Project:

Measuring Zeta Potential

Zeta potential determination of nanoparticles in aqueous dispersions by PALS

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1 Introduction

Zeta potential values provide an indirect measurement of the net charge on the nanoparticle (NP) surface. Among different approaches to characterize the superficial properties of NPs in liquid state, zeta potential measurement is one of the most accessible. For this reason, the measurement of zeta potential can be routinely used as a pre-screening technique to control batch to batch consistency.

The surface charge of NPs influences their physical state in liquids (e.g. stability, absorption of proteins) and, thus, their interactions with biological systems. Therefore, zeta potential values are particularly interesting to characterize NPs intended for biomedical applications. Zeta potential values can be used as a criteria to determine particles tendency to aggregate in aqueous media and may give useful information to correlate NP physical-chemical properties to their *in vitro* and *in vivo* activity (e.g. NPs-cells interactions). In specific cases, changing the environmental conditions of the dispersive media (e.g. media composition, titration *vs.* pH) allows studying the NP physical-chemical properties in different conditions, e.g. to mimic their mechanism of action in *in vivo* systems.

This protocol deals with the measurement of zeta potential of NPs dispersed in aqueous solutions, thought the determination of their electrophoretic mobility. Guidelines for making successful zeta potential measurements are provided, as well as a discussion of relevant standards for quality control and criteria for data analysis. The SOPs to be use in PALS system produced by Malvern (Nano series) are described in details. The protocol has to be slightly modified to be applicable to other systems.

2 Principle of the Method

When charged NPs are dispersed in a liquid, a layer of ions of opposite charge strongly bound to their surface forming a charged thin layer, known as the "Stern layer" (see Fig. 1). The Stern layer induces the formation of a second diffuse outer layer, composed by loosely associated ions that is called "diffusive ion layer". These two layers are collectively called "the electrical double layer". When the NPs move in the liquid phase (due to gravity, kinetic energy, and/or under an applied electrical field), there exists a boundary between the ions in the diffuse layer that move with the particle and ions that remain with the bulk dispersant. The electrostatic potential at this "slipping plane" boundary is the zeta potential (Fig. 1).

During a zeta potential measurements, an electrical field is applied across the sample, inducing the movement of charged particles. The ratio between the NP velocity and the external applied field, known as electrophoretic mobility (μ_e), is then measured and converted to the zeta potential (z) using the Henry equation:

$$\mu_e = \frac{2 \cdot \epsilon \cdot z \cdot f(k \cdot \alpha)}{3 \cdot \eta} \quad (1),$$

where ε and η are respectively the dielectric constant and the absolute zero-shear viscosity of the medium [1-3]. $f(k \cdot \alpha)$ is known as "the Henry function", where α is the radius of the particle and k is known as the Debye parameter, which represent the thickness of the electrical double layer (see Fig. 1B) that depend on the ionic strength of the medium and on the temperature of the sample, being $1/k$:

$$1/k = \sqrt{\frac{\epsilon_0 \cdot \varepsilon \cdot k_b \cdot T}{2000 \cdot e^2 \cdot I \cdot N}} \quad (2),$$

where k_b is the Boltzmann constant, T the temperature of the sample, e the electronic charge constant, N the Avogadro number and I the ionic strength of the media.

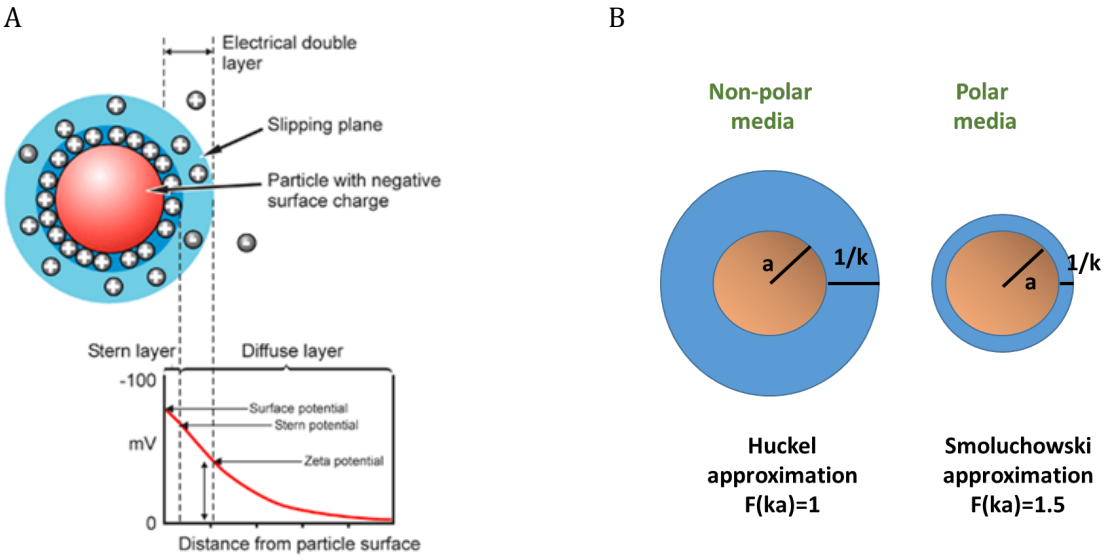

***Figure 1*: (A) Schematic representation of zeta potential definition and (B) of the approximation of Henry function in different media.**

For NPs in a polar media (e.g. NaCl >10 mM) $f(k \cdot \alpha)$ is approximate to its maximum value, $f(k \cdot \alpha) = 1.5$, which is known as the Smoluchowski approximation (Fig. 1). On the other hand, for non-polar media $f(k \cdot \alpha)$ can be approximated to its minimum value, $f(k \cdot \alpha) = 1$ (Hukel approximation). The literature indicate intermediate values for $f(k \cdot \alpha)$ in intermediate conditions [3]. However, in most water based media relevant for biological application the value 1.5 is the most appropriate.

