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Clarification of methodical questions regarding the investigation of nanomaterials in the environment

Development of a decision support tool for the investigation of nanomaterial's environmental behaviour based on dispersion behaviour and dissolution in relation to various environmental parameters
Short report

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Clarification of methodical questions regarding the investigation of nanomaterials in the environment

Development of a decision support tool for the investigation of nanomaterial's environmental behaviour based on dispersion behaviour and dissolution in relation to various environmental parameters

Short report

by

Dr. Philipp Kozin, Dr. Frank von der Kammer
University of Vienna, Vienna

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Umweltbundesamt
Wörlitzer Platz 1
06844 Dessau-Roßlau
Tel: +49 340-2103-0
Fax: +49 340-2103-2285
info@umweltbundesamt.de
Internet: www.umweltbundesamt.de

/umweltbundesamt.de
/umweltbundesamt

Study performed by:
Department of Environmental Geosciences
University of Vienna
Althanstraße 14 UZAII
1090 Vienna
Austria

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Summary

During the OECD *Working Party on Manufactured Nanomaterials* meeting in Berlin in January 2013 (OECD 2014) experts agreed on the necessity to develop two new OECD Test Guidelines (TG) and a corresponding guidance document (GD) required to perform the tests on the dispersion stability and dissolution behavior of nanomaterials in relevant environmental aquatic media. The developed TGs shall provide the experimental routine to test dispersion behavior and dissolution rate of such materials in aquatic media to estimate their possible fate in and impact on the environment and the joint GD shall answer the question of how nanomaterials should be tested under consideration of their nanomaterial specificities in further environmental tests. The current scientific report summarizes the experimental data obtained while developing the TG on dispersibility and agglomeration behavior of nanomaterials in different aquatic media as well as first considerations on the corresponding GD. Besides that a review on nanomaterials transformation reactions in environmental aquatic media is included in this report.

Engineered Nanoparticles (ENPs) may behave differently compared to solid bulk (› 1µm) materials and dissolved ions or macromolecules due to their specific surface interactions. It has been accepted that some of the existing guidelines on testing of chemicals for their environmental behavior and eco-toxicity are not applicable or require adaption for nanomaterials (OECD 2014). It is therefore required to determine if a material which has been classified as being a nanomaterial or containing nanomaterials has to be tested with specific guidelines or under consideration of specific GDs which take the differences in behavior into account. The experimental data described in this scientific report were produced in support of the development of the related TG on Dispersion Stability of Nanomaterials in Simulated Environmental Media.

Sub-micron sized particles form thermodynamically unstable dispersions in aqueous systems. However, a pseudo-stable situation might be observed when an energy barrier prevents the attachment of particles. This energy barrier, which prevents particles to agglomerate, is influenced by the particles surface chemistry and the composition of the surrounding aqueous medium. The agglomeration behavior of the particles will further determine their transport, environmental distribution, uptake by biota and final fate. While it is impossible to cover the whole complexity of environmental conditions in a laboratory test, it is possible to test the impact of major driving forces on the behavior of the particles. Currently the concentrations of manufactured nanomaterials, which will enter the environment, are expected to be far lower than the concentration of natural (nano-) particles. Therefore the assessment of environmental behavior needs to take into account the presence of natural particles and the heteroagglomeration of manufactured nanomaterials with natural particles will be a dominant pathway. However, for the decision if a material has and retains nanoparticulate properties in OECD standardized test systems the dissolution and homoagglomeration are the critical characteristics. Approaches to test ENPs for their heteroagglomeration are still poorly developed, mainly due to the fact that the counterpart for heteroagglomeration, particulate entities or surfaces of natural origin are extremely diverse and analysis of the agglomeration state of heteroagglomerates is still challenging (Hendren, et al. 2015). The approach that is presented in this report represents a simplification of extensive particle testing under various environmental conditions (von der Kammer et al. 2010; Ottofuelling e t al. 2011; Liu et al. 2013). The parameters that influence particle agglomeration behavior/dispersion stability are shortly described below:

Ionic strength –When particles with the similar polarity of surface charge are under investigation, high electrolyte concentration promotes their agglomeration and sedimentation.

pH –For particles with variable charge surfaces (e.g. metal oxides), with organic coatings with amphoteric character or groups which can protonate/deprotonate (e.g. carboxylic groups), the pH pays a ma-

jor role for their colloidal stability. Dispersions of electrostatically stabilized particles are most unstable at the pH where this electrostatic repulsion is minimal. In the absence of any specific adsorption of electrolytes from the dispersing medium this is the point of zero charge (PZC), when the surface charge density is zero. This is a material-related, intrinsic parameter. Often, instead of the PZC, the isoelectric point (EIP) is determined, what is the pH at which the particles do not move in an electric field. In absence of specific adsorption this IEP equals the PZC, if specific adsorption takes place the IEP is unequal the PZC and becomes an extrinsic parameter which is determined by the surrounding medium.

Concentration of Dissolved Organic Matter (DOM) –Particle dispersions generally become more stable due to adsorption of NOM on the surfaces of the particles. NOM surface adsorption may increase negative surface charge, induce charge reversal or, in cases of positively charged particles, may lead to reduction of net positive charge (up to neutralization) and therefore can also induce agglomeration. In the presence of cations strongly complexed by NOM (as Ca^{2+}) dissolved NOM may reduce the activity of the cations which will increase colloidal stability of negatively charged particles.

Particle concentration –Larger number of particles increases the number of collisions per time which leads to faster agglomeration and subsequently the sedimentation of particles.

Particle size – Large particles sediment faster. Thereby, also agglomeration directly leads to faster particle settling. Diffusion counters sedimentation, but much less for larger particles.

Particle density – Density of particles is the significant factor influencing their sedimentation rate, especially at the stage of sample's centrifugation.

Presence/absence of CO_2 in the atmosphere – Presence of CO_2 in the atmosphere above the particle dispersion can influence the stability of dispersion through changing the pH of dispersion and the properties of EDL at high pH values (above pH=5), where CO_2 is increasingly soluble in aquatic media. Adsorption of carbonate ions to the surface of the particles changes their point of zero charge.

The endpoint described in the present TG is agglomeration behavior presented as dispersion stability and dispersibility.

Dispersion stability – is the tendency of the particles in a dispersion to agglomerate and settle. It describes the behavior of the dispersion over time and how much of the particles is still dispersed after a period of time. It is in a way linked to an agglomeration rate. The agglomeration rate reports on the number of successful particle collisions per unit time, while dispersion stability is reporting the percentage of dispersed particles over time.

Dispersibility – describes how well a nanomaterial can be dispersed under defined conditions of a dispersing method. In a defined procedure the amount of dispersed particles after a given time period is measured, given a pre-set threshold (e.g. 1, 10 or 50%) the material is then termed "dispersible" or "non-dispersible".

For the test system described herein, it may be necessary to quantify nanoparticle concentrations at ppb-levels. Therefore, prior to the investigations of agglomeration behavior of nanomaterials, the detection limits of various nanoparticle detection techniques were assessed. Detection limits of ICP techniques, UV-VIS, Nephelometry and an industry-standard dispersion stability test system (Formulaction Turbiscan LAB) were tested.

Period of investigation and number concentration - The agglomeration behavior of nanomaterials can in principle be investigated by testing the sample's dispersibility. In case of such dispersibility check, only 2 samplings/ measurements have to be done. One has to be done directly after sample preparation (0 h time point) while another at the experimental endpoint (6 h time point). The time period of 6 hours was chosen empirically, as the most convenient time period to overview the particle agglomeration in the described conditions. However, the test must be performed with a certain particle number

concentration in the sample. Dispersibility testing is quite convenient since it is less time-consuming, and provides a simple “Yes” or “No” answer to the question of particle agglomeration and sedimentation. However, it can cause numerous difficulties in understanding the origin of obtained data since it does not overview the dynamics of particle agglomeration/sedimentation. Such opportunity is provided by dispersion stability testing used in the presented test guideline. During dispersion stability test a number of experimental points are obtained in accordance to the particle concentration measurements performed every hour (0-6 hours). Built experimental curves can originate from similar experimental factors, but have different character. Such approach readily allows to visually overview the reasons of particle sedimentation, however it does not allow to quantitatively evaluate the data.

