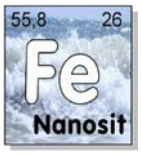
Responsible partner for this SOP: Fraunhofer IKTS	

Dispersion of Carbo-Iron® Suspensions for Ecotoxicological Testing

AUTHORED BY:	DATE:
Tobias Meißner	27.09.2013

REVIEWED BY:	DATE:
Dana Kühnel	02/10/2013

APPROVED BY:	DATE:
Annegret Potthoff	02/10/2013

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1. PURPOSE

This SOP describes the preparation and application of Carbo-Iron suspensions to be used in subsequent ecotoxicological tests. Dispersion is considered a crucial step in toxicity testing, hence, the preparation of a nanomaterial stock suspension has to follow a reproducible procedure in order to ensure e.g. stability of the dispersion over the anticipated test duration.

2. OBJECTIVE

Standardized toxicological testing of suspended nanomaterials involves the preparation of stock suspensions from nanomaterial powder in a reproducible manner. Carbo-Iron is a nanostructered composite material made of zero-valent iron and activated carbon. It is applied in groundwater remediation by injecting a carboxymethyl cellulose (CMC) stabilized Carbo-Iron suspension into the aquifer. Based on this technical approach, Carbo-Iron stock suspensions are stabilized with CMC before transfer into the test media. CMC is increasing the colloidal stability of the particles and prevents their agglomeration. Due to the need of oxygen for the organisms in ecotoxicological tests, it has to be accepted, that zero-valent iron as part of Carbo-Iron oxidizes and loses its reactivity while dispersing. Ecotoxicological studies are therefore carried out on aged material. Suspension preparation prior to ecotoxicological testing is based on a two-step approach. First, dispersion of Carbo-Iron is conducted by ultrasonication in order to achieve a deagglomerated stock suspension. Second, serial dilutions are prepared from the stock suspension by adding a CMC-water suspension. These dilutions are added to the respective test media in a fixed ratio.

3. REGULATORY BASIS, REFERENCE DOCUMENTS

Substantive guidelines and norms that serve as basis for this SOP
DIN ISO 14887:2010-3 Sample preparation - Dispersing procedures for powders in liquids (ISO 14887:2000)
OECD Safety of manufactured nanomaterials No. 36 - ENV/JM/MONO(2012)40 Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials
ISO 22412:2008 Particle size analysis - Dynamic light scattering (DLS)
ISO 13320:2009 Particle size analysis - Laser diffraction methods

Description of calorimetric method to determine delivered acoustic energy and specific energy / energy densitiy
Taurozzi JS, Hackely VA, Wiesner MR (2011) Ultrasonic dispersion of nanoparticles for environmental, health and safety assessment - issues and recommendations. Nanotoxicology 5:711-729

Multi-step dispersion methods for the testing of nanomaterials
Bihari P, Vippola M, Schultes S, Praetner M, Khandoga A et al. (2008) Optimized dispersion of nanoparticles for biological in vitro and in vivo studies. Part Fibre Toxicol 5:14
Meißner T, Potthoff A, Richter V (2009) Physico-chemical characterization in the light of toxicological effects. Inhal Toxicol 21:35-39
Meißner T, Kühnel D, Busch W, Oswald S, Richter V, Michaelis A, Schirmer K, Potthoff A. (2010)

Physical-chemical characterization of tungsten carbide nanoparticles as a basis for toxicological investigations. Nanotoxicology 4:196-206
Taurozzi JS, Hackely VA, Wiesner MR (2012) A standardised approach for the dispersion of titanium dioxide nanoparticles in biological media. Nanotoxicology 7:389-401

Test media to be used are described in the OECD Guidelines for the Testing of Chemicals.

4. RELATED DOCUMENTS

Table 1 : References to documents needed to proceed according to this procedure

Document ID	Document Title

5. DEFINITIONS

Table 2: Glossary of Terminology used in the SOP

Term	Description
CMC	carboxymethyl cellulose
OECD	Organisation for Economic Co-operation and Development

6. PROCEDURE

a) Short description:

Carbo-Iron stock suspensions with a concentration of 1 g/L and 20 wt% CMC (200 mg/L) are prepared by ultrasonication. Suspensions are placed on ice and treated with an ultrasonic horn. Exposure solutions for toxicological testing are prepared by adding stock suspension or diluted stock suspension in the test media. For this, a fixed ratio of 10 vol% suspension and 90 vol% test media is used. Before adding to media, Carbo-Iron stock suspension is diluted with water containing 200 mg/L CMC. Final CMC concentration in the toxicity test is hence is 20 mg/L.