During a zeta potential measurement, the electrophoretic mobility is quantifying by measuring the small frequency shift of the light of a coherent laser source (laser) scattered by the charged NPs that are moving in an external electric field. The shift in frequency is measured by a Doppler interferometer. The Zetasizer Nano from Malvern uses the phase analysis light scattering (PALS) technique, which consist in the sequence of two different mobility measurements [4]. First, the fast field reversal (FFR) mode is applied. It consists in quickly reversing the external electric field, allowing to measure the true particle mobility without the interference of electroosmotic phenomena. In this way an average value of the zeta potential is calculated. Then, the slow field reversal (SFR) mode is applied, which consists in slowly reversing the field, reducing electrode polarization. It allows calculating a distribution of zeta potential values.

3 Applicability and Limitations

3.1 Nanoparticle concentration

NP concentration should be maintained in a range that allows to obtain a high signal to noise ratio, avoiding multiple scattering interferences (see section 6). If the NP dispersion is too concentrated, although a movement can be detected and measured, it may provide interpretation issues while converting electrophoretic mobility to zeta potential values due to multiple scattering phenomena and to the limitation of NP mobility (no Brownian motion) [3].

3.2 Nanoparticle size

Zeta potential measurements are relevant only in samples with size the sub-5µm region [3]. If sedimentation or significant aggregation of the sample occurs before or during the measurement, the system is not suitable for zeta potential measurements, since NP electrophoretic mobility is strongly compromised. The lower limit for the measurement of electrophoretic is determined by the signal to noise ratio, which is a complex function of size, concentration and refractive index of the NP dispersion. Therefore is impossible to give an unambiguous statement of the lower size measurable [3].

3.3 Liquid medium

A minimum level of conductivity of the medium is required in the solution, so that an electric field can be applied in the cell without inducing electrode polarization, which causes voltage irregularities. For this reason ISO and ASTM standard guides for zeta potential measurements [1,3] suggest 10 mM NaCl or similar media (e.g. PBS 0.1 x or PB 10 mM).

Mobility or zeta potential measurements should not be performed in deionized water due to its low conductivity. On the other hand, measurements in biological relevant media, such as PBS or cellular culture media, are critical due to their very high ionic strength. The very high ionic conductivity during the measurement increase the joule heating, often inducing the blackening of the electrode and/or sample degradation. Measurements in these conditions are possible, taking some precautions. First, the voltage should be manually reduced to improve the quality of the measurements. Second, the specific mode for measuring in very high ionic strength media provided by Malvern software, known as the Monomodal mode, should be used instead of the General Purpose mode.

Measure of zeta potential should be limited to aqueous system. In non-polar dispersant (e.g. organic solvents) the Henry function cannot be applied. A complex understanding of the position and the thickness of the double layer is needed to model these particular cases. However organic solvents are not relevant for nanoparticles intended for medical purposes. Therefore, they won't be considered in this protocol.

3.4 Measurement of pH and conductivity

As shown in equation (1), zeta potential is a function of the particulate system as a whole (NPs + environment) and varies with the characteristics of the dispersive medium. Zeta potential values for a specific sample strongly depend on the pH and on the conductivity of the dispersive medium [3]. Therefore, pH and conductivity values should always be measured (see specific

SOPs) and reported in association with each zeta potential measurement. Zeta potential values, without the description of the detailed SOPs used and/or without reporting pH, ionic composition and electrolyte concentration of the medium, are to be considered meaningless.

3.5 Temperature control

To applied Henry equation, the knowledge of the viscosity and of the temperature of the medium are required. Therefore, during the measurement the temperature of the cuvette needs to be stable. For this reason, an equilibration step of the temperature of the cuvette is introduced prior to the measurement.

4 Equipment and Reagents

4.1 Equipment

- ZetaSizer Nano ZS or similar equipment (Malvern Instruments)
- Folded capillary cell (referred to as zeta cells, polycarbonate cell with gold-plated
- electrodes; Malvern Instruments, DTS1060C or DTS1017)
- Caps for zeta cells (2 per cell)

4.2 Reagents

- Zeta Potential Negative Transfer Standard (Carboxylated polystyrene latex dispersed in pH 9.22 buffer; Malvern distributors, reference DTS1235)
- NIST-SRM 1980 - Positive Electrophoretic (+μE) Mobility Standard (NIST site or from lgcstandards)
- 10 mM Phospate buffer (PB) at pH 7.4 or 10 mM NaCl are the highly recommended dispersants. Other buffers, such as 0.1x PBS at pH 7.4, can be used but the condition of measurement should be checked and varied accordingly to their ionic strength.

