square Cell".

The default Temperature = 23°C (73°F).

Scattering angle is always -13.5°.

Fiber angle is always -18.13°.

Select either "Constant Voltage" or "Constant Current" mode of operation. Constant current is recommended for samples with a high number of counter ions such as CMP slurries.

E-field strength: Varies with sample, see Table 1 for suggestions.

Electrode spacing: 0.4 cm for standard cell.

Measurement Mode: Phase.

Initial Delay: Apply electric field for this many seconds before initiating the measurement.

Stop Condition: Default is Stop After selected time in minutes/seconds. Less than 5 minutes is not recommended.

Physical Properties: Typically set at the default values shown for low concentration aqueous samples: Refractive Index = 1.33, Viscosity = 0.933, Liquid: Water, Dielectric Constant: 78.5.

Only change these values when working with a different solvent/diluent.

Give the protocol a name and click "Save Protocol".

SAMPLE MEASUREMENT PROCEDURES

Prepare the sample cuvette by filling it about $\frac{2}{3}$ full of sample, Figure 7. The liquid sample level should be adjusted so that the top surface of the liquid lies a little bit below the plastic piece that holds the electrodes. This will prevent capillary "creep" of the liquid up the inside surface of the cuvette and onto the outer surface, where it can distort the optical quality of the cuvette surface. Insert the electrodes and clean the cuvette surface with a lint free wipe, Figure 8. Make sure there are no bubbles in the measurement zone that is $\frac{3}{4}$ from the bottom of the cell.

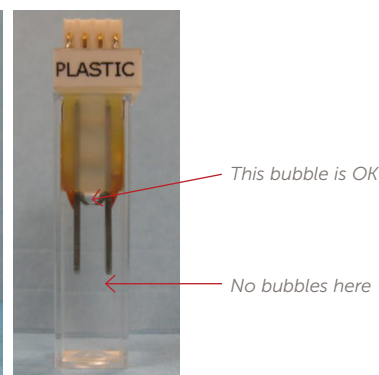


Figure 7.

Figure 8.

Insert the cuvette with electrode assembly into the sample cell holder with the pins of the electrical connector pointed toward the back of the unit. Attach the zeta power cable to the plug, Figure 9, and orient the cell so the label "PLASTIC" faces the front of the system.

Note: If the PLASTIC label faces to back of system the (±) calculated charge will be reversed.

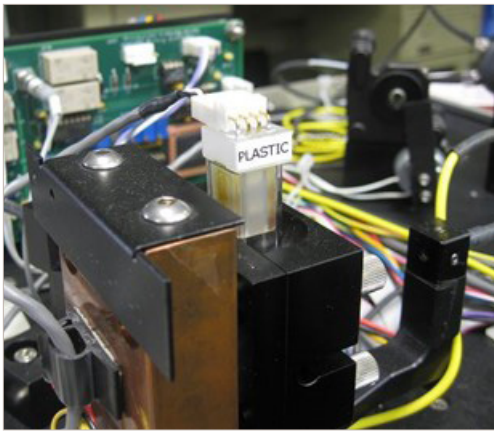


Figure 9. Zeta potential cell orientation.

Close the sliding sample door to the unit, and on some units slide the bar to the zeta position. Wait 2 minutes for the sample temperature to come to equilibrium.

For ZPW software, click the “G” icon to begin the measurement.

For Nicomp.NET software, click “Run, Manual Zeta” to begin the measurement.

The instrument will adjust the ND filter until scattering intensities are optimized for the zeta measurement. A small window will appear stating this action is taking place. If the error “sample too concentrated please dilute” appears, dilute the sample by 50% and attempt the measurement again. If the error persists, try initializing the ND filter. If the zero point on the ND filter has been lost by the software the full range of the filter is no longer available and the instrument assumes that the reason the scattering intensities are too large is due to the sample concentration.

A window will appear stating that data collection has begun with the E-Field off. The reference signal is being measured and stored. This step takes approximately 1 minute. The small window towards the bottom of the screen will show the number of cycles and runs to be completed. It will also display a countdown of the time remaining between runs.

