# Consolidated pre-validated guidance document on zeta potential determination

## DELIVERABLE 4.5

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## Abstract

The objective of Task 4.1 of RiskGONE project is to provide guidance documents for the techniques used to characterize the physicochemical properties of engineered nanomaterials (ENMs), that will be later available to regulatory agencies, such as OECD. In this way, the goal of this deliverable is to provide a consolidated pre-validated guidance document on zeta potential determination by dynamic light scattering (DLS) of the applied ENMs in the RiskGONE project. Round Robin (RR) exercises were organized with task partners to demonstrate the validity and reproducibility of the proposed guidance document.

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# List of abbreviations

DMEM - Dulbecco's Modified Eagle Medium

DLS - dynamic light scattering

ENMs - engineered nanomaterials

FBS - fetal bovine serum

OECD - Organization for Economic Co-operation and Development

PBS – phosphate buffered saline

RR – Round Robin

# 1. Technical & Scientifical progress

## 1.1 Introduction

When a material is in contact with a liquid acquires an electronic charge on its surface. Zeta potential (ζ –potential) is the property that defines the charge of the material surface. This property can be used to predict and control the stability of materials in suspension or emulsion (Jiang, 2008). It can be experimentally determined, and it is related to the electrostatic repulsion between the particles in suspension. The zeta potential has proven to be extremely relevant for the study and control of colloidal stability and flocculation processes (Dixit, 2017).

**Table 1: Stability behavior of the colloid based on zeta potential value.**

| Z potential value (mV) | Stability behaviour |
|---|---|
| **0 to ±5** | Flocculation or coagulation |
| **±10 to ±30** | Incipient instability |
| **±30 to ±40** | Moderate stability |
| **±40 to ±60** | Good stability |
| **Greater than ±60** | Excellent stability |

## 1.2 Principles of the method

The zeta-potential is an important parameter associated with the surface functionality and/or with the stability of dispersed particles. The main focus of zeta potential analysis is obtaining information on the surface charge of a material. Electrostatic repulsion between particles depends on the value of zeta potential. The higher the zeta potential, the stronger the repulsion, the more stable the system becomes

The surface of a charged particle attracts a thin layer of opposite charged ions, these ions are strongly bound, and this thin layer is named the Stern layer. When the particle diffuses in solution an outer diffuse layer, where ions are less firmly associated, appear creating an electrical double layer. The composition of this diffuse layer is dynamic and varies depending on a variety of factors e.g., pH, ionic strength, concentration, etc.

When an electric field is applied to such dispersion, the charged particles move towards the opposite electrode. At the edge of the diffuse layer there is a hypothetical plane (slipping plane) which acts as the interface between the moving particles and the layer of surrounding liquid. The Zeta potential can be defined as the voltage at this particle-fluid interface.

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Zeta-potential values are typically in the range of +100 to −100 mV but the dividing line between stable and unstable suspensions is generally taken as +30 or -30 mV, particles having zeta potentials outside of these limits normally considered stable.

As result of the electric field application, the charged particles move with a velocity V, determined from the frequency shift. The application of this external voltage induces that the positive charged particles move to the cathode, whereas the negative particles move to the anode. An optical detection system, as an electrophoretic light scattering system, is frequently used to detect the particles movement. The collected signal of the particles' movement is shifted to higher or lower frequencies depending on their charge. The obtained frequencies are then mathematically converted to electrophoretic mobilities, velocities, and finally zeta potentials.

The obtained velocity (V) is the product of electrophoretic mobility (μ) and the applied electrical field (E):

$$V = \mu \times E$$

Henry's equation relates the electrophoretic mobility (μ) to zeta potential through Henry's function, the dielectric constant of the dispersant and the sample viscosity:

$$\frac{U}{E} = \frac{2\varepsilon\zeta F(\kappa a)}{3\eta}$$

where, **U/E** is the electrophoretic mobility ($m^2\ s^{-1}\ V^{-1}$), **$\zeta$** is the zeta-potential (V), **$\varepsilon$** is the solvent dielectric permittivity (or constant) ($kg\ m\ V^{-2}\ s^{-2}$), **$\eta$** is the viscosity ($kg\ m^{-1}\ s^{-1}$), and **F**(*κa*) is Henry's function (dimensionless).

