A common European approach to the regulatory testing of nanomaterials

Standard Operating Procedure

Title	***Protocol for the determination of the Dispersibility***
Subtitle	The protocol for the measurement of dispersibility was developed and tested with several NANoREG powders.
NANoREG Work package/task:	WP2 Synthesis, supplying and characterization
Owner and co-owner(s)	
Date finalised	
Document name	NANoREG D2.09 SOP 04 Protocol for the determination of the Dispersibility
Key words:	

Version	***Date***	***Reason of change***

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New proposed TG for determination of " Dispersibility"

The following protocol for the measurement of dispersibility was developed and tested with several NANoREG powders.

Protocol for the determination of the Dispersibility

A. 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
- Excel template for the dispersibility determination (HH template)

B. 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 the probe-sonicator comparison 'Template for Probe Sonicator Calibration'

C. Preparation of the batch dispersion

- Prepare a stock suspension of 2.56 mg particles/mL in MilliQ water.
- Adjust the pH of the suspension to be within the electrostatic stabilization domain of the particles.
- Carry out DLS measurements, as described in the NANoREG SOP for probe-sonicator calibration of delivered acoustic power and de-agglomeration efficiency for in vitro and in vivo toxicological testing (Note: the medium of the suspension is water and not serum, as described in the NANoREG protocol).

D. Determination of the dispersion curve

- Use the stock suspension of particles in MilliQ water.
- Use the method described in the NANoREG ENPRA dispersion protocol.
- Instead of sonicating for 16 min, as described in the protocol, sonicate the suspension for 1 min.
- Carry out DLS measurements, as described in the NANoREG SOP for probe-sonicator calibration of delivered acoustic power and de-agglomeration efficiency for in vitro and in vivo toxicological testing.
- Then, replace the suspension in the 10 mL beaker with fresh suspension (from the batch suspension) and sonicate the suspension for 2 min.
- Carry out DLS measurements.
- Repeat steps 5 and 6 with 3, 4, 5, 10, 15, 20, and 30 min of sonication.

E. Determination of the dispersibility of the powder

- Use the Excel template for dispersibility determination to calculate the dispersibility/agglomeration factor of the powder (Annexe 1).
- **Calculation of the 'Dispersibility' using 'Excel template for the dispersibility determination'.**
- **Definitions:**
- Dispersibility is a measure of the strength of the agglomerates of the powder. It is expressed as D_{BET}/D_{DLS}, where D_{BET} is the primary particle diameter calculated from the BET value and D_{DLS} is hydrodynamic diameter (taken as the z-average) of the agglomerate after ultrasound treatment at a fixed power and time. In the case of a very good dispersibility and weak agglomerates, the dispersibility value can reach 1 (particle and agglomerate sizes are the same). The following definitions could be useful:

- **Dispersibility value equal to 1** corresponds to an ideal powder with no agglomerates. In principal, this value cannot be reached because D_{DLS} is larger than D_{BET}.
- **Dispersibility value of 0.5–1** corresponds to a powder with very good dispersibility, with a maximum of 4–5 primary particles forming an agglomerate (loose packed configuration) and an agglomerate diameter corresponding to 2 particles.
- **Dispersibility value of 0.3–0.5** corresponds to a powder with good dispersibility, with around 5–15 primary particles forming an agglomerate and an agglomerate diameter corresponding to 3 particles.
- **Dispersibility value of 0.25–0.3** corresponds to a powder with medium dispersibility, with an agglomerate diameter that is 3–4 times larger than the primary particle (15–32 primary particles in an agglomerate).
- **Dispersibility value of 0.1–0.25** corresponds to a powder with bad dispersibility, with an agglomerate diameter that is 4–10 times larger than the primary particle (32–500 primary particles in an agglomerate).
- **Dispersibility value of <0.1** corresponds to a powder with very bad dispersibility, with an agglomerate diameter that is more than 10 times larger than the primary particle (more than 500 primary particles in an agglomerate with a loose-packed configuration).

- **Note**: The agglomerate factor, which is the inverse of dispersibility, is often used in ceramics.

Annex 1

1.1 Example of the determination of the Dispersibility: NM-103

INPUT:

- **Cell B1**: Name of the powder and other important information.
- **Cells C5 – L10**: The measured agglomerate diameter in nm. The values have to be measured following the SOP's and has to be given in DLS z-average.
- **Cell C15:** BET in m^2/g (you can use the BET values given in the Technical Data Sheet or measured following the corresponding SOP).
- **Cell C16**: Density in g/cm^3.
- **Cell C18** Diameter of the primary particles in nm (you can use the D_{TEM} values given in the Technical Data Sheet or measured following the corresponding SOP). This value is only for comparison. The diameter of the primary particle measured by TEM or calculated from BET and Density must be similar.