To choose the right dispersant, it is suggested to check NPs size in the condition used to measure zeta. It is important to exclude buffers which induce NPs aggregation effects. A buffer which allow control of pH at 7.4 (e.g. PB 10 mM at pH 7.4) is highly suggested, if NPs stability is maintained.

All reagents should be made using de-ionized (18.2 MΩcm, 273K) extracted by a high specification laboratory deionizer and filtered to 0.22 um membrane prior to their use.

4.3 Preparation of the standards

The negative transfer standard from Malvern does not need any preparation step prior to the measurement. It can be directly loaded into the cuvette as indicated in the next section. The pH of the standard should be 9.22. Not to be used after the expiration date.

NIST-SRM1980 positive standard can be tricky to handle. It should be prepared as indicated by NIST guidelines [5]:

- “Shake the SRM bottle vigorously for 1 minute with wrist action, then
- transfer a 10 mL aliquot by pipette to a 100 mL volumetric flask and

- make to volume with deionized water.
- Mix the resulting suspension ("the sample") thoroughly and
- transfer to a polyethylene (non-glass) bottle.
- Ultrasonicate for 1 minute at 40 W.
- After cooling to 20 C to 25 C, measure the sample pH. The pH should fall within the range of 3.5;
- if not, adjust the value using 0.1 mol/L nitric acid or 0.1 mol/L sodium hydroxide. Shake the sample mildly with wrist action before each analysis."

4.4 Preparation of the sample

For routinely analysis disperse the sample in the chosen buffer (e.g. 10 mM PB, 10 mM NaCl or 0.1 PBS could be good choice, depending on NPs stability) and measure of zeta values at 25°C. To select the right buffer, always perform size analysis of the NPs in the chosen buffer before performing zeta potential measurement. NPs should be stable in the chosen buffer and no aggregation phenomena should be induced.

Ionic conductivity of the buffer should be below 5 mS/cm. Dispersants with higher conductivity may be used, but changes in the reported SOPs are needed.

NPs concentration to obtain optimal measurements conditions is highly sample dependent. Metallic nanoparticles scatter light more strongly than hydrocarbon-based 'soft' nanoparticles such as liposomes, lipid nanoparticles and polymers. The optimum sample concentration is determined by the nature (light scattering properties) of the analyte NPs, and it has to be defined case by case. Sample concentration with allow to reach an attenuator factor of 6-7 are highly recommended. Always first check NPs size at the concentration chosen for zeta potential measurements to check NPs are well dispersed and stable in those conditions.

4.5 Preliminary analysis of the sample

Prior to the zeta measurement perform the preliminary analysis, shown in Fig. 2:

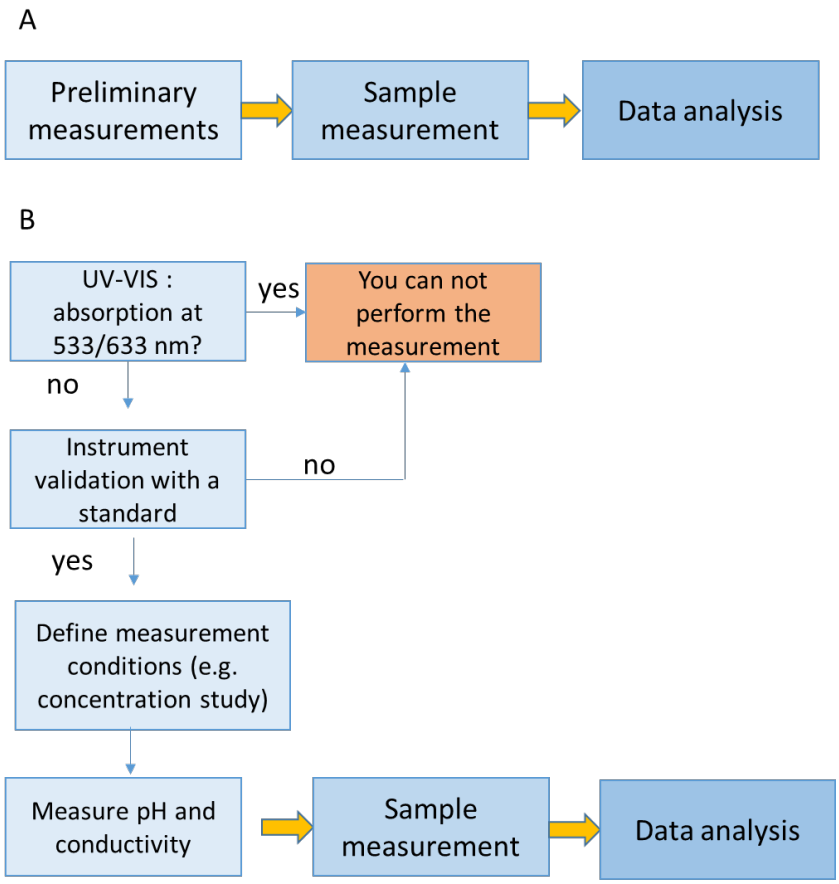

***Figure 2*: (A) Schematic representation of zeta potential measurement (B) and the details flow of the preliminary analysis.**

