



Nano-silver induces dose-response effects on the nematode *Caenorhabditis elegans*

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ARTICLE INFO

Article history:

Received 9 January 2012

Received in revised form

6 March 2012

Accepted 7 March 2012

Available online 3 April 2012

Keywords:

Dose-response

Nano-silver

Nematode

Caenorhabditis elegans

Nanoparticle

ABSTRACT

Toxicity of nano-formulated silver to eukaryotes was assessed by exposing nematodes (*Caenorhabditis elegans*) to two types of silver nanoparticles (AgNPs): with average primary particle diameters of 1 nm (AgNP1) and 28 nm (AgNP28, PVP coated), respectively. Tests were performed with and without presence of *Escherichia coli* to evaluate how the presence of a food bacterium affects the AgNP toxicity. A pre-exposure experiment was also conducted with nematodes pre-exposed to 0 and 1 mg AgNP L⁻¹, respectively, for 20 h prior to exposure at higher concentrations of AgNP. Both AgNP1 and AgNP28 showed adverse dose-response effects and mortality on *C. elegans*. LC₅₀ for AgNP28 was lower than for AgNP1 and, hence, at the present test conditions the PVP-coated AgNP28 was more toxic than AgNP1. Including *E. coli* in the test medium as a food source increased AgNPs toxicity towards nematodes compared to when bacteria were not present. Pre-exposure to a low-level AgNP1 concentration made the nematodes slightly more sensitive to further exposure at higher concentrations compared to no pre-exposure, indicating that nematodes have no efficient physiological ability to counteract nano-silver toxicity by acclimation. The amount of dissolved Ag⁺ was 0.18 to 0.21 mg L⁻¹ after 20 h at the highest AgNP1 (10 mg L⁻¹) and AgNP28 (3 mg L⁻¹) doses in the exposure medium, respectively. The upper limit of Ag⁺ solubility cannot immediately explain the dose-response-related toxic effects of the AgNP nor the difference between AgNP1 and AgNP28. Higher toxicity of AgNP28 than AgNP1 may be explained by a combination of effects of coating, Ag-solubility and higher uptake rates due to agglomeration into µm-size agglomerates in the exposure medium.

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1. Introduction

The number of consumer products containing engineered nanoparticles (ENP) is presently increasing at high speed and prospects foresee a dominating role of nanoproducts within the next decades. The Woodrow Wilson inventory on nanoproducts contains over 1000 consumer products where more than 25% contain nano-silver (Woodrow Wilson International Center for Scholars, 2010). Among these products are bandages, wound dressings, clothing, food containers, laundry machines, algicides, refrigerators, vacuum cleaners as well as milk bottles and chew toys for infants. The main purpose for applying nanosilver is its antibacterial properties (Lok et al., 2007; Fabrega et al., 2011). Another common group of products containing colloidal nanosilver is categorized as food supplements with alleged anti-inflammatory effect (see Woodrow Wilson International Center for Scholars, 2010).

This very diverse group of nanosilver-containing products will in daily use and disposal lead to different routes for nano-silver to enter the environment, e.g. via solid garbage and down-the-drain

disposal. Benn and Westerhoff (2008) found that the accumulated release of silver from a pair of nanosilver-doped socks can exceed 1 mg after only a few washes. Nano-silver will therefore be transported to wastewater treatment plants (WWTPs), where it may adsorb to biosolids and enter the environment via sludge deposition or end up in surface waters (Benn and Westerhoff, 2008).

Nano-silver has been shown to cause bacterial growth inhibition (Morones et al., 2005) and cell death (Sondi and Salopek-Sondi, 2004), while in higher organisms, DNA impact has been reported for two types of mammalian cells: mouse embryonic stem cells and fibroblasts (Ahamed et al., 2008). Furthermore, nano-silver exposure caused abnormalities and mortality in zebrafish embryos (Asharani et al., 2008) and induced reproductive arrest in nematode *Caenorhabditis elegans* (Roh et al., 2009). However, our knowledge on nano-silver toxicity is still very limited and in order to obtain a more complete understanding we have to consider the whole spectrum of AgNPs already in use in combination with a range of relevant exposure scenarios.

