A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***Protocol for effective density measurements of nanoparticles***
Subtitle	The following SOP describes the experimental set-up and sample preparation for the determination of the effective density of nanoparticles.
NANoREG Work package/task:	WP2 Synthesis, supplying and characterization
Owner and co-owner(s)	Dr. Aurélie Walter, FOPH (EFPL)
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Version	***Date***	***Reason of change***

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TG 109 „Density“ including “true density” and “effective density” (or agglomerate density), EPFL

The following SOP describes the experimental set-up and sample preparation for the determination of the effective density of nanoparticles. This SOP was developed in addition to the different densities defied in OECD TG 109. The methods is based on the following publication: Deloid G, Cohen JM, Darrah T, Derk R, Rojanasakul I, Pyrgiotakis G, Wohlleben W, Demokritou P. Estimating the effective density of engineered nanomaterialsfor in vitro dosimetry. Nat Commun. 2014, oi:10.1038/ncomms4514 . Effective density is a important parameter for dasage prediction in in vitro experiments

Protocol for effective density (agglomerate density) determination

A. Method Version February 26th 2016 / Dr. Aurélie Walter

This method determines the agglomerate density of powders suspensions.

B. Equipment

- 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.
- PCV tube, 1 mL.
- Easy-read measuring device for PCV tubes.
- Centrifuge (Eppendorf, 5702R), 4 × 85 mL swing bucket rotor with round buckets (rotor diameter: 14 cm).

C. Protocol

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.
- Use the Excel template for probe-sonicator comparison ‘Template for Probe Sonicator Calibration’.
- Prepare the batch dispersion.
- Prepare a stock suspension of X mg/mL in MilliQ water.
- Adjust the pH of the suspension to be within the electrostatic stabilization domain of the particles. Normally, a pH value 2–3 units below or above the pH of the IEP, but still far enough from the pH at which the solubility of the investigated powder increases, is used (see examples in Table 2-2).

Table 2-1 Examples of powder preparation for agglomerate density measurements

Sample	Bulk density	pH for measurement
NM-101	1.9 mg/mL	pH 9
NM-103	4.5 mg/mL	pH 4

Powder dispersion

- Use the stock suspension of particles in MilliQ water.
- Use the method described in the NANoREG ENPRA dispersion protocol, i.e. sonicate for 16 min, as described in the protocol.
- As soon as the sonication is finished, pour 1 mL of the suspension into the PCV tube. Prepare 3 replicates.

Centrifugation

- Set centrifuge at 20 °C.
- Centrifuge the tubes for 1 h at 4000 rpm with the in-house centrifuge or at 2504 *g*.
- Determine V_{pellet}.

Determination of the agglomerate density

- Use the excel template (see Annexe 1).

It is important to note that this method works only for particles that settle under the given experimental conditions. This was not the case for material NM-203, which means that the dispersion is very stable and/or the nanoparticles are too small and diffusion is the main transport mechanism.

Annex 1

Excel file for the determination of agglomerate density

Density Calculations

$$\rho_{EV}=\rho_{media}+\left[\left(\frac{M_{ENM}-M_{ENMsol}}{V_{pellet}SF}\right)\left(1-\frac{\rho_{media}}{\rho_{ENM}}\right)\right] \quad (5)$$

Reference: DeLoid et al. 2014

Input parameters

$M_{ENM}=c_{ENM}*V$			
Density Di water (at 20°C)	0.998205	g cm^{-3}	
Density NP	3.6079	g cm^{-3}	measured density
Volume (tube)	1	mL	
SF (random stacking)	0.634		

c_{ENM} [g/mL]	ρ_{ev} for ISDD	Vpellet (mL)	ρev	Comment	Date
	NM in DI water				
4.50E-03	4.5 mg/mL	0.003725	2.3764684540		
		0.003125	2.6410950372	Aurélie	
		0.003475	2.4756240982		
		0.003205			
		0.0037		Sophie	
		0.00385			
		0.003305			
		0.0033		Sophie	
		0.003305			
	4.5 mg/mL	0.003443333	2.498		

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Lausanne 01.09.2016