SAMPLE PREPARATION PROCEDURES

Take care to avoid changing the surface chemistry of the sample during the measurement procedure. Remember that the measured zeta potential is valid only for the specific chemistry (pH, salt concentration, surfactant concentration, etc.) experienced during the analysis. Any changes in dispersion chemistry could easily lead to a change in zeta potential.

The system can measure a broad range of concentrations, but sometimes sample dilution is necessary. A general guideline is that you should be able to easily see through the sample or the concentration is too high. The ideal diluent to use is the supernatant liquid of the sample without any particles present. If this is not possible it is better to use 10 mM KCl than using DI water.

Data Interpretation

Like all particle characterization measurements, always test repeatability. It is better to take 10, one-minute measurements than one, ten-minute measurement. Zeta potential measurements inherently include occasional outliers. Many customers will take 5 – 10 measurements, discard outlier results (more than 15% away from the mean result) and then report the average value.

The zeta potential magnitude required to stabilize a dispersion depends on many things, including the nature of the sample itself. See Table 2 for estimated zeta potential values required to achieve stability for different sample types.

Sample type	Zeta potential
Metal sols	>40 mV
Metal oxides	>30 mV
Polymers	>20 mV
Emulsions	>10 mV

Table 2. Stability requirements.

SYSTEM VERIFICATION

Each new Nicomp system is shipped with a bottle of the Entegris ZRS standard. This is the sample we recommend using to verify system performance on a regular basis. The procedure for analyzing the standard is supplied with the bottle and is outlined below.

Power up the Nicomp system according to the user manual. The Nicomp system must be powered up for a minimum of ten minutes prior to making a measurement.

Sample Preparation:

Place 0.05 g of the ZRS sample in 100 mL DI water and stir for 3 minutes. Measure the pH of the prepared sample to verify that the pH is between about 6 – 7.

Note: this sample is an emulsion and will only be stable enough for use in verification for ~48 hours. After this time a new standard should be prepared.

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 and the "PLASTIC" label faces front.

Instrument Settings:

Using either ZPW or Nicomp.NET software, configure the measurement using the following parameters:

Temperature	23°C (73°F)
Viscosity	0.933 cP
Refractive index	1.333
External fiber angle	-19°
Scattering angle	-14° or -13°
Phase analysis (PALS)	checked on
Dielectric constant	78.5
Electrode spacing	0.4 cm
E-Field strength	4 V/cm
Smoluchowski limit	checked on (ZPW only)

Configure the software to make at least three, five-minute measurements.

Data Interpretation

The last result shown is the average zeta potential over the course of the measurement. Compare this value to the information provided on the certificate of analysis (CofA) for the ZRS batch being used. The acceptable ranges are shown as a range such as -35 to -55 mV.

If three out of the three zeta potential values are within the range defined on the certificate of analysis supplied with the ZRS standard, then it can be said that the Nicomp system is accurate.

If three of the three collected zeta potential values are within 10% of their average, then it can be said that the Nicomp system is repeatable.

Repeat measurement again with the same aliquot of the ZRS standard if any of these conditions are not met. If on the second group of measurements any of these conditions are still not met, then prepare a new dilution of the ZRS standard.

APPENDIX I: SMOLUCHOWSKI VS. HUCKEL MODEL

The equation for calculating zeta potential from the measured electrophoretic mobility includes a function $f(\kappa a)$ that models the relationship between the particle radius a and the electric double layer distance $1/\kappa$.

Particle radius = a

Distance from particle surface to edge of the double layer = $1/\kappa$

Product is κa

If $\kappa a > 1$, use Smoluchowski

If $\kappa a < 1$ use Huckel

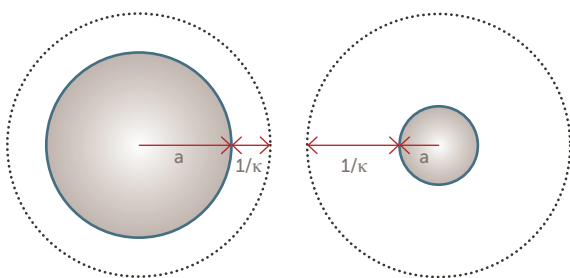


Figure 10. Smoluchowski vs. Huckel models for κa .