- The right dispersant for zeta potential measurement should be chosen. The dispersant should be selected according to a few criteria. First, NPs should be stable in the dispersant chosen (no aggregation effects). This has to be checked by DLS analysis prior to zeta potential measurements. Second, it would be advisable to use a buffer that would allow to maintain pH in the physiological range. For this reason 10 mM PB or 0.1x PBS at pH 7.4 may be the first choices to check. As an alternative if NPs are not stable in these two buffers, 10 mM NaCl can be used. Avoid buffers with high ion conductivity (20 mS/cm or above).
- NPs concentration for zeta potential measurements should be selected. A preliminary concentration study is suggested before the first analysis of unknown samples. To check if the dilution is in the right concentration during the measurement, be sure that the average counting rate and the attenuation factor are within the manufacture specifications (values of 6-7 are suggested).
- An UV-VIS spectra of the dispersion should be recorded between 400 and 800 nm in the conditions used to measure zeta potential to check the non-absorbance of the dispersion in the laser wavelength of the Malvern instrument (usually 633 nm).

- Instrument performances should be validated by measuring electrophoretic mobility and/or zeta potential value of a standard prior to start a new measurement session (section 6.1).
- pH and conductivity should be measured and reported along with the zeta potential measurement (in the same conditions). Zeta potential measurements are strongly affected by small quantities of ions or organic impurities contained in the dispersion. Therefore, contamination of the solution due to the contact with electrodes to measure pH and conductivity is to be avoided. For this reason, it would be advisable to measure the pH and conductivity on a different aliquot of the same dispersion (see specific SOPs). pH and conductivity values should be measured at the same temperature selected for the zeta measurements (usually 25°C).

5 Procedure

Follow the procedure reported in this session (Fig. 3), first to perform the instrument validation by measuring the standard of choice and then to measure the zeta potential of the sample (e.g. preliminary concentration study and final zeta potential analysis). For the criteria to follow for instrument validation, for the acceptance criteria for zeta measurements and for data analysis see section 6.

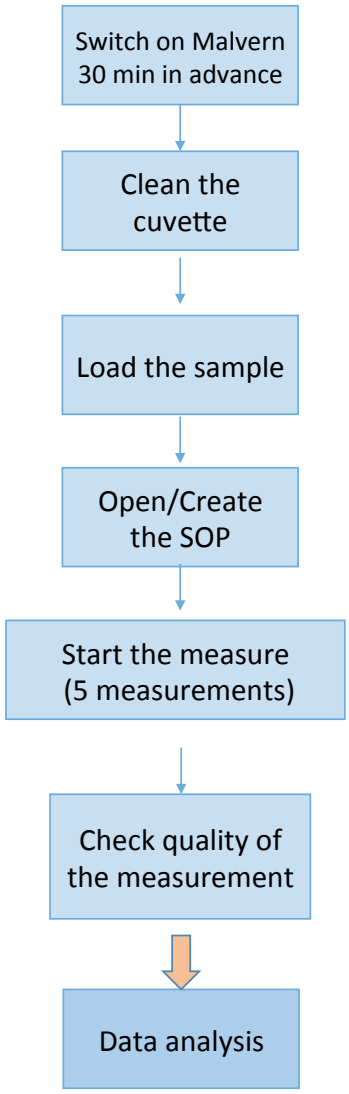

***Figure 3*: Flow chart of measurement procedure.**

5.1 Cleaning of zeta cells

Use a new disposable zeta cell for each measurement. Clean the new zeta cell according to the procedure recommended by US-NCL [2].

- Filter de-ionized water and ethanol with 0.2 um pore size membrane filters.
- Rinse the zeta cells with filtered water, followed by filtered ethanol, and finally by water again. It is recommended to flush a minimum of 1.0 mL of each rinsing solvent through each port to thoroughly rinse each electrode. This can be achieved by using a 1 mL disposable syringe. During the last washing with water is recommended to flush a minimum volume of 4 mL.
- After the zeta cells are rinsed, visually check the electrodes both inside and outside of the cell and the measuring window for any manufacturing defects such as scratches on or non-transparent measuring windows, dirty or non-homogenous surface coating of electrodes (electrodes are gold-plated), or any residual polycarbonate (from the

manufacturing process) in the cell or on the electrodes. New zeta cells should not be used if any of these defects are found.

- The zeta cells, after rinsing, should be dried using a gentle stream of nitrogen attached to a filter to remove any remaining solvent and physically re-examined as above. If you do not have a filter stream of nitrogen, just flux a 1 mL of the filtered dispersed media you are going to use during the measurement (e.g; 10 mM NaCl). Do not use un-filtered stream of nitrogen/complex air, which can introduce some dust into the cell.