OUTPUT

- **Cells C11-L11:** Mean diameter of the agglomerates (nm) for each time point of dispersion.
- **Cells C12- L12**: Standard deviation for each time point of dispersion.
- **Cells C13-L13:** Standard deviation in % of the mean diameter for each time point of dispersion.
- **Cells C23-L23**: Dispersibility for each time point of dispersion calculated from D_{BET}/D z-average (Dz-avarage is taken from Cells C11-L11).
- **Cells D24-L24**: Dispersibility rate (1/min) is the rate how fast the agglomerate size is changing.
- **Cells C25-L25:** The agglomerate factor is the invers of Dispersibility. Agglomerate factor of 1 means primary particle size = agglomerate size, 10 means the agglomerate diameter is 10 times large than the primary particle size.
- **Cell F27:** Dispersibility after 30 min ultra sound treatment. This value has to be given in the Result template "Dispersibility "of Nanoreg. For your own purpose, you can change the dispersion time in cell E27 to see the effect of dispersion time on de-agglomeration.

Figures

- **Right side top**: Your measured data plus the calculated mean diameter.
- **Right side middle**: The calculated Dispersibility (**Cells C23-L23**).
- **Right side bottom:** Dispersibility rate (Cells D24-L24).

- **Bottom left:** Dispersibility, double logarithmic presentation, used for the calculation of the ”dispersibility” Cell F27
- **Bottom right:** Dispersion rate in double logarithmic presentation (values not used so far, but could be helpful for a further classification of the materials)

Dispersion of NM-103 in water

Please fill in your values only into the green cells!!

Measured Agglomerate size (DLS, z-average value in nm)

Time (min)	0	1	2	3	4	5	10	15	20	30
Run 1	221.0	148.2	139.8	135.4	131.2	128.5	122.1	120.7	117.9	116.3
Run2	203.8	147.1	140.0	134.9	130.9	129.8	123.0	119.7	117.8	115.2
Run 3	201.2	146.7	138.8	135.3	132.4	130.1	123.8	122.4	119.8	116.7
Run 4										
Run 5										
Run 6										
Mean	208.7	147.3	139.5	135.2	131.5	129.4	123.0	120.9	118.5	116.1
SD	8.81	0.62	0.53	0.21	0.65	0.71	0.72	1.12	0.93	0.65
SD %	4.22	0.42	0.38	0.16	0.50	0.55	0.58	0.92	0.78	0.56

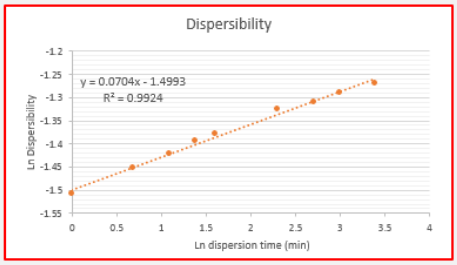
BET (m^2/g)	51	Value from technical data sheet of from own measurment (SOP and template BET, Nanoreg)
Density primary particle (g/cm^3)	3.607	Value from technical data sheet of from own measurment (SOP and template Density, Nanoreg)
D_{BET} (nm)	32.6	Calculated as D_{BET}= 6/(densityxBET) density in g/cm3, BET in m2/g. The result is multiplyed by 1000 to get D_{BET} in nm
D_{TEM} (nm)	25	Value from technical data sheet of from own measurment (SOP and template TEM size measurement, Nanoreg). D_{BET} and D_{TEM} should be similar

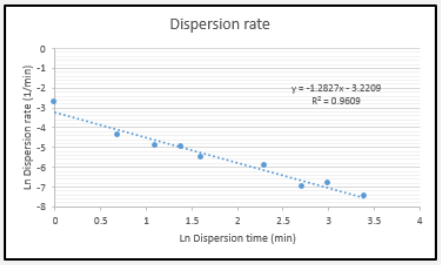
Dispersibility — Dispersibility is given by D_{BET}/D_{DLS} — Agglomeratefactor is 1/Dispersibility

Dispersion of NM-103 in water

Time (min)	0	1	2	3	4	5	10	15	20	30
Disbersibility	1.56E-01	2.21E-01	2.34E-01	2.41E-01	2.48E-01	2.52E-01	2.65E-01	2.70E-01	2.75E-01	2.81E-01
Dispersion rate (1/min)		6.51E-02	1.24E-02	7.50E-03	6.75E-03	3.95E-03	2.66E-03	9.02E-04	1.10E-03	5.78E-04
Agglomerate factor	6.4	4.5	4.3	4.1	4.0	4.0	3.8	3.7	3.6	3.6

Dispersibility	A	B	Dispersion time (min)	Dispersibility	
Disp =exp[A+Bln(t)]	-1.499271075	0.0704445	30	2.84E-01	Value which has to be transferd to the result template Nanoreg





Dispersibility calculation	Time	0	0.6931472	1.09861229	1.3862944	1.6094379	2.3025851	2.7080502	2.9957323	3.4011974
	Ln Dispersion rate	-2.73206882	-4.39152	-4.8929697	-4.99892	-5.533216	-5.931087	-7.010498	-6.814958	-7.45619
	Ln Dispersibility	-1.50783963	-1.453419	-1.4218438	-1.39427	-1.378456	-1.327101	-1.310234	-1.2901	-1.269323