A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***Protocol for IEP determination using zeta potential versus pH titration***
Subtitle	The SOP describes the experimental set-up and sample preparation for the experimental evaluation of a new OECD TG on IEP determination using a zeta potential versus pH titration. The following protocol was applied to NANoREG NM-212 and NM-101.
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TG 112 (dissociation constant in water) and TG 108 (complex formation in water), EFPL, UdL

The following SOP describes the experimental set-up and sample preparation for the experimental evaluation of a new OECD TG on IEP determination using a zeta potential versus pH titration. The following protocol was applied to NANoREG NM-212 and NM-101.

Protocol for the determination of TG 112 dissociation constant in water) and TG 108 (complex formation in water)

This method can be employed to identify the IEP and the complex formation (adsorption) with a trace metal of NM powders dispersed in water by ultrasonic dispersion.

A. Equipment

Materials to produce a stock dispersion for the zeta potential versus pH titration

- All equipment required for the NANoREG SOP for probe-sonicator calibration of delivered acoustic power and de-agglomeration efficiency for in vitro and in vivo toxicological testing
- Excel template for the probe-sonicator comparison 'Template for Probe Sonicator Calibration'
- All equipment required for the NANoREG ENPRA dispersion protocol
- DI water
- NaCl
- HCl, 0.1 M
- NaOH, 0.01 M
- pH meter
- Beakers, 30 mL
- Magnetic stirrers and stir bars
- Micropipettes and tips
- Syringe (1 mL) to fill the folded capillary cell
- Folded capillary cell plus caps
- Zeta potential transfer standard

Materials for the measurement of complex formation in water (metal adsorption)

- Potentiostat and polarographic stand
- Mercury capillary drop electrode
- Ag/AgCl reference and glassy carbon auxiliary electrodes
- Glass-jacketed thermostatic cell
- Water recirculating thermostatic bath
- pH meter
- water-saturated N_2/CO_2 (99.999% purity) gas mixtures for the removal of dissolved O_2
- Micropipettes and tips

B. Calibration

Probe-sonicator calibration

- Use the NANoREG SOP for probe-sonicator calibration of delivered acoustic power and de-agglomeration efficiency for in vitro and in vivo toxicological testing
- Employ the Excel template for probe-sonicator comparison 'Template for Probe Sonicator Calibration'

AGNES calibration

- Use the NANoREG SOP for AGNES measurements.

C. Measurement of the zeta potential

Planning of the experiment and practical considerations

- Establish the MNM concentration that will be used and the pH points to measure.
- Based on the MNM concentration selected, the pH points considered, and the technique/techniques that will be used to analyse the samples, estimate the total volume of stock dispersion that should be prepared.

Build-up of SOP for the size measurement that will be run before and after the zeta potential measurement

- include the NM refractive index
- select water as the solvent
- set the temperature to 25 °C and the equilibration time to 60 s
- select the folded capillary cell
- select automatic for all the measurement conditions
- set up one size measurement

Build-up of SOP for the zeta potential measurement

- include the NM refractive index
- select water as the solvent
- select the Smoluchowski approximation
- set the temperature to 25 °C with no equilibration time before the measurement
- select the folded capillary cell
- select automatic for all the measurement conditions
- set up three zeta potential measurements with 60 s of equilibration time between each measurement
- set up a combination of three SOPs (SOP play list): SOP size, SOP zeta potential, and SOP size

Preparation of the stock dispersion

- Prepare a stock suspension of 2.56 mg particles/mL in MilliQ water, following the NANoREG ECOTOX dispersion protocol for producing reproducible dispersion of manufactured NM in environmental exposure media.
- Measure the size distribution of the stock dispersion using DLS according to the SOP developed in the NANoREG ECOTOX dispersion protocol.

Preparation of samples for zeta potential versus pH titration measurements

- Rinse the folded capillary cell by flushing with water, then rinse the cell with 50% EtOH in water (v/v), and finally rinse again with water. Dry the cell under a nitrogen flow. Visually check the electrodes and cell for the presence of defects.
- Dilute the stock dispersion with an appropriate amount of NaCl solution to obtain a final concentration of 10 mM NaCl in the samples for titration.
- According to the starting pH and the pH points to measure, divide the sample into two aliquots which allows the pH changes in acid and basic directions. Store the aliquot that will be measured later in a closed bottle or vial and perform the titration in the shortest time possible.
- Start the first titration leg versus acidic or basic pH.
- Under magnetic stirring, add the proper volume of HCl/NaOH to reach the selected pH point. Let the sample stir until the pH stabilizes.
- Use the syringe to remove a 700 µL aliquot of the sample and insert it into the capillary cell, taking care that no bubbles remain in the cell or attached to the electrodes.
- Run the SOP playlist.
- After the measurement, discard the sample and clean the cell by flushing with water several times.
- Dry the cuvette under a nitrogen flow and check visually that no residue is left in the cell.
- Repeat point 5 and follow the procedure described from that point to reach the following pH point of the titration.

Determination of the NM IEP

- Plot the zeta potential measured at each pH point of the titration and determine the NM IEP as the pH point at which the zeta potential is equal to zero.