For a low F(κa), particle size small compared to the Debye length (1/κ), the Henry-function approaches the value to 1 (Hückel approximation, non-polar systems) (Hückel, 1924). For large F(κa), particle size large compared to the Debye length, it approaches to 1.5 (Smoluchowski approximation, ionic media as water) (Somoluchowski, 1921). Smoluchowski and Hükel approximations are usually used for the calculation of zeta potentials of dispersed colloidal systems containing spherical particles. This poses no problems for larger particles (*large* F(κa) *value)* because the electrophoretic mobility becomes independent of shape, but shape may be important when small particles are considered (low F(κa)).

Many particle properties (*e.g.,* charge density) and solution properties (*e.g.,* ionic strength and composition) affect extremely to the Z-potential measurement, some particles can change the surface charge (becoming either positive or negative) when they are dissolved in a desired media. The pH of the media used to disperse the particles very often determine the particle´s charge. At low pH, surface hydroxyl groups can become protonated and positively charged, whereas at high pH these sites are negatively charged. The isoelectric point is the value of pH at which a molecule is neutral, perform a Z-potential measurement at this pH will generate uncertainty in the measured zeta-potential because particles tend to aggregate when they are uncharged. The presence of both monovalent (*e.g.,* $Na^+$) and

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polyvalent ions (*e.g.,* $Ca^{2+}$) also affects the double layer and alters the electrophoretic mobility and consequently the calculated Z-potential.

## 1.3 Applicability and limitations

The determination of Z potential and hydrodynamic diameter by DLS systems is limited to spherical particles in the size range from 0.3 nm to 10 µm, depending on the available instrument. Very small sized particles have high mobility and low light scattering properties and very big particles can precipitate easily. Non-spherical and high aspect ratio micro or nanoparticles are not easily measured because the evaluation of the data is based on a spherical approach.

Micro- and nanomaterials must be dispersed in a media prior to the measurement. The sample concentration should be adjusted, and the scattering of the sample should be stronger than the solvent scattering.

A high particle concentration will cause multiple scattering and particle-particle interactions that can affect the diffusion coefficient, hindering the data analysis. On the other hand, too low concentration will induce weak scattering of the light and decrease measurement efficiency. This will invalidate the autocorrelation function due to fluctuations on the particle number. Thus, autocorrelation function should be measured using as much as possible diluted dispersions.

Particles movement can be also affected by the absorption of the incident light, due to local heating of the sample. It is always recommended to record a UV-vis spectra of the sample previously to Z potential measurement. In case that the sample absorbs the wavelength from the incident light, a different technique should be used (Xu, 2002) (Stetefeld J, 2016).

The sample pH can also affect the Z potential measurement. Also, high conductivity samples and the presence of organic compounds (in order to help sample dispersion, as detergents) can negatively affect the Z potential measurement.
Some sample features (Dixit, 2017) in order to guarantee an adequate zeta potential measurement are:

a. Highly monodispersed.
b. Optimum concentration for an effective light scattering (633 nm wavelength).
c. <1 mS/cm (low salt concentration) in conductivity.
d. Well-dispersed in a polar dispersant.

## 1.4 Materials

### 1.4.1 Reagents

- ENM dispersions of RiskGONE project
- Ultrapure resistivity water (resistivity 18 MΩ.cm, for example, Invitrogen) or other dispersant of your choice, such as DMEM + 10% vol/vol FBS, PBS, etc.