5.2 Measure procedure:

- As first step, remember to switch on the instrument 30 minutes before starting the measurement to warm up the laser.
- Load the zeta cell following the procedure reported below.
    - Connect a syringe of 1 mL pre-loaded with the sample to a port of the cell.
    - Orient the cell upside-down (ports oriented down) and then
    - slowly inject the sample reaching half of the loop formed at the bottom of the cell, checking that no bubbles are formed into the cell.
    - Return the cell in the vertical position (ports up) and
    - continue to inject the sample form the syringe.
    - Fill to the maximum level of the cell (total volume 0.8 mL or 0.75 mL depending on the cell). In DTS1070 cells the maximum level is marked by a "MAX FILL line sign". DTS1060 should be filled completely.
    - Then check that the electrodes are completely immersed in the liquid and there are no bubbles in the cell.
    - Remove the syringe and
    - cap the filled cells with its two caps.
    - Insert the cell into the instrument according to the instruction of the manufacture. If using DTS 10701 cells Malvern logo should be oriented towards the front of the instrument. If using DST1060 cells the narrower part of the cell defined by a weld line should face the front side of the instrument.

NIST-1980 positive standard is known to be tricky to handle. It will adhere of the surfaces and it is difficult to clean it from the metallic surfaces. Follow NIST suggestion to pre-conditionate the electrophoresis cell with the sample for 1 minute [5]. Then, you can introduce a fresh sample of the standard for analysis and allow equilibrating at least for 10 s to 15 s, prior to the measurements. Never re-use the cell where you have loaded the positive standard, especially if for samples of other nature.

- Open a new measurement file in the Malvern software by opening File->new->measurement file, then select the zeta potential option
- Create the SOP using the software from File->new->SOP->zeta potential or open an existing one from File->Open->SOP->zeta potential to select an existing SOP file.

  For routinely analysis disperse the sample in 10 mM NaCl and measure of zeta values at at 25°C. For 10mM NaCl and for Malvern negative standard, follow the SOPs described in table 1 and reported step by step in this session.

Table 1: SOP for zeta measurement with 10 mM NaCl and for Malvern DTS1235

Parameter	Value
Equilibration time	>300s (1°C/min)
Number of measurements	5
Number of runs	Automatic. For some samples it may be necessary to increase the number of runs to a fixed value (20-40).
Delay between measurements	300 s
Volume inside the cuvette	According to the manufacture
Model for F(ka) selection	Smoluchowski (1.5)
Automatic attenuation selection	yes
Automatic voltage selection	yes (around 150 V), if sample degrade switch to manual mode and start at lower voltage (80 V)
Analysis mode (Malvern)	General Purpose/Auto Mode

Always perform a measure of size by DLS, first before, and then after the measurements of zeta potential.

To create a new SOP for zeta potential measurements follow these steps:

1. Select zeta potential from measuring type in "instrument configuration".
2. Indicate the physical-chemical properties of the sample and of the medium in "material" and "dispersant" session of the sample description. For the sample you must indicate the refractive index and the absorption at the laser wavelength of your instrument (633 or 532 nm). For the dispersant you must indicate the viscosity (at the T of the measurement), the refractive index and the dielectric constant, which would allow to calculate zeta potential from the electrophoretic mobility by Henry equation. The physical properties for 10 mM NaCl and for the medium of Malvern DTS1235 are reported in table 2. Instrument validation by NIST-RSM1980 positive electrophoretic standard is performed by measuring electrophoretic mobility and not zeta potential values (see instrument validation session). Therefore, viscosity, refractive index and dielectric constant of the dispersive media are not needed for this measurement [5].

Table 2: properties of 10 mM NaCl and of the Malvern DTS1235 standard dispersive media

Aqueous medium	Absolute viscosity (mPa s)			Diectric constant	Refractive index
10 mM NaCl	20°C	25°C	37°C	78.6	1,332
	1.003	0.891	0.693		
Malvern DTS1235	Na	0.8872	Na	78.5	1,330

Na= not available

3. Select the model to be used to calculate zeta potential from the electrophoretic mobility of the sample in "sample/general option". For samples dispersed in 10 mM NaCl and for Malvern negative standard the Smoluchowski approximation should be applied (f(k·α)=1.5).

4. Select the temperature of your measurement and the time for temperature equilibration in "sample/temperature". For routinely analysis T should be set at 25°C. Equilibration time prior to the measurement should be set at least at 300s. If the temperature of the sample differ ± 5°C from the temperature of the measurement (e.g. from RT to 37°C), leave the sample to equilibrate for more time (rate of 1°C/min).
5. Indicate the type of cuvette you are using in "sample/cell".
6. Set the number of measurements, runs for each measurement, and delays between measurements in the "Measurement" session of the SOP. Perform at least 5 measurement for each sample and let the instrument to define automatically the number and duration of the runs for each measurement. For critical samples (e.g. neutral ones) it would be necessary to increase the number of runs to a fixed value (e.g. 20-30) to get good frequency and phase plots. Bear in mind that increasing the number of runs you are prolonging the NPs contact with currents. The more current passes thought the particles, the more heating the will experience due to joule effects. A compromise should be found in this case, choosing a number of runs that allow to obtain good quality data, without degrading the sample.
7. Delay between each measurement should be set to "300s". During the measurement, sample heating due to the current flowing in the cells increase the temperature of the sample near the electrodes, inducing bubble formation, sample degradation and increasing conductivity of the solution. Sample heating due to joule effects may cause significant increase of zeta potential within the measurements and favors sample degradation. A delay time helps to reduce sample heating, allowing sample to recover 25°C between two measurements. This will reduce degradation of critical samples and avoid increase of zeta potential with sequential measurements.
8. The measurement should be performed in automatic mode. When creating the SOPs, select the automatic attenuation option and the automatic voltage selection in the "measurement/advance" session of the SOP.
9. Data analysis should be performed using the General Purpose mode. You have two choices: either to select the automode, which will allow the software to select the measurement mode according to the conductivity of the sample or to directly select the general purpose mode in "data processing" section of the SOP. In automode the software will select the general purpose if conductivity <5 mS cm-1 or the Momomodal mode if conductivity is >5 mS cm-1. Since for 10 mM NaCl a conductivity of 1 mS cm-1 is expected the instrument should automatically select the "General purpose mode" which would allow to measure both an average numerical value and a distribution of zeta potential. For samples characterized by very high conductivity (>5 mS cm-1), e.g. PBS, the Monomodal mode should be selected instead of the General Purpose mode. Note that in the Monomodal mode no zeta potential distribution is given, but only an average value is calculated. To avoid electrode degradation and heating due to the joule effect, in high conductivity samples the voltage should be reduced manually to very low values (start by using 10-20V).

