Protocol(s) for size-distribution analysis of primary NM particles in air, powders, and liquids
Deliverable 2.10

Introduction

The implementation of the EC-definition of a nanomaterial across various regulatory fields requires a detailed detection and characterization of manufactured nanomaterials by appropriate, validated testing methods. Transmission Electron Microscopy (TEM) is one of the few techniques that can identify nanoparticles according to the current definitions.

In this deliverable, SOPs for quantitative TEM analysis in the context of the EC definition are proposed and applied and validated on a series of nanomaterials, by intra-laboratory and inter-laboratory validation.

Description of Work

Standard operation procedures (SOPs) and guidelines were developed, applied and combined as shown in figure to analyse the size and shape of manufactured nanomaterials:

- EM samples are prepared bringing the nanomaterial in dispersion using a modification of “The generic NANOGENOTOX batch dispersion protocol for *in vitro* studies”
- Specimens are prepared bringing a representative fraction of the material on an EM-grid.
- Representative and selected EM images are recorded.
- The nanomaterials are described following guidelines for qualitative characterization of nanomaterials in dispersion in a regulatory framework based on representative and selected EM-micrographs
- Colloidal nanomaterials are characterized quantitatively by an image analysis method which includes detection, classification and measurement of primary particles.
- Aggregated, fractal-like nanomaterials are characterized by an image analysis method which includes detection, classification and measurement of primary particles in aggregates.
- Data analysis and representation of measurement results are performed according to relevant ISO-norms.

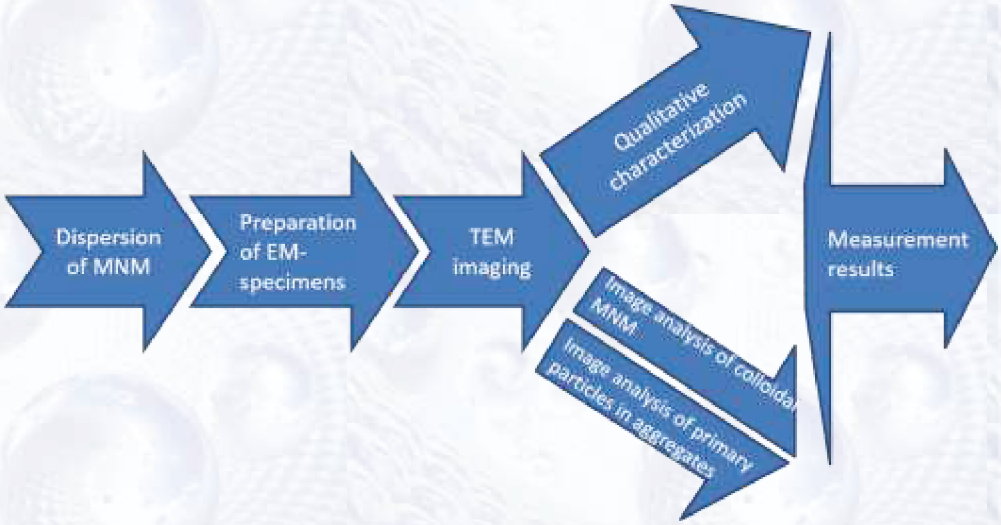

Figure 1: Schematic overview of the steps included in a complete TEM analysis to measure the size and shape of the particles of a MNM.

All protocols were performance tested and validated for application in a regulatory context using reference and representative nanomaterials in intra- and inter-laboratory validation approaches.

Main Results

The SOPs presented in the deliverable are the following:

- The SOP to prepare a TEM specimen suitable for qualitative and quantitative analysis from a dispersed NM ensures that the NM samples are suitable for TEM imaging and analysis. The examined materials were evenly distributed over the grids and the fraction of the attached NM represents the dispersed NM optimally.

- The SOP to record a set of calibrated transmission electron micrographs showing NM that are representative for the NM on the EM grid ensures that the number of particles and the magnification of the micrographs are suitable for subsequent descriptive and quantitative image analyses.