The choice of “dispersibility” or “dispersion stability” approaches depends on the aims of the test, and requires the understanding of advantages and disadvantages of each approach, as described above. “Dispersibility” and “dispersion stability” experimental approaches are strongly bounded to each other and are both applicable for the needs of presented TG. Thus, in certain cases, the 7-point measurements can be substituted by 2 point measurements, where measurements have to be performed at 0 and 6 hours points. Such simplified 2-point approach can be applied for the primary assessment of nanomaterial stability in aquatic media. If dispersed material reveals more than 90% (fully dispersible) or less than 10% (non-dispersible) of initial material concentration in the dispersed form after 6 hours of experimental time the 2-point measurements of material dispersibility can be considered sufficient and no further testing is required. In case the concentration of material in the dispersion after 6 hours of experimental time stays in the range of 10-90%, it is recommended to perform the full 7-point testing, it will lead to a better understanding of particle agglomeration behavior, and its reasons. Thus Figure 2 shows the possible characters of particle agglomeration (sedimentation) behavior and explains its possible reasons. Curve of (a) type would correspond to a material that sediments mainly due to the density factor. As can be observed, sedimentation mainly occurs during the last hour measurement, when particle dispersion undergoes centrifugation that facilitates sedimentation. It could mean that low density particles (as e.g. polystyrene sulfonates) agglomerate to larger clusters but still float to a large extent as they are settling slowly in the gravitational field. Centrifugation would account for this low density by applying respectively high g-forces. Given the particles have agglomerated to clusters larger the centrifugation particle size cut-off, they will sediment out in this step and an abrupt decrease in supernatant concentration is found. Curve (b) would be referred to the systems that undergo continuous agglomeration followed by particle sedimentation. This is a classic case of particle sedimentation caused by formation of agglomerates. In turn, case (c) would correspond to a dispersion containing rather heterogeneous particles. As can be seen from curve (c) the largest particles would sediment during the 1-2 hours of observations, while the smaller ones will remain the constant concentration in the dispersion. It could also mean that a fraction of 2/3 of the sample agglomerates fast and settles, while 1/3 of the particles possess higher repulsive forces and stay stably dispersed.

It has to be acknowledged that a substance may exist in several nanoforms of the same chemical identity; the different nanoforms may possess variable properties. The multi-point testing helps to reveal this heterogeneity of a substance in its different nanoforms.

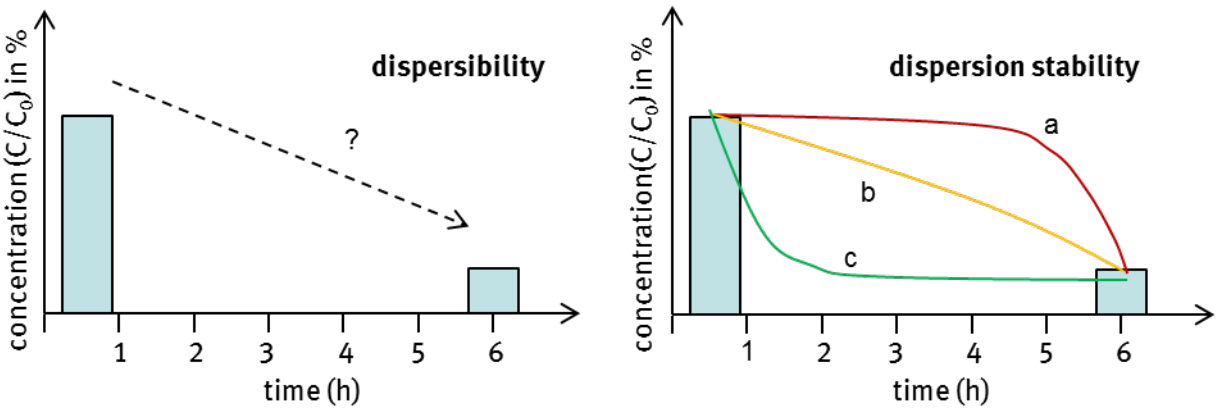

Figure 1: Possible types of particle agglomeration behavior in dispersibility test and in dispersion stability test.