- Start the measurement. A new window opens. Click on "Start" (large green triangle), at which point the green light will start flashing. The black bar at the bottom of the SOP

window contains information on the progress of the experiment. At the end of the experiment, the instrument beeps three times.

- Perform data analysis as indicated in the next section.

Precautions to avoid sample degradation. During each run, always check the phase and the frequency plot, as indicated in the quality criteria section. If during the measurement you see changes in the phase plot or in the frequency plot, it may indicate that sample degradation is occurring.

Sample degradation can be check by measuring the size before and after the zeta measurement to see if there was a change in NP size distribution.

If sample degradation occurs, you may reduce the number of runs, or manually reduce the voltage. A compromise should be found to get good frequency and phase plots, without degrading the sample. The optimal solution to select will be case by case dependent. A suggestion is to start by using the automatic mode with delay time of 300s between each measurement. If sample degradation occurs, then proceed either by reducing the numbers of run (if less runs are enough to get good data) or by reducing the voltage (if reducing the number of runs is not possible).

If automatically selected, the value of the voltage should be around 150V. To avoid sample degradation, in specific cases voltage can be manually reduced to 80V. For delicate samples a preliminary study changing the voltage from 80V to 150V can be performed to find the best measurement conditions (stable phase and frequency plots during the whole measurement) to avoid sample degradation.

After the best measurement conditions have been defined repeat the measurement in the right conditions as indicated above. Each sample can be measured only one time, performing at least 5 measurements.

5.3 Data report

The steps for reporting data are summarized in Fig. 4.

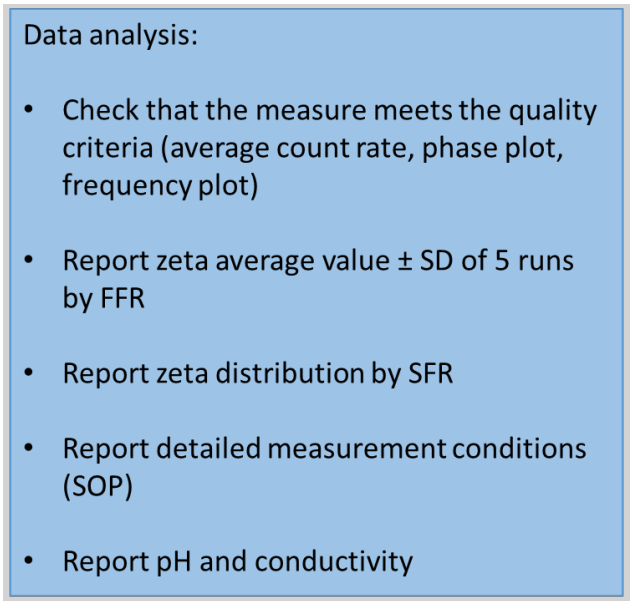

Figure 4: Brief outline of the data analysis.

For each measurement, the average zeta potential value and the standard deviation of the 5 runs should be calculated and reported, as in table 3.

Always describe the specific SOPs used for the measurement (number of runs, approximation of Henry function used, mode utilized for data acquisition and analysis). Always report the pH of the sample, the temperature of the measurement, the conductivity measured by Malvern instrument and the conductivity measured by an external conductivity meter.

If measuring in general purpose, the zeta distribution plot derived by SFR mode should also be reported.

Table 3: Reporting data

Sample	Zeta potential	Media	T	pH	Conductivity	Att. Fact.	Analysis mode	Aprox. of Henry equation
Malvern DTS1235								
Zeta potential distribution								

6 Quality Control, Quality Assurance, Acceptance Criteria

6.1 Instrument verification

Zeta potential measurements are based on first principles and hence no calibration is required. However, the instrument should be verified routinely by running an appropriate quality control standard. For this reason is recommended to run a commercial standard along with the samples. It would be advisable to run at least one standard at the beginning of each measurement session.

