
Standard Operation Procedure for static solubility testing of NM suspension

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1. Scope

This Standard Operating Procedure (SOP) describes the preparation procedure for static solubility testing of NM in aqueous solvent (deionized water and cell media).

Note: Not described in detail is the chemical element analysis.

2. Basics

The solubility is the property of a (nano-)material to dissolve in a solvent. Nowadays, the solubility is a common characterization parameter for NM and becomes especially of interest because the dissolution of NM has been shown as a characterization parameter which can be linked to potential toxicity. In consequence, defined and harmonized solubility tests are necessary to investigate the amount of ions which can be released from a NM.

2.1. **Materials**

The following materials and chemicals are required:

- Deionized (dH₂O) or HPLC Grade water (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% FBS).
- Nanomaterial (NM)
- Spatula
- Pipette
- Centrifuge vial 50 ml
- Rack for centrifuge vials
- Disposable syringe (B. Braun Inject 10 ml)
- Syringe filter (0.22 microns Cameo 30N Syringe Filter Nylon, GE Water & Process Technologies, PA 0.20 µm nylon, Roth KC84; 0.20 µm syringe filter Ø 33 mm, Rotilabo KY61.99)

or alternatively applicable instead of the filtering procedure products (in cursive) are

- ultrafiltration centrifuge vials including filtration membranes (e.g. VIVASPIN 15R, Sartorius, 5kD)

2.2. Instruments

The following instruments are required:

- Overhead-shaker (60 rev/m)
- Centrifuge (5 G)

Note: The usage and maintenance of the instruments will be not described in this SOP. Please refer to the manual.

3. Experimental procedure

Briefly, NM is weighted in a vial with a certain concentration before overhead-shaken for a defined time period. Afterwards the sample is centrifuged and the supernatant filtered before chemical mass determination by Inductive Coupled – Optical Emission or Mass Spectrometry (ICP-OES/ICP-MS).

3.1. Suspension preparation

The NM is weighing in a correspondingly labeled vial and the mass is documented. Target value for the concentration of the suspension is 1 g/L with a volume of at least 20 ml (for nanoGRAVUR). This results in a test sample of 20 mg of the solid NM. After weighing, 20 ml of the medium is added to the centrifuge tube.

3.2. Shaking

The sealed tubes are fixed in an overhead shaker and shaken for a certain period (e.g. 4 h, 24 h, 72 h) at 60 revolutions / minute. Meanwhile, the room temperature is recorded (1 hour-values) and the pH is measured at the beginning and after the shaking periode, since the temperature as well as the pH also influences the solubility. After shaking the sample is immediately transferred to the centrifuge.

3.3. Centrifugation and filtration

The centrifuge vials (without filter membrane) are placed in the corresponding inserts of the centrifuge and centrifuged for 30 minutes at 5.000 revolutions / min. Please wait until the centrifuge has completely stopped without slow down process (break)

before remove the samples. After the centrifugation the vial is removed from the centrifuge and transported in an upright position until filtration.

For the filtration the supernatant of each sample is taken by a disposable syringe (B. Braun Inject 10 ml) and filled in a fresh labelled vial via a syringe filter (dependent on the size of the particles or agglomerates – 0.02µm Whatman Anotop 25 inorganic membrane filter Ø 25 mm, 0.22 microns Cameo 30N Syringe Filter Nylon, GE Water & Process Technologies, PA 0.20µm nylon, Roth KC84; 0.20 µm syringe filter Ø 33 mm , Rotilabo KY61.99).

Alternatively when centrifuge vials with a filter membrane are used (e.g. Sartorius Vivaspin 15R – 5kDa) the filtration step is obsolete and the supernatant can directly be used for element analysis to quantify the Ion concentration of the specific NM.

4. Further sampling handling

The filtered sample is according to the suggested further analysis (for example chemical identification via ICP-OES/MS) stored or processed.

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.