

CEINT/NIST PROTOCOL

REPORTING GUIDELINES FOR THE PREPARATION OF AQUEOUS NANOPARTICLE DISPERSIONS FROM DRY MATERIALS

Ver. 2.0

July 8, 2010



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Scope

This document provides *general guidelines* for reporting relevant conditions and critical parameters relating to the aqueous dispersion of nanoparticles, by application of ultrasonic energy or other mechanical dispersion methods (section A), and an example presented as a materials and methods section that can be adapted as needed for different nanoparticle sources (section B). It applies specifically to source engineered nanomaterials (ENM) obtained in dry powder form or as concentrated stock suspensions. This document is subject to future changes as additional information becomes available.

A. General Guidelines

General guidelines consist of a list of parameters that should typically be reported in *Materials and Methods* sections (or alternatively as part of the Supporting Documentation) of scientific publications, as well as a list of key characteristics of the final nanoparticle dispersion. These guidelines relate exclusively to the dispersion process; additional parameters or experimental conditions relating to the synthesis and/or testing of the source material may be necessary in order to reproduce the experiment in its entirety.

1. Minimum parameters that should be specified in reporting the preparation of nanoparticle dispersions:

- Description of powder “as received”
 - primary particle size and shape, and method of measurement (e.g. TEM)
 - specific surface area and method of measurement
 - identity of coatings or surface functionality if known
 - for commercial sources, product tradename or catalog number together with batch/lot number
- Description of suspending medium used to disperse particles
 - composition including ionic strength or molar salt concentration
 - pH
 - dispersing agent(s) (e.g., surfactant) and concentration(s), if used
 - order in which components were added (where appropriate)
 - source and quality of water if prepared or diluted on-site (e.g., ultrapure, resistivity value, endotoxin removal, organic carbon removal, final filter pore size)
 - for commercial sources, product and batch number(s) as applicable
- Description of sample container used during dispersion procedure
 - flat, conical or round bottom
 - nominal container volume/capacity
 - material type (e.g., glass, Teflon, polyethylene, etc.)
- Conditions of sonication (preferred method; if used)
 - mass of powder initially added to specified volume of dispersion medium

- volume of sample treated
- type of sonicator used (bath, probe immersion, inverted cup)
- power input to sample (as calibrated: see CEINT/NIST protocol “*Preparation of Nanoparticle Dispersions from Powdered Material using Ultrasonic Disruption.*”)
- total cumulative treatment time
- operation mode (continuous or pulsed), if pulsed indicate pulse duration
- tip/probe immersion depth (if used)
- temperature before and after sonication
- whether or not cooling bath was used
- sonicator make, model, maximum power output, probe type/diameter if used
- Conditions of treatment other than sonication (secondary method; if used)
 - describe method (e.g., ball milling, high intensity vortexing, centrifugation, filtration, etc.) and any relevant details pertaining to device or method used including operating viscosity range, mill media composition, post-dispersion dilutions
 - volume and concentration -or mass- of sample treated, as applicable, and where appropriate whether the powder sample was added initially, continuously, or intermittently
 - total cumulative treatment time
- Post-treatment storage conditions prior to testing (container material, temperature, exposure to light, exposure to oxygen, time)
- Presence of endotoxins or procedures applied to avoid/remove endotoxins (if suspension to be used for toxicity testing)
- Conditions of filtration and/or centrifugation used (where appropriate)

2. Key characteristics of the final suspension to be reported should include, where possible:

- Post-treatment mass concentration of particle phase
- Particle size distribution and method of measurement in the relevant suspending medium including steps taken to ensure that the particles size distribution is stable from the point treatment is suspended to the point of measurement, and throughout the duration of relevant tests to be conducted with the material.
- pH

Additional characteristics that may be relevant, especially to subsequent biological testing:

- For commercial sources, steps should be taken to ensure the as-received material reflects the manufacturer’s description
 - Comparison of powder characterization to nominal specifications and typical values, e.g. moisture content by TGA
 - Evaluation of hydrophilicity/wettability
 - Purity determinations
- Post-treatment molar concentration of dissolved species
 - dissolved constituents, e.g. metal or metal oxide species, or total organic carbon for carbon-based species
 - loss of suspending medium constituents, e.g. nutrients

- fate of dispersing aids (free and adsorbed concentrations)
- Description of particle shape, structure, aspect ratio
- Crystallinity and phase composition (typically determined by x-ray diffraction)
- Electrophoretic mobility or other measure related to the net nanoparticle surface charge and correlation to stated particle composition; and isoelectric or zero point of charge value where applicable (note: conditions of measurement, including pH and ionic strength, must be identified)

B. Example procedure for the preparation of a Ag nanoparticle aqueous dispersion, presented in the form of a Materials and Methods section

The following example is presented solely for illustrative purposes and does not reflect an actual procedure. Accordingly, product and manufacturer names are fictitious.

The silver nanoparticles (Product No. 0047, Lot 123) used in this work were obtained as a black-colored powder from NanoSilver, Inc. (New York, NY). The primary particles as initially received had a number-average diameter of (10 ± 4) nm and a roughly spherical shape based on TEM image analysis (Electro II, Electron Applications, Tokyo, Japan). A cubic crystalline structure was confirmed by X-ray diffraction (XR500, XRDCorp, Baltimore, MD) and a specific surface area of (11 ± 3) m²/g was determined by nitrogen adsorption using the BET method (AccuSorpII, Micro-Surf Corp, Toronto, Canada). The powdered material was stored in a desiccator until used. A powder mass of 10 mg was dispersed in 200 mL of ultrapure water (resistivity > 18 MΩ·cm, pH 5.8) in a 7 cm diameter, 250 mL, flat bottom borosilicate glass beaker, using a Sonomix 400 sonicator (SonoCorp, Durham, NC) to create a stock dispersion of Ag NPs. The sonicator was fitted with a flat tip, ½ inch probe and had a maximum power output of 600 W at an operating frequency of 20 kHz. Sonication was performed in continuous mode at a delivered power of 90 W for 20 min. The beaker was immersed in an ice bath to reduce the effect of heating during sonication. The measured temperature increase during sonication was 4 C. Power delivered by the probe was verified calorimetrically following the method detailed in the CEINT/NIST protocol “*Preparation of Nanoparticle Dispersions from Powdered Material using Ultrasonic Disruption.*”

The sonicated dispersion was then manually filtered using a 0.2 μm anodized alumina membrane syringe filter (AluPore, Lansing, MI) to remove any residual poorly dispersed materials. The Ag particles in the final dispersion had a hydrodynamic volume-weighted diameter of (15 ± 5) nm as measured by dynamic light scattering at a 90° scattering angle (Nanosizer III, NanoSizeInc, Germany). A zeta potential of (-22.5 ± 1.38) mV was calculated at pH 6.5 using the Henry equation for conversion of the measured electrophoretic mobility (ZetaNano, London, UK). The concentration of Ag NPs in the final dispersion was 212 mg/L as determined using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Spectro 5000, Wonder Labs, Phoenix, AZ) after digesting the nanoparticles in 5 % HNO₃ for 24 h.

Because these particles were intended for 24 h *in vitro* studies, the particle size distribution

of silver nanoparticles suspended at relevant concentrations in DMEM (the in vitro cell culture medium) was monitored for 24 h (see “e.g. *Results*” section for further details). All glassware and materials used to prepare suspensions were first sterilized with alcohol and samples were prepared in a sterile particle-free hood. The ultrapure water used to create the stock suspension was additionally treated with an endotoxin removal filter (Biopack, Millipore). The prepared dispersions were stored in amber glass vials for no longer than 48 h after production and before use.