C. elegans is a well-known model species widely used in toxicity testing of metals to eukaryote organisms (Donkin and Williams, 1995; Shen et al., 2009; Wang and Yang, 2007; Williams and Dusenbery, 1990) and also of ENPs (Ma et al., 2009; Roh et al., 2009). Besides enabling assessment of basic toxicology on a cellular

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and organism level, the *Caenorhabditis* model offers information about ecotoxicological aspect as nematodes are important members of the terrestrial mesofauna with a crucial role as grazers of microorganisms (Ma et al., 2009). As nematodes inhabit both sediments and soils, they are relevant for assessment of ENP ecotoxicology in both terrestrial and aquatic environments. The existence of numerous toxicology tests, a deep knowledge about its stress response at a genetic level, as well as a short life cycle, and general ease of maintaining in the laboratory made *C. elegans* an obvious choice for the present study.

The aim of the present study was to assess the toxic effects of nano-silver on nematodes, using *C. elegans* as a eukaryote model organism, by: (i) estimating toxicological endpoints (LC₅₀ and EC₅₀) of two types of nano-silver (differing with respect to particle size and formulation); (ii) examining how the presence of bacteria in the test system affects the toxicology of nano-silver; (iii) evaluating if pre-exposure of nematodes to a low (sub-lethal) concentration of nano-silver influence the toxicity at a subsequent exposure to higher concentrations of nano-silver. Regarding the latter, we hypothesized that *C. elegans* would show an increased resistance to nano-silver via physiological adaptation in the pre-exposure phase.

2. Materials and methods

2.1. *C. elegans* culture conditions and synchronization

The nematode strain used was *C. elegans pha-1(e2123ts)* which grows normally at 15 °C, but does not proliferate above 25 °C (Schnabel and Schnabel, 1990). Likewise, in our laboratory we did not observe nematode proliferation above 22 °C at which temperature experiments was incubated. This feature prevented the disturbance by new offspring during the time of incubation (up to 96 h). The nematodes were maintained on NGM agar plates, seeded with *Escherichia coli* OP50 as food source at 15 °C according to Stiernagle (2006). Age-synchronization of nematode cultures was achieved by washing NGM plates with sterile MilliQ water removing all nematodes

and leaving only eggs and bacteria attached to the plates. Eggs were left to hatch for 72 h at 15 °C. Microscopic inspection of the plates was performed to ensure that worm stages beyond hatching were removed. Indeed such worm stages were very easy to see and remove from the plates by washing. When tested in our laboratory, we found this method as reliable as — and less time consuming than standard synchronization method using bleach and NaOH. Before testing, worms in L2 stage were washed of the plates using K-medium and collected in sterile tubes. The K-medium was replaced twice with fresh K-medium to rinse the worms of bacteria.

2.2. Nano-silver test materials

We tested the toxicity of two types of commercial nano-silver (AgNP1 and AgNP28). They were selected primarily because they are both freely available on the market (AgNP1 is sold as a health-improving tonic), but also to investigate the difference of very small and intermediate nanoparticle size. Besides, the AgNP28 was coated with polyvinyl pyrrolidone (PVP, 0.2% of total weight) to enhance dispersability. PVP is not expected to have any toxic effect of its own (Kvítek et al., 2008; Meyer et al., 2010) and therefore differences in toxic effects may be caused by differences in size of the nanoparticles and by PVP enhancing their dispersion.

AgNP1: according to the supplier, the particle diameter is approximately 1 nm and delivered as a suspension in pure water (MesoSilver, Purest Colloids, Westampton, NJ, USA). Measurements of this stock suspension showed that it contained $23.4 \pm 1.2 \text{ mg L}^{-1}$ total Ag. Inspection with Dynamic Light Scattering (DLS) (Malvern Nano ZS, Malvern, UK), using a measured viscosity (SV-10 Vibro Viscometer, A&D Company Ltd., Japan) of 1.441 cP and optical indices for water, showed a strong primary mode with a peak size at 1.3 nm in both number and volume sizes (Figs. 1a and b). Similar sizes in number and volume indicates that the amount of material in the coarser size modes, which could be seen in intensity plots, is negligible. Using the Smoluchowski model, the average zeta-potential was found to be highly negative ($-61.9 \pm 2.2 \text{ mV}$), but still with a spread from -14.6 to -124 mV (Suppl. Fig. 1).

