ERANET-SIINN Project NanoToxClass

Establishing nanomaterial grouping/ classification strategies according to toxicity and biological effects for supporting risk assessment

Standard Operation Procedure - Preparation of nanoparticle suspensions by cup horn sonication

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Note: The SOP is based on nanOxiMet and nanoGRAVUR investigations/SOP´s

Information version numbering: Version x.y
0.X is used for internal versions, 1.0 for the first external release.
Example: Version number 1.4 denotes the 4th (intermediate and internal) version after the 1st submission (contributing to an upcoming 2nd external release).
2.0 is used for the second submission to the EC.

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1 Scope

This Standard Operating Procedure (SOP) describes the nanoparticle suspension preparation within the NanoToxClass project by using indirect probe sonication with a cup horn.

2 Basics

Bringing nanoparticles into suspension is always a challenge and several different protocols are currently used within several scientific studies. However, a dispersion procedure often has to be adapted to the specific material, experiments especially if several, parallel investigations are planned. Consequently, there is not a unique procedure to be applied, only a compromise of the minimal common base, acceptable for the different investigations.

The aim of this Standard Operating Procedure is the description of the in NanoToxClass applied suspension procedure. The procedure was tested prior on a P25 titanium dioxide suspension and revealed an appropriate stability of the suspension. An appropriate stability is declared as a constant hydrodynamic particle diameter (z.average) and polydispersity index (PDI) with a standard variation ≤ 10 % between the three internal measurements.

Stability criteria are:

- Visual observation (no visible sedimentation of the particles)
- Size distribution (mean z.average and PDI of the three internal measurements of one sample/analysis) of the particles in the suspension

Depending on the NM in suspension the duration of the size analysis can vary significantly from ~2-40 min.

In the follow the SOP describes suitable steps for preparing a nanoparticle suspension in this project.

3 Materials and Instruments

3.1 Materials

The following materials and chemicals are required:

- Deionised (dH_2O) / ultrapure water, e.g. HPLC grade water (CAS 7732-18-5) or

Millipore-filtered (electrical resistance 18.2 Ω)

Or

- Cell culture media (e.g. Ham's F-12K Medium or Dulbecco's Modified Eagle's medium, DMEM containing 10% FCS).

  e.g. Gibco F-12K + L-Glutamine (Product number 21127-022); Gibco DMEM (Product number 31053-028) or Pan Biotech (Product number P-04-01159)

Caution: FCS is added after sonication!

- Nanomaterial (solid/powder or in dispersion)
- Clean spatula
- Pipette
- Plastic or glass (for the pigment materials glass vials are essential!) vial of suitable size (e.g. 50 mL)

Note that for cell culture all equipment needs to be sterile.

3.2 Instruments

Comparable equipment as the mentioned instrument is required:

- Ultrasonication equipment (e.g. Bandelin Sonoplus HD2200 ultrasonic homogenizer 200 Watt rated power, Bandelin Cup Horn BB6)
- Note: The usage and maintenance of the instruments will be not described in this SOP. Please refer to the manual.

4 Experimental procedure

4.1 Suspension preparation

- Note that for cell culture purposes all handling steps should be performed in a sterile environment, e.g. in a laminar flow cabinet
- For preparing the nanomaterial suspension dH_2O or cell culture media is used
- a defined amount of the nanomaterial – here ~10 mg – is weighed in a 50 ml plastic centrifuge vial (a variance of 10% is accepted), smaller amounts can be adopted as long as the effective sonication energy is similar (see below, then different sizes of centrifuge vials may be used instead)
- each of the centrifuge vials is filled up to ~20 ml (concentration 0.5 mg/mL) with dH_2O, smaller volumes or different concentrations can be adopted

  or

Cell culture media [(e.g. Ham's F-12K Medium; or Dulbecco's Modified Eagle's medium, DMEM containing 10% FCS)]. FCS is added to a final concentration of 10% after sonication! FCS is pipetted into the dispersion (sterile!) and then the suspension is briefly vortexed (roughly ~ 5 sec. at 2000 rpm) before analysis.

