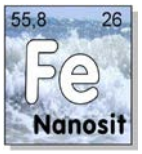
Responsible partner for this SOP: Fraunhofer IKTS	

Dispersion of Zeolite Suspensions for Ecotoxicological Tests

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1. PURPOSE

This SOP describes the preparation and application of zeolite 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 against agglomeration.

2. OBJECTIVE

Zeolite Fe-ZSM-5 and its nanoparticle-loaded variant Magnetite/Zeolite are used for waste water treatment. Magnetite/Zeolite is a development of the Helmholtz Centre for Environmental Research GmbH - UFZ. In the case of an unintended release, particles can enter the environment. Therefore, an ecotoxicological assessment is needed.

Standardized toxicological testing of suspended nanomaterials involves the preparation of stock suspensions from nanomaterial powder in a reproducible manner. Investigations have shown that suspensions containing these zeolite materials can be stabilized by polyaspartate. The dispersant polyaspartate is increasing the colloidal stability of the particles and prevents their agglomeration. Suspension preparation prior to ecotoxicological testing is based on a two-step approach. First, dispersion of zeolite is conducted by ultrasonication in order to achieve a deagglomerated stock suspension. Second, serial dilutions are prepared from the stock suspension by adding a polyaspartate solution. 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 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
OECD	Organisation for Economic Co-operation and Development

6. PROCEDURE

a) Short description:

Zeolite stock suspensions with a concentration of 1 g/L and 1 wt% polyaspartate (10 mg/L) are prepared by ultrasonication.

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, zeolite stock suspension is diluted with water containing 10 mg/L polyaspartate. Final polyaspartate concentration in the toxicity test is hence is 1 mg/L.

b) Materials and devices:

-
- zeolite powder (Fe-ZSM-5 or Magnetite/Zeolite from UFZ)
- sodium of polyaspartic acid (CAS 181828-06-8; examined for MW 2000 - 3000; Baypure DS 100, Lanxess)
- rotary sample divider for large quantities of zeolite powder
- ultrasonic dispersion device equipped with an ultrasonic horn
- scale
- beaker glass
- pipettes
- deionized or distilled water
- test media
- 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 zeolite 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:

Polyaspartate solution

A polyaspartate solution (10 mg/L) is prepared by dissolving sodium polyaspartate in water. It is recommended to prepare a higher concentrated polyaspartate stock solution that is diluted to 10 mg/L before usage. The stock solution can be stored for a maximum of 7 days at 4 °C.

Zeolite stock suspension

A quantity of 40 mg zeolite powder is put in a glass beaker, and 40 mL of 10 mg/L polyaspartate solution is added. This suspension is dispersed with an ultrasonic horn and a total transferred specific energy of 10^6 kJ/m³. 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 zeolite 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 10 mg/L polyaspartate 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

Zeolite 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 zeolite stock suspension has to be validated by means of particle size measurements. This can be performed by using laser light diffraction. Particle size of the zeolite suspension should always be checked before application.

Also, the particle size in the test medium should be examined. Due to the size in the micrometer range, sedimentation of the particles occurs. After a certain time and without prior homogenization, particle size cannot be measured then. It is up to the user and the question if the sample is homogenized before size measurement. 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 laser light diffraction, the median particle diameter x_{50} should be used to assess the particle size of the zeolite material (for details see ISO 13320).

g) Testing errors:

Besides sedimentation, flocculation or agglomeration could occur. The causes may be manifold: contaminated polyaspartate, different batches of polyaspartate or zeolite with variations in properties, contamination of stock suspension or test media and others. Moreover, stability and behavior of polyaspartate-stabilized Fe-ZSM-5 and Magnetite/Zeolite was confirmed in selected test media (Elendt M4, Grimme & Boardman, reconstituted water according to OECD 203). Behavior in other media may vary and instability can occur.

7. SCOPE/AREA OF APPLICATION

Area 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 Elendt M4, Grimme & Boardman, and reconstituted water according to OECD 203.

8. ATTACHMENTS

1 Gonzalez-Olmos R, Kopinke, FD, Mackenzie K, Georgi A (2013) Hydrophobic Fe-zeolites for removal of MTBE from water by combination of adsorption and oxidation Environ Sci Technol 47: 2353-2360 (dx.doi.org/10.1021/es303885y)

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.