APPENDIX II: DIELECTRIC PERMITTIVITY VALUES

Solvent	Dielectric constant
Acetic acid	6.15
Acetone	20.7
Acetonitrile	37.5
Anisole	4.33
Benzene	2.27
Bromobenzene	5.17
Carbon disulfide	2.6
Carbon tetrachloride	2.24
Chlorobenzene	5.62
Chloroform	4.81
Cyclohexane	2.02

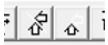
Solvent	Dielectric constant
Dibutyl ether	3.1
o-Dichlorobenzene	9.93
1,2-Dichloroethane	10.36
Dichloromethane	8.93
Diethylamine	3.6
Diethyl ether	4.33
1,2-Dimethoxyethane	7.2
N,N -Dimethylacetamide	37.8
N,N -Dimethylformamide	36.7
Dimethyl sulfoxide	46.7
1,4-Dioxane	2.25
Ethanol	24.5
Ethyl acetate	6.02
Ethyl benzoate	6.02
Formamide	111
Hexamethylphosphoramide	30
Isopropyl alcohol	17.9
Methanol	32.7
2-Methyl-2-propanol	10.9
Nitrobenzene	34.82
Nitromethane	35.87
Pyridine	12.4
Tetrahydrofuran	7.58
Toluene	2.38
Trichloroethylene	3.4
Triethylamine	2.42
Trifluoroacetic acid	8.55
2,2,2-Trifluoroethanol	8.55
Water	78.5
o-Xylene	2.57

APPENDIX III: ADJUSTING ALIGNMENT

Warning: Please call your local Entegris representative if you are concerned the system requires alignment. Performing this procedure on your own may void warranty or could lead to expensive repairs.

The zeta potential reference beam values should be greater than around 2 MHz for optimum operation. The reference beam value can be seen by clicking on the “reference beam on” icon.

In ZPW software the reference beam on and off icons are to the far right of the top control bar:

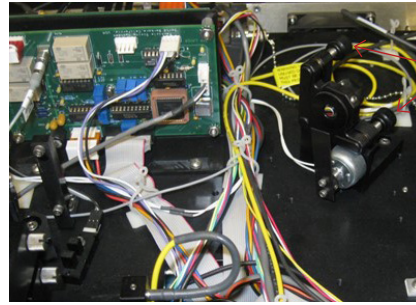


In Nicomp.NET software, click the Reference Beam icon to toggle the reference beam on or off. When the icon is red, the beam is on.



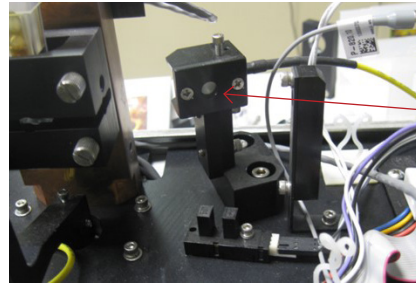
The shutter that blocks the reference beam during normal sizing operation of the instrument will move and the PEM will start to vibrate. One can always ensure that the measurement is progressing by the sound this PEM makes.

Upon installation of the instrument, an Entegris representative may optimize the alignment by carefully adjusting the thumbscrews located on the two-dimensional tilting stage, on which the PEM unit is mounted. There are not many reasons that the user will have to readjust this alignment. In any case, the tilting stage should be adjusted to produce a total detected intensity of approximately 2000 – 4000 kHz. Any value higher than 4000 kHz should be avoided by slightly “de-tuning” the tilt stage.



Turn these knobs until the count rate is ~3500 kHz

Figure 12. Alignment adjustment knobs.



Maximize the brightness at the center of this spot

Figure 13. Zeta potential detector.

FOR MORE INFORMATION

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