- a centrifuge vial is placed in the middle of the Cup Horn. The bottom of the centrifuge vial is placed 0.5 - 0.9 cm above the sonication unit (Figure 1)
- the cup horn is filled with 240 mL deionised water; differs on diameter and size of the cup horn and provider please adapt to get finally the effective sonication power (see below)
- the suspension is sonicated in the Cup with a final effective sonication power of 6 W measured by calorimetry (see Taurozzi et al. 2010); a variance of 5 % for the total sonication power is accepted
  - for Bandeline Sonoplus HD 2200, this power is reached by 200 Watt with permanent sonication (100% duty cycle, 100 % power) for 23 min.; Vial placed 0.9 cm above sonication unit.
  - for Branson Digital Sonifier 450 (400 Watt) with Disruptor Horn (101-147-048) this power is reached by duty cycle 100%, output control 4 for 9 min 50 sec.; Vial placed 0.9 cm above sonication unit.
  - heating of the sample by more than 2°C is to be avoided (e.g. cooling or changing water, do not use ice!)
  - With the Bandeline Sonoplus, for sonication the vial with the suspension is cooled by cold water in the cup horn to minimize the heating of the suspension during the sonication; also possible is a continuously flush of the cup horn
  - With the Branson Cup Horn Sonifier, continuously flush the cup horn by fresh water or use as well cold water (exchange after each sonication)

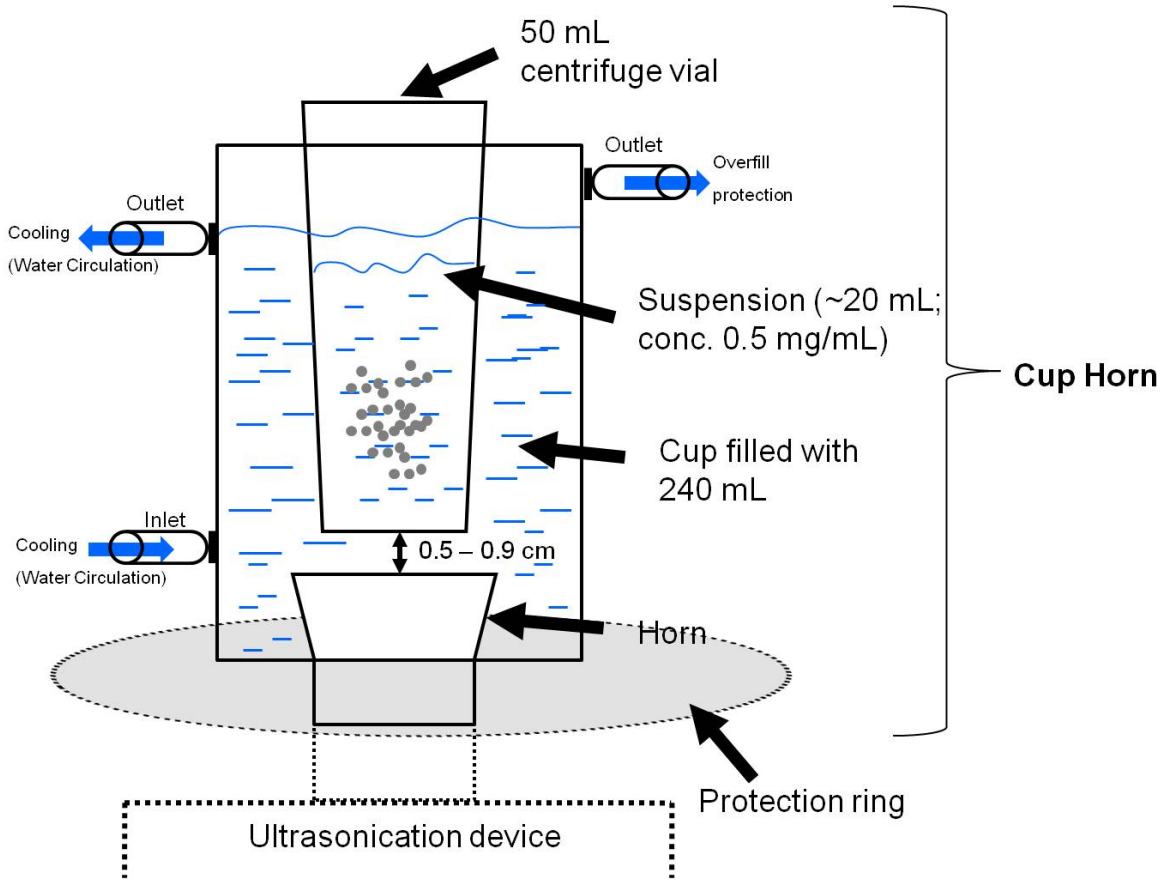

Figure 1. Scheme of the experimental setup (here for Bandelin Cup Horn BB6)

5 Safety precautions

Please follow the safety information and regulations of the working laboratory as well of the materials provider. In general handle with care, wear protective clothing and suitable gloves at any time and labelling the material.

6 Waste disposal

Please follow the disposal advice of the material provider, if available.