### 1.4.2 Materials and Equipment

- ZetaSizer Nano ZS (Malvern Instruments) or similar equipment

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- Disposable cells (disposable Z potential polycarbonate cell with gold-plated electrodes; Malvern Instruments, DTS1060C or DTS1070)
- Pipette (to transfer variable volumes - from 100 µL to 1 mL).
- Laboratory vortex mixer, with speed range 300-3500 rpm, touch mode

## 1.5 Procedure

1. Turn on the equipment 30 minutes before use to warm up the laser and start the software.
2. Preparation of the sample:
   -From stock solution, disperse the sample in ultrapure water at 100 µg/mL, vortex for 30 seconds.
3. Clean the disposable cell three times with ultrapure water and then store in a dust-free environment prior to use. After rinsing, it should be dried using a gentle stream of nitrogen to remove any remaining solvent. Measuring window of the zeta cells and the electrodes should not be touched. Use of gloves is highly recommended to avoid any residual oil deposits.
4. Loading Sample into Zeta Cells, the zeta cell can accommodate a minimum of 750 µL of sample using a 1000 µL pipette to one of the ports on the zeta cell. Fill the zeta cell with sample avoiding bubbles during the filling.
5. Introduce the setting described in Table 2:

**Table 2: Settings for Z potential measurements using a ZetaSizer Nano ZS (Malvern Instruments).**

| Parameter | Value |
|---|---|
| **Equilibration time** | 120 s (1°C/min) |
| **Scattering angle** | 173° |
| **Laser wavelength** | 633 nm |
| **Number of measurements** | 10 |
| **Number of runs** | Automatic |
| **Run duration** | Automatic |
| **Delay between measurements** | 0 s |
| **Positioning method** | Seek for optimum position |
| **Extend duration for large particles** | No |
| **Automatic attenuation selection** | Yes |
| **Analysis mode** | General Purpose |

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6. Data report
    a. Check if the measurement meets quality criteria.
        i. The count rate is useful for monitoring the sample quality. The scattering intensity should be within the acceptable range. The mean counting rate of scattering light reported during the measurement should be between 100 and 500 k counts per second (kcps). The count rate plot should be stable over time. Presence of dust, agglomeration or sedimentation cause great variations in the count rate plot. The instrument attenuator should be between 4 and 9.
        ii. Check autocorrelation function. The value of the intercept should be around 1 (>0.9) (Calzolai, 2016).

### 1.7 Quality control and quality assurance

Although no calibration is required for Z potential measurements, the instrument should be verified by using a quality control standard.

In addition, it is important to check if all the measurements are carried out under operational qualification of the instrument.

### 1.8 Safety warnings

To minimize exposure to the nanomaterial, handle the samples with care. Use appropriate protective gear, such as lab coat, gloves, googles and masks. Further information on handling the nanomaterials and the safe handling of the used equipment is described in materials data sheet and user manuals developed by manufacturers, respectively.

After the measurements, please dispose of the dispersions in a suitable container for nanomaterials.

## 2. Deviations from Description of Action – impact/how you cope with them

No major deviation to report until now.

## 3. Conclusions and next steps

The use of stable nanoparticle dispersions is necessary to correlate the physicochemical properties of nanoparticles with their toxic potential. The criterion for preparing a stable dispersion is to increase the repulsive forces between the particles to suppress such agglomeration or at least to slow it down kinetically. Electrostatic stabilization, i.e. adjusting the pH to increase the surface charge of the particles, can increase the repulsive force between the particles. However, in toxicological tests, the pH has to be limited to a range appropriate for the healthy functioning of cells and test organisms. In this case, the surface charge can alternatively be controlled by the use of a dispersing salt, which can dissociate into multiple charged ions. If a particle is ionic or has highly polar bonds, the multi-

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charged ions can be adsorbed in an aqueous environment, leading to an increase in the surface charge of the particle and the zeta potential. Examples of such salts are those containing polyphosphate, hexametaphosphate, pyrophosphate and polysilicate anions. (Jiang, 2008). Unfortunately, this approach is only effective at low ISs, below 0.1 M, but steric stabilization, achieved by coating the nanoparticles with polymers such as PEG, PEG-NH2, PEG-COOH or proteins, is possible at all IS in solution.

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