AgNP28: according to the supplier, the particle diameter is in the 30–50 nm range and with a purity of 99.5%, delivered as a dry powder where particles are coated with polyvinyl pyrrolidone (PVP, 0.2% of total weight) to enhance dispersal (Nanostructured & Amorphous Materials Inc., Houston, USA). AgNP28 particles were brought into aqueous suspension in ultrapure water (MilliQ) following a sonication-filtering procedure according to the work of Bilberg et al. (2010), using exactly the same type of nanomaterial, as well as procedures for measuring total Ag concentration in the stock suspensions. Stock suspensions contained $5.9 \pm 0.3 \text{ mg L}^{-1}$ total Ag. DLS analysis of the AgNP28 dispersion using the Malvern DLS Nano ZS using a measured viscosity of 0.887 cP and optical indices

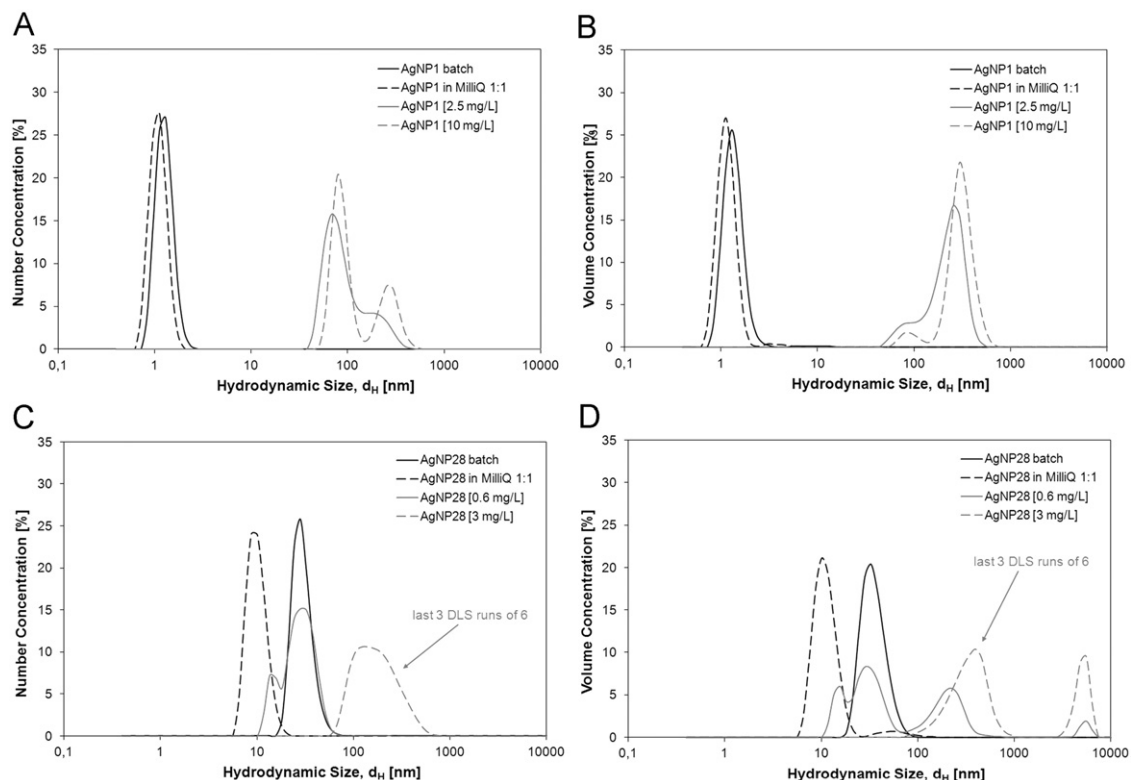


Fig. 1. Results from DLS analyses of AgNP batch dispersions, AgNP batch dispersion diluted in MilliQ-filtered water, as well as a low and a high dose AgNP in K-medium diluted 1:1 with MilliQ-filtered water. (A) Derived number size-distributions for AgNP1. (B) Derived volume size-distributions for AgNP1. (C) Derived number size-distributions for AgNP28. (D) Derived volume size-distributions for AgNP28.

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