Preparing suspensions of nanoscale metal oxides for biological testing

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Preparing suspensions

1. Initial Considerations

This document is intended to provide information as to the methods developed and currently used for the preparation of suspensions of nanoscale metal oxides for biological testing within the NanoCare project. The described methods are not meant to be comprehensive, but rather should provide the opportunity to prepare suspensions in a manner consistent with that performed within the NanoCare project. This should allow improved reproducibility of experimental results as well as easier comparison between different studies. This does not necessarily imply that in all instances the procedures should be used. It should be recognized that there may be certain modifications required to give considerations to the diversity of nanoscale material. Therefore, the reader needs to carefully examine the procedures to determine if they are adequate for the selected test material.
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1. Preparing suspensions for toxicological tests

1.1. Pre-wetting the test material

Test material should only be pre-wetted if simple stirring in water or test medium (according to section 1.2) is not possible.

The test material is weighed into a 10-mL snap-on lid glass (diameter approx. 20 mm, height 40 mm) and covered with the wetting agent. The glasses should be filled to a maximum of 6 mL[1]. The test substance is stirred for one hour, lid closed, using an appropriate magnetic stir bar at 300 rpm and room temperature.

For the purpose of pre-wetting the test material is pasted/mixed with either DMSO (0.5 % DMSO final concentration in test medium) or BSA solution.

1.2. Preparing the stock suspension

The mass concentrations of stock suspensions normally range between 1 and 10 g/L. To prepare the stock suspensions the test substance is weighed into a 10-mL snap-on lid glass (diameter approx. 20 mm, height 40 mm) and covered with the solvent. The glasses should be filled to a maximum of 6 mL. The test substance is stirred for one hour, lid closed, using an appropriate magnetic stir bar at 900 rpm[1] and room temperature.

The respective test medium is used to prepare the stock suspensions.

For the purpose of diluting, characterizing or testing aliquots are taken during stirring.

Before characterization (see below) the stock suspension is stirred for 24 hours, lid closed, at 900 rpm and room temperature. The stock suspension is stirred also 24 hours (instead of 1 hour), if it is used directly for testing (if there are no dilutions to prepare).

1.3. Preparing the diluted suspensions

Diluted suspensions are prepared of the stock suspension. For this purpose an aliquot is taken out of the stock suspension while stirring. This aliquot is added to the – already stirring - solvent (10-mL snap-on lid glass with a teflon magnetic stir bar, filled to a maximum of 6 mL at 900 rpm).

Before farther using, the dilutions are stirred for 24 hours.

For dilution the respective test media are used:

Aliquots for application or characterization are taken while stirring.

2. Characterization of the suspensions

Photo documentation

The suspensions (stock suspension and dilutions) to be located in a snap-on lid glass are photographed in front of a suitable background, while stirring.

Physical characterization

The need for characterizing the particle dimensions in the suspensions is discussed before starting the study.

The stock suspensions and the final dilution (test media) are characterized.

We define: ultra-fine fraction (<100 nm), fine fraction (100 nm – 1 µm) and agglomerates (>1 µm).
The characterization takes place either by analytical ultra-centrifugation (especially ultra-fine and fine fraction), or by laser diffraction and dynamic light scattering (especially fine fraction and agglomerates). Typical particle concentrations for an authentic characterization are in the range of 0.01 – 10 mg/mL, favoured in the range of 0.1 – 1 mg/mL.
If the classification in degrees of fineness cannot reflect the significant differences between differing preparations, alternatively D_{10}, D_{50}, D_{90} is indicated. These sections quote 10 - 50 – 90 weight by weight of the integral particle dimensions.

[1] Due to unbalance, glasses filled with less than 5 mL will topple over at more than 900 rpm.