DOSSIER ON TITANIUM DIOXIDE
- PART 1 - NM 105
ANNEX 2

Series on the Safety of Manufactured Nanomaterials
No. 54

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A. Appendix A.
SOP for surface charge and isoelectrical point by zetametry (CEA)

General description

The SOP is developed by CEA and it is different from the Nanogenotox SOP. Dispersion state and stability of suspensions are governed by an equilibrium between attractive (mainly van der Waals) and repulsive (electrostatic or steric) interactions. A stable suspension is obtained if the repulsive interactions overcome the attractive ones, responsible for aggregation and subsequent sedimentation. Zeta potential is a good indicator of the magnitude of repulsive interactions between charged particles. The charge at the very surface of the particles is not accessible and Zeta potential corresponds to the potential at the shear plane. This is the boundary between the bulk dispersant and the double layer of solvent and ions moving together with the particles, see Figure A.1. The reciprocal Debye length, $\kappa^{-1}$, represents the thickness of this double layer. The zeta-potential varies with pH due to protonation-deprotonation of the material surface. From colloid science, a suspension of small particles is considered stable if the zeta-potential exceed $|30|$ mV.

For low pH, the surface of metal oxide (MO) materials is protonated (MOH$_2^+$), i.e. positively charged, and for high pH the deprotonation results in negatively charged particles (MO$^-$. The pH-value at which the charge is reversed determines the so-called isoelectric point (IEP) where the dispersion is unstable. IEP can be determined by titration, but can also be measured from manually prepared different dispersions displaying the same ionic strength for various pH. The zeta potential can be highly influenced by the properties of the medium, such as ionic strength (by compression of the double layer), or adsorbing molecules or ions (especially multivalent ions).

The zeta potential ($\zeta$) is not directly measurable and is calculated from the measurement of electrophoretic mobility $U_E$ using Henry’s equation:

$$U_E = \frac{2 \varepsilon \zeta f(\kappa a)}{3\eta}$$
$\varepsilon$: dielectric constant of medium

$\eta$: viscosity

$k$: inverse of the Debye length, $a$: radius of a particle

$f(ka) = 1.5$ for aqueous suspensions in the Smoluchowski approximation

In practice, the sample is exposed to an electric field which induces the movement of charged particles towards the opposite electrode.

**Chemicals and equipment**

- HNO$_3$ (analytical grade)
- NaOH (analytical grade)
- NaNO$_3$ (analytical grade)
- Purified water (MilliQ or Nanopure water)
- Ultrasonic probe Sonics & Materials, VCX500-220V, 500 W, 20 kHz equipped with a standard 13 mm disruptor horn, or equivalent.
- pH-meter with standard pH probe
- Zetasizer Nano ZS (e.g, Malvern Instruments), equipped with laser 633 nm
- Autotitrator (Malvern MPT-2) – *optional for automatic determination of IEP*
- Malvern software (DTS 5.03 or higher) installed on a computer to control the Zetasizer
- Clear, disposable zeta cells (DTS1061 - DTS1060C)

**Sample preparation**

**Summary**

Samples for zeta potential measurements are prepared as aqueous suspensions of 1 g/L for SiO$_2$ nanomaterials with constant ionic strength of 0.036 mol/L (monovalent salt) and controlled pH. They are prepared by dilution of concentrated sonicated stock suspensions of 10 g/L into pH and ionic strength controlled “buffers” prepared by addition of HNO$_3$, NaOH and NaNO$_3$ in various proportions.

**Stock suspension preparation**

20 mL of stock suspensions of 10 g/L NM in pure water are prepared as follows:

200 mg of NM are weighed and introduced in a 20 mL gauged vial (with protective gloves, mask and glasses, and damp paper towel around the weigh-scale).

- The 20 mL gauged vial is completed with ultrapure water (MilliQ)
- The suspension is transferred into a flask suitable for sonication (a 40 mL large-neck glass flask of internal diameter 38 mm was used, height of 20 mL liquid 20 mm), making sure that all the settling material is recovered.
- The suspension is dispersed by ultrasonication for 20 min at 40% amplitude in an ice-water bath. Probe, sample and bath are placed in a sound abating enclosure, and inside a fume hood.

**Preparation of “buffer” solution**

Denominated “buffer” solutions are aqueous ionic solutions of Na\(^+\), H\(^+\), NO\(_3\)\(^-\) and OH\(^-\), designed to display the same ionic strength with a modulated pH.