Such approach, combining analysis of dispersibility and dispersion stability of investigated material can help to assess the obtained experimental data and estimate the need in further analysis with the minimal measurement cost and time spending.

Special attention has to be paid to reporting of material concentration values obtained during the measurements. The above mentioned percentage values (such as 90% or 10% of the material amount) shall be calculated in assumption that 100% of the material concentration is the amount of material measured at the initial (0 hours) measurement. This initial concentration will sometimes however be lower than the calculated (expected) material concentration, due to the material losses during experimental preparations. The difference between the calculated concentration value and the initial material concentration value (0 hours) shall be determined and reported too. The calculated concentration value shall be either derived from the amount of material (by weight) placed in the dispersion, or by full digestion of analyzed dispersion. High losses during preparation indicate that some of the instruments or lab ware used is not compatible with the dispersion. Some types of plastics do support the attachment of particles to the surfaces and promote losses.

When the direct measurements of particle concentrations during each hour is not possible due to the unavailability of suitable instruments or by some other reasons, the continuous measurements of particle concentration in the dispersion can be performed using UV-VIS spectrometry. Usage of UV-VIS spectrometry comes with certain limitations for the analysis that has to be considered during the experiments and interpretation of the results. Typical UV-VIS measurement allows a measurement of only one sample at a time. Thus the measurement of each sample will demand a continuous work of UV-VIS spectrometer for 24 hours. No replicate measurements would be possible during such measurement. However, the number of measurements over time is dramatically increased (a one measurement per 30 seconds would be a typical acquisition rate) and can be easily regulated to the desired value. This even increases accuracy due to a large number of replicates which differ only very little in their acquisition time point. Here also an improved time-resolution is obtained, which might sheed even more light on the agglomeration process than the 7-point testing. Particle concentration can be monitored by UV-VIS spectrometer based on light scattering or light absorption by the particles, depending on the properties of the particles. Here NM300K and NM105 have been monitored in absorption because both particles have true absorption bands, the silver particles from the plasmon resonance and the TiO_2 particles from the UV-filter properties of the material. Results and sensitivities reported here will differ significantly from materials like SiO_2 nanoparticles where only weak scattering will produce a (small) signal in the photometer. The signal to mass ratio for light scattering is typically much lower than for true absorption. Detection wavelengths have to be chosen with care and a spectral scan would be advised before selecting the wavelength. The final results are presented in the form of relative to the first measurement absorption, (A/A0).

The development of the experimental procedures was based on three different particle types. They each represent a positive and negative control as well as a borderline case. Ag-NPs (NM300K, OECD material) has been used as positive control that shows good dispersion stability under almost all environmental conditions considered in the test, NM400 carbon nanotubes have been chosen as a negative control with vanishing dispersion stability under almost all conditions and NM105 TiO2-NPs have been employed as a borderline case which shows good and no stability, depending on the water chemistry.

The parameters in the aquatic media in which the stability is tested have been selected according to database analysis (Salminen, et al. 2005) and current literature on NP stability in aqueous media (Hammes, et al. 2013, von der Kammer, et al. 2010, Zhu, et al. 2014).It has become evident that if comparing the 90% percentile of surface water concentrations of stability controlling ions with their de-stabilizing potential, the major role is played by divalent cations, NOM and pH. These three parameters have been introduced into the experimental procedure in different concentrations or values. NOM is only applied in two concentrations (0 and 10 mg/L, depending on the surface area of the particles).

The OECD TG developed based on the research presented in the report was adopted by the OECD council in October 2017 and is available at the webpage on OECD Guidelines for the Testing of Chemicals: http://www.oecd-ilibrary.org/environment/test-no-318-dispersion-stability-of-nanomaterials-in-simulated-environmental-media_9789264284142-en

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