A suitable negative standard is available from Malvern Instruments (Zeta Potential Transfer Standard, DTS1235). It is characterized by a zeta potential value of -42 ± 4.2 mV at 25°C (pH=9.2). It is recommended to use it when measuring negative-charged samples. If the average zeta potential value measured after 5 runs is within the 10% of the expected value, then the instrument performances are successfully verified.

A suitable positive charged standard is the NIST-SRM1980. It is recommended for instrument verification when measuring positive-charged samples. After 5 consecutive runs at 25°C, the average value of the positive electrophoretic mobility measured should be (2.53 ±0.12) μm·cm/V·s. If the average value measured is within this range, then the instrument capability is successfully validated [5].

The physical properties and the temperature validity range of these two standards are summarized in table 4.

Table 4: physical data of the standards

Property	NIST SRM1980	Malvern DTS1235
Electrophoretic mobility	2.53 ±0.12 µm·cm/V·s	Na
Zeta potential	Na	42±4.2 mV
Temperature range	20-25°C	25°C

Na= not available

6.2 Quality criteria to be checked during the measurement

Sample concentration. Check that the scattering intensity is within the acceptable range. For unknown samples, a preliminary concentration dependent study is advisable. The mean counting rate of scattering light reported during the measurement should ideally be between 100 and 500 k counts per seconds (kcps). The instrument attenuator value should be between 4 and 9.

Count rate plot. It reports the number of photons reported per second. The count rate plot should be stable over time (Fig. 3), and can be used to check dispersion stability during the measurement. If the dispersion is not stable, sample aggregation is possibly occurring. Instability may also be caused by the creation of bubbles by electrolysis, especially in dispersants with an elevated salt concentration.

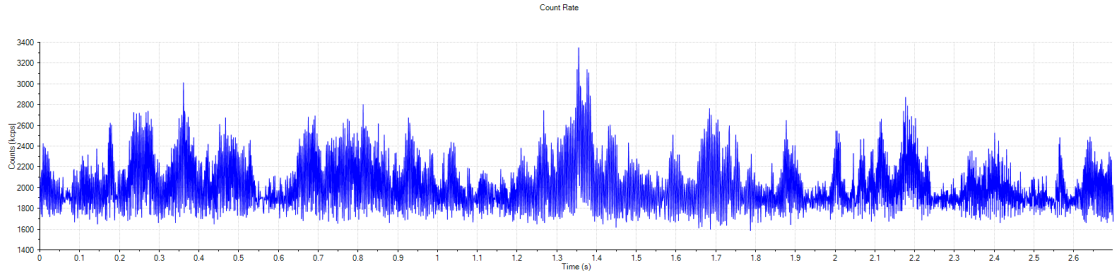

Figure 3: Count rate plot during the measurement of Malvern standard DTS1235

Phase plot. It shows the difference in phase between the measured beat frequency and a reference frequency as a function of time. In general purpose mode, the phase plot should be well defined in each run. The first part of the graph should presents small alternating slopes with time which corresponds to the application of the FFR mode used for the determination of the mean zeta value. Then either by positive or by a negative peak should follow, which correspond to the SFR mode analysis for the determination of the zeta potential distribution. An example of a good defined phase plot is showed in Fig. 4. The peak should be well defined and without noise.

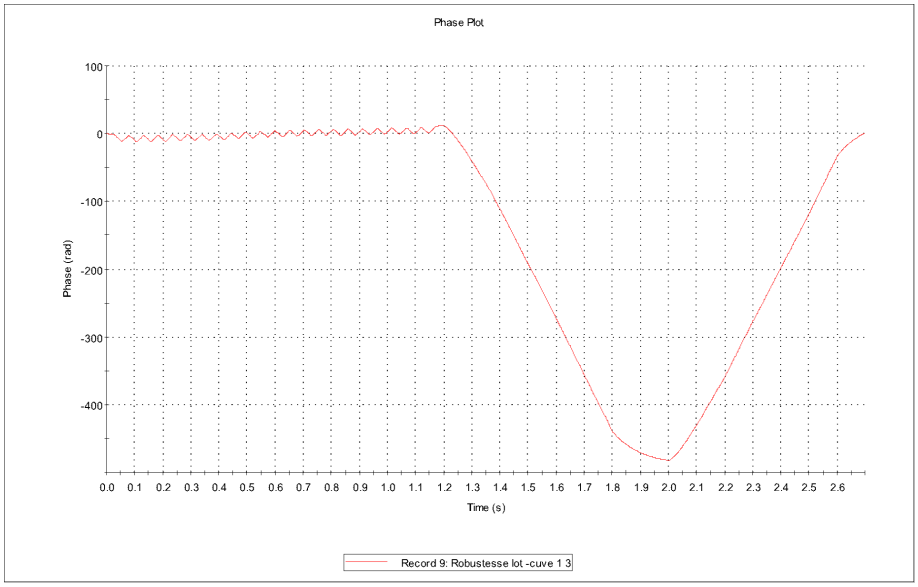

Figure 4: Phase plot of Malvern standard DTS1235

Frequency plot. In SFR mode frequency plot is used to calculate the electrophoretic mobility distribution and, thus, the zeta potential distribution. An example of a frequency plot is reported in Fig. 5. The baseline should be smooth, the peak should be sharp.