- A first set of concentrated buffer solutions (0.1 mol/L of salt, various pH) are prepared by addition of HNO\(_3\), NaOH and NaNO\(_3\) in various proportions in ultrapure water.
- Then 20 mL of these concentrated buffers are poured into 50 mL gauged vials completed with ultrapure water, giving a new set of buffers with a salt concentration of 0.04 mol/L and a pH ranging from 1.5 to 12.5. The combination of the two buffers gives access to the necessary intermediate pH.
- By this procedure, acidic buffers contain 0.04 mol/L of NO\(_3\)\(^-\) and various ratios of Na\(^+\)/ H\(^+\) as counter ions; likewise, basic buffers contain 0.04 mol/L of Na\(^+\) and various ratios of NO\(_3\)\(^-\)/OH\(^-\).

**Preparations of suspensions for zeta potential measurements and determination of isoelectric point**

In this SOP Zeta potential measurements are performed on 1 g/L suspensions for SiO\(_2\) samples. 10 g/L suspensions of the SiO\(_2\) samples are used right after sonication. Series of samples are prepared by addition of 400 µL of concentrated NM suspension and 3.6 mL of 0.04 mol/L buffer solutions in a 5 mL glass flask. This leads to samples of 1 g/L SiO\(_2\) and a constant ionic concentration of 0.036 mol/L in monovalent salt.

For each NM, an additional sample is prepared in MilliQ or Nanopure water with the same NM concentrations, i.e. by addition of 400 µL of concentrated NM suspension and 3.6 mL of water.

**Measurements and data treatment**
**Summary**

For each suspension of known pH, fixed ionic strength and fixed NM concentration, the measurements for determining the zeta potential are performed on a general purpose mode with automatic determination of measurement parameters. Three measurements are performed and averaged for reporting. For unstable samples, measurements are performed on supernatants. Zeta potentials are then plotted against pH to determine the stability domains and isoelectric points (IEP).

**Measurements**

Equilibrium pH of the suspensions are measured and considered as pH values for the reported results. The suspension to be characterized by zetametry are inserted in Malvern patented folded capillary cells with gold electrodes (volume 0.75 to 1 mL), DTS1061. Zeta measurements (electrophoretic mobility) are performed on the “general purpose” mode at 25°C with automatic optimization of laser power, voltage settings, the number of runs (10 - 100) and run duration, and repeated 3 times with no equilibration time as the sample is already at ambient temperature.

The Smoluchowski model (F(κa)=1.5) was used, considering the high polarity of aqueous solvent, and hence a thin double layer around the particles. For the dispersant, the refractive index $R_i$, absorption $R_{abs}$, viscosity and di-electric properties considered are the ones of pure water. The parameters used for dispersant and material properties are listed in the following table.

<table>
<thead>
<tr>
<th></th>
<th>Water (STP)</th>
<th>TiO$_2$</th>
<th>SiO$_2$(amorphous)</th>
</tr>
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<tbody>
<tr>
<td>$R_i$</td>
<td>1.33</td>
<td>2.49</td>
<td>1.5</td>
</tr>
<tr>
<td>$R_{abs}$</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Viscosity [cP]</td>
<td>0.8872</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Data treatment**

Electrophoretic mobility is measured by a combination of laser Doppler velocimetry, a technique based on the phase shift of the laser beam induced by the movement of particles under an electric field, and phase analysis light scattering (patented M3-PALS technique). In this “mixed mode measurement” (M3), the measurement consists of the application of an alternative electric field in two modes, a fast field reversal mode, and a slow field reversal mode. The light scattered at an angle 17° is combined with the reference beam and the resulting signal is treated by the computer.
During the fast field reversal mode, the electro-osmose effect is negligible, allowing to determine an accurate mean zeta potential, whereas the slow field reversal mode helps modelling the distribution of potentials.

**Figure A.2:** Simplified scheme of optical configuration for zeta potential measurement on Zetasizer NanoZS.

An example of the main data plots returned by DTS software from zeta potential measurements is shown on figure A.3 (phase plot and corresponding electric field applied, mean zeta potential and zeta potential distribution).

**Figure A.3:** Data plots retrieved from zeta potential measurements on Nanosizer ZS, example of 3 consecutive measurements on a suspension of NM-104 (TiO₂) at 0.5 g/L in pure water.

More details on the results of zeta potential measurements with the M3-PALS technique are available in the documentation from Malvern Instruments and can be downloaded from http://www.malvern.com, application library section. The reported value is the average of zeta potential values from the 3 measurements (determined during the fast field reversal step), with possible exclusion of diverging data.