b) Materials and devices:

- Carbo-Iron powder
- CMC (CAS 9004-32-4; examined for molecular weight of 70,000 g/mol)
- rotary sample divider for large quantities of Carbo-Iron powder
- ultrasonic dispersion device equipped with an ultrasonic horn
- scale
- beaker glass
- pipettes
- deionized or distilled water
- NaOH
- test media
- 0.45 µm filter and syringe
- stirrer

c) Sample preparation:

A representative sampling is a prerequisite for meaningful analysis results. Therefore, a sample division becomes necessary for large quantities of powders that tend to unmix. Division of the Carbo-Iron powder on a rotary sample divider provide representative sub-samples, thus ensuring the reproducibility of the analysis. After the division, the sample aliquot can be taken for the preparation of the stock suspension.

d) Detailed description of the procedure:

CMC solution

A CMC stock solution (2 g/L) is prepared by dissolving CMC in 10^{-4} M NaOH and stirred overnight. Subsequently, stock solution is filtered with a 0.45 µm filter and stored for a maximum of 7 days at 4 °C. Before use, CMC stock solution is diluted with deionized or distilled water (no NaOH) to 200 mg/L.

Carbo-Iron stock suspension

A quantity of 40 mg Carbo-Iron powder is put in a glass beaker, and 40 mL of 200 mg/L CMC solution is added. This suspension is dispersed with an ultrasonic horn and a total transferred specific energy of 10^6 kJ/m^3. A description for determination of the specific energy is given by Taurozzi et al. (2011). During sonication, the sample is cooled in a water bath with ice. Breaks are conducted during the sonication process, allowing the sample to cool down. The sample should not be warmer than lukewarm.

Dilution of stock suspension

The maximum Carbo-Iron concentration subjected to toxicity tests is 100 mg/L. All test concentrations below 100 mg/L require a dilution of the stock suspension (1 g/L) with the 200 mg/L CMC solution. For example, a test concentration of 50 mg/L means a dilution of the stock suspension from 1 g/L to 500 mg/L.

Transfer in test media

Carbo-Iron suspension or diluted suspension is always transferred in test media with a fixed ratio of 10 vol% suspension to 90 vol% test media. For this, the sample is slowly added dropwise to the media. The entire step should be done under continious stirring to homogenize the sample quickly.

e) Controls:

Reproducibility in size of the Carbo-Iron stock suspension has to be validated by means of particle size measurements. This can be performed by using dynamic light scattering or laser light diffraction. Particle size of the Carbo-Iron suspension should always be checked before application. Also, the particle size in the test medium should be checked regularly. The time intervals for measuring the size should be selected according to the duration of the ecotoxicity test.

f) Data analysis:

For the evaluation of the measurements with DLS, the average particle diameter xDLS and the polydispersity index PI is used (see ISO 224412). When using the laser light diffraction, the median particle diameter x50 should be used to assess the particle size of Carbo-Iron (for details see ISO 13320).

g) Testing errors:

Flocculation or agglomeration could occur. The causes may be manifold: contaminated CMC, different batches of CMC or Carbo-Iron with variations in properties, contamination of stock suspension or test media and others. Moreover, stability and behaviour of CMC-stabilized Carbo-Iron was confirmed in selected test media (ADaM, Elendt M4, Elendt M7, Grimme & Boardman, reconstituted water according to OECD 203). Behaviour in other media may vary and instability can occur.

7. SCOPE/AREA OF APPLICATION

Areas of application are ecotoxicological tests such as those described in the "OECD Guidelines for the Testing of Chemicals". Most widely stability against agglomeration is given for the test media ADaM, Elendt M4, Elendt M7, Grimme & Boardman, and reconstituted water according to OECD 203.

8. ATTACHMENTS

1 Mackenzie K, Bleyl S, Georgi A, Kopinke FD (2012) Carbo-Iron – An Fe/AC composite – As alternative to nano-iron for groundwater treatment. Water Res 46:3817-3826 (doi:10.1016/j.watres.2012.04.013)

2 Bleyl S, Kopinke FD, Mackenzie K (2012) Carbo-Iron®—Synthesis and stabilization of Fe(0)-doped colloidal activated carbon for in situ groundwater treatment. Chem Eng J 191:588-595 (doi:10.1016/j.cej.2012.03.021)

9. HEALTH, SAFETY AND ENVIRONMENTAL CONSIDERATIONS

Standard safety aspects and local laboratory rules have to be considered. Personal protective equipment (lab coat, gloves, etc.) has to be worn.