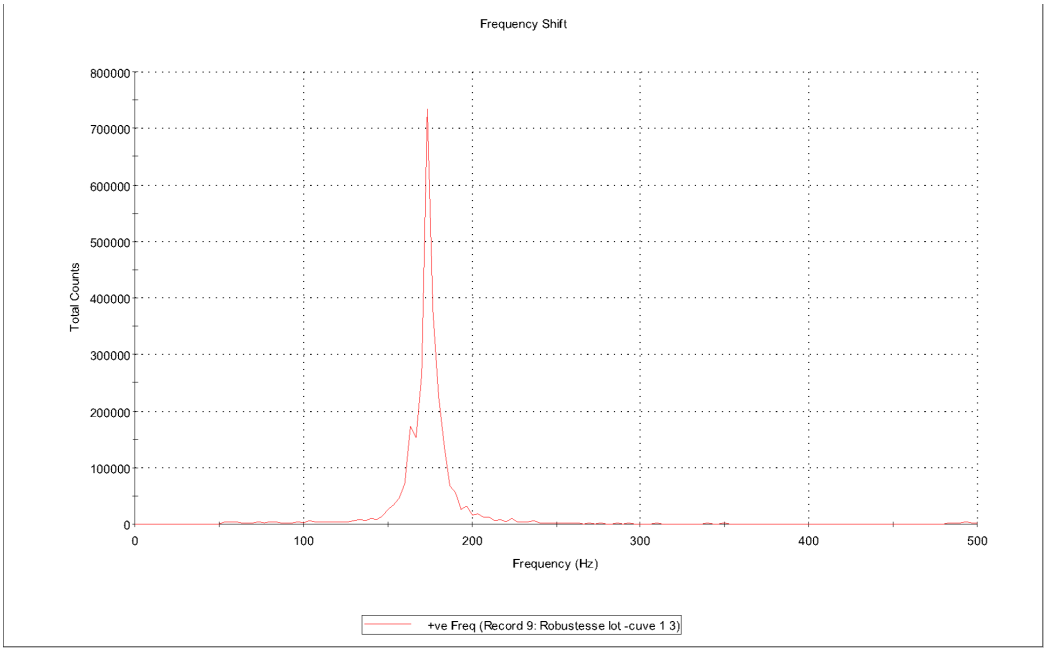

Figure 5: Frequency plot of Malvern standard DTS1235

Conductivity. Check that the conductivity values reported by the instrument to see if they are in line with the one expected/measured for your samples. The conductivity of NaCl 10mM should be around 1 mS cm^{-1}. Samples with a conductivity > 5 mS cm^{-1} should be measured and analyzed with the Monomodal mode. The higher limit of conductivity accepted in Malvern ZetaSizer is 200 mS/cm^{-1}.

6.3 Acceptance criteria for comparison of zeta values

A few round robin reported in literature have demonstrated that zeta potential measurement are very sensitive to small number of impurities, to minor changes in experimental procedures and to many hard-to-define parameters [3,6]. According to the ASTM and to the ISO standard guidelines [1,3], a maximum variation of 10% relative standard deviation in the reported zeta potential numerical value is acceptable when comparing data measured in the same conditions, if at least 5 measurements are compared. If the measurement is performed in General purpose Mode, in addition to the statistical comparison of numerical values, a qualitative comparison of zeta potential distributions is advisable.

7 Health and Safety Warnings, Cautions and Waste Treatment

Samples should be prepared in a biological safety hood to protect the sample from particulate and to minimize exposure; appropriate safety precautions and protective gear such as gloves, lab coat and goggles must be worn. After the measurement the samples should be discharged as appropriate for nanomaterials.

8 Abbreviations

FFR: fast field revers

NP: nanoparticles

PALS: phase analysis light scattering

SFR: slow field revers

9 References

[1] ISO 13099-2:2012, Colloidal system- Methods for zeta potential determination-part 2: Optical methods, June 2012.

[2] NCL method PCC-2, Measuring Zeta Potential of Nanoparticles, April 2008, revised November 2009.

[3] ASTM E2865-12, Standard Guide for Measurement of Electrophoretic Mobility and Zeta Potential of Nanosized Biological Materials, ASTM International, West Conshohocken, PA, 2012, www.astm.org.

[4] Malvern technical note: Measuring zeta potential using phase analysis light scattering (PALS). http://www.malvern.com/en/support/resource-center/technical-notes/TN101104PhaseAnalysisLightScattering.aspx.

[5] Certificate of SRM 1980 - Positive Electrophoretic (+μE) Mobility Standard, https://www-s.nist.gov/srmors/view_detail.cfm?srm=1980.

[6] G. Roebben, S. Ramirez-Garcia, V.A. Hackey, M. Roesslein, F. Klaessig, V. Kestens *et al.*, *J Nanopart Res*, 2011, *13*, 2687.

10 Annex

11 Related Documents

Document ID	Document Title
	Malvern DTS1235 Certificate
	NIST1980 certificate
EUNCL-PCC-001	*Measuring Batch Mode DLS*
EUNCL-PCC-013	*Measuring the pH of Nanoparticle Suspensions*