- The method for characterizing the primary particles and aggregates of a NM by describing their physical properties based on TEM micrographs provides a step-by-step guide for the descriptive characterization of nanomaterials.

- The SOPs to analyze the 2D properties of the primary and aggregated/agglomerated particles on EM micrographs ensure that the primary particles are detected and that size and shape measurands are determined quantitatively. A modified version allows to measure the size and shape properties of the aggregates/agglomerates.

Data were analyzed and represented according to relevant ISO-norms.

The performance of the methods and concepts established in this work was shown in intra- and inter-laboratory validation studies, such that they are ready to be considered for adoption into guidance documents for physico-chemical characterization of nanomaterials applied in various fields.

An intra-laboratory validation approach comparing the measured size with the (certified) reference size values for a panel of colloidal, near-spherical nanomaterials, spanning a size range from 8.9 to 202 nm, demonstrated that the TEM size measurement was accurate. Estimation of the combined intra-lab measurement uncertainties of a series of 23 measurands for all examined materials demonstrated that size and shape measurements of the primary particles of colloidal and fractal-like MNM, and of the aggregates of fractal-like MNM, were precise.

An inter-lab validation approach (figure 2) demonstrated that the developed SOPs could be implemented successfully by the participants, such that the primary particles and the aggregates could be measured precisely.

- Each participant estimated his intra-lab uncertainties, as a basis for his validation of these SOPs.

- Comparison of the size and shape measures of the participants demonstrated that the SOPs can be implemented to define and characterise colloidal and fractal-like aggregated MNM in a regulatory context.

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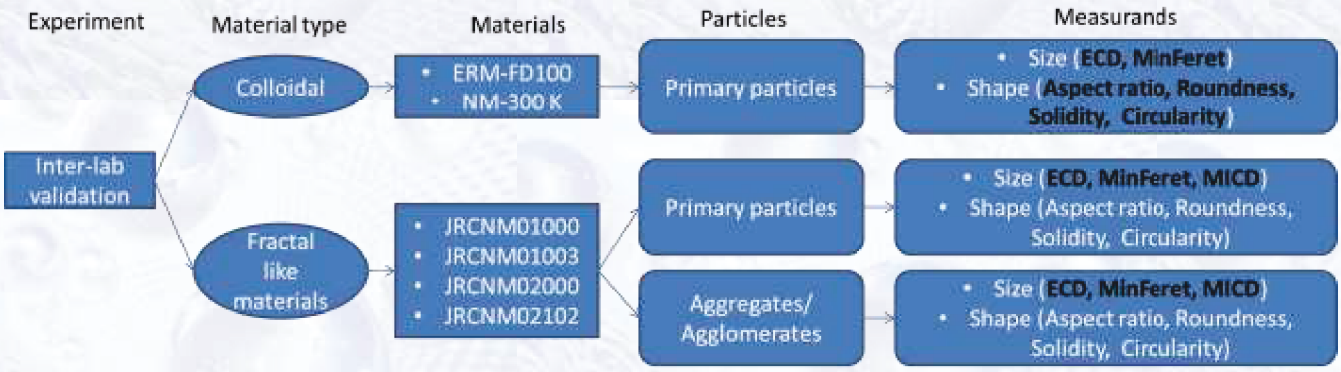

Figure 2 Schematic overview of the between-laboratory validation studies (ILC) indicating the material types, materials and particles tested and the measurands measured.

The EM-based results were related to the results obtained with alternative methods. These include ensemble techniques based on light scattering, such as dynamic light scattering (DLS) and particle tracking analysis (PTA), and single particle inductively coupled plasma-mass spectrometry (SP-ICP-MS).

The results illustrate that the size measurands measured with the different techniques are method-defined and cannot be directly compared without prior knowledge. The hydrodynamic radius of near-spherical colloidal NM assessed by DLS and PTA is for example only comparable with the ECD value obtained by TEM when the colloidal suspension is perfectly stable and no aggregation occurs.

For more details about NANoREG please visit the official website **www.nanoreg.eu**.

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