A common European approach to the regulatory testing of nanomaterials

Revised OECD methods for determination of physicochemical NM properties Deliverable 2.09

Introduction

The OECD Guidelines for the Testing of Chemicals are a collection of internationally accepted and relevant test methods for determining the safety of chemicals and chemical preparations, including pesticides and industrial chemicals. They are a unique tool for assessing the potential effects of chemicals on human health and the environment.

In D2.03 '*Experimental evaluation of OECD methods for analysis of physicochemical MNM properties*', important OECD guidelines relevant for particle characterisation were assessed for their applicability to nanomaterials (NM). Some of the tested guidelines were found not to be applicable for nanomaterials (TG 106 and TG 107/117/123). Others needed modification to made them fit for NM testing.

Deliverable 2.09 follows on deliverable 2.03; it presents proposals for adaptation of Guidelines TG 105, 108, 109, 110 and 112 to make them fit for testing nanomaterials. On top of this measurement of dispersibility, zeta potential and water dissolution rates have been addressed.

Description of Work

Based on the evaluation of Technical Guidelines reported in deliverable 2.3, proposals for adapted or new guidelines were experimentally developed and evaluated up to a stage where they are ready for validation in interlaboratory tests.

Main results and evaluation

The table below gives a summary of the results reported in the deliverable:

OECD Technical Guideline	Appropriate for NMs	Modified protocol established	Future work
TG 109 (relative density)	Partially	Yes; New protocols for true and effective (agglomerate) density	Validation
TG 110 (granulometry)	Partially	Yes, protocols for CLS, DLS and SEM (image analysis) were established	Validation
TG 106 (sorption-desorption)	NO	From a thermodynamic point of view not possible	
TG 105 (water solubility)	Partially	Yes; A revision of the TG to address dissolution is proposed, and a protocol was established and demonstrated.	Validation
TG 115 (surface tension of aqueous solutions	YES	No modification necessary beside the replacement of solution by solution/suspension	
TG 107/117/123 (*n*-Octanol-water partition coefficient)	NO	From a thermodynamic point of view not possible	
TG 112 (dissociation constant in water)	NO	Protocols were developed for other more relevant endpoint such as isoelectric point.	Validation
TG 108 (complex formation in water)	Partially	A new protocol for the determination of the complex formation was included in the SOP for determination of isoelectric point (TG 112	Validation
Dispersibility	Yes	New protocol developed	Validation

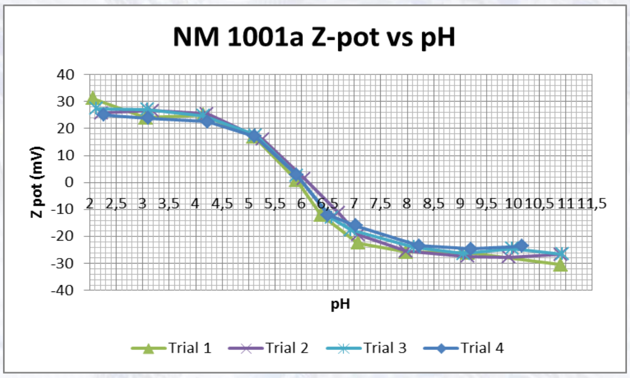
For *TG 109 (relative density)*, additionally to the existing types of densities, a method for the determination of effective density (density of the agglomerates or skeletal density) was added. This effective density is of high importance for the characterisation of the nano powders in liquid dispersions especially for the prediction of dosage. Also an alternative method for measuring the true density has been developed (He-Pycnometer).

TG 110 (Granulometry) focusses on the determination of the hydrodynamic diameter of NM. The work in WP 2 shows that dynamic light scattering (DLS) is the most convenient method, but other methods based on the determination of the sedimentation rate are also useful. In all cases, it is of utmost importance that the NANoREG protocol for powder dispersion is applied when preparing the suspension.

TG 105 (water solubility). The two methods described in this guideline have been evaluated. Due to practical reasons, the column method is rejected. Application of the Flask method to NM is possible with some modification/refinement. It is proposed to replace the term “dissolved” by “dispersed”. A proposal for adaption of the Flask method is included in the Deliverable as well as an alternative method using a stirred batch reactor. For both methods an experimental evaluation was carried out.

TG 115, surface tension of aqueous solutions. Based on detailed literature study it was concluded that this TG needs only very minor modification to be applicable to nanoparticles. The term “solution” has to be replaced by “suspension”. Additionally for preparation of the suspension the protocols of NANoREG have to be applied.

TG 112 (dissociation constant in water) is not applicable to nanoparticles but the Zeta-potential (surface potential at the shear plane of particles in suspension) is as important as the dissociation constant of molecules in water. A protocol for the determination of the isoelectric point was therefore established. This parameter is of high importance to understand colloidal properties as well as adsorption of biomolecules.



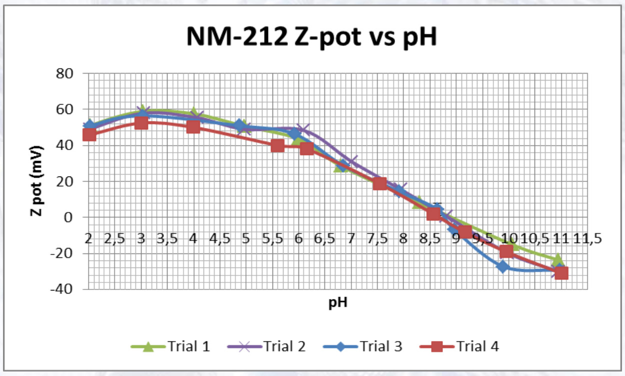


Figure: Following the above-mentioned protocol, the zeta potentials of NM-1001a and NM-212 were determined as a function of pH. The measured zeta potentials are very reproducible, and the pH values of the IEP fit well with published values for these types of materials (6–7 for anatase and 8–9 for ceria)

TG 108 (complex formation in water) also requires modifications, the most important of them being the replacement of the complex formation stability constant by the determination of the adsorption capacity and affinity of nanoparticles for dissolved heavy metal ions. The adsorption capability for heavy metals is important to understand possible secondary effects regarding toxicity measurement (reduced content of soluble heavy metals).

The results reporting in Deliverable 2.09 will -where possible- feed into the OECD harmonisation programme.

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

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