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Acute toxicity of Ag and CuO nanoparticle suspensions against *Daphnia magna*: The importance of their dissolved fraction varying with preparation methods

Hun Je Jo, Jae Woo Choi, Sang Hyup Lee, Seok Won Hong*

Water Research Center, Korea Institute of Science and Technology, Hwarangno 14-gil 5, Seongbuk-gu, Seoul 136-791, Republic of Korea

HIGHLIGHTS

- Toxicity of Ag and CuO nanoparticles is highly dependent upon preparation methods.
- Preparation methods cause differences in dissolved concentrations of nanoparticles.
- Dissolved fractions of Ag and CuO nanoparticles govern their toxicity.

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ABSTRACT

A variety of methods to prepare nanoparticle suspensions have been employed for aquatic toxicity tests, although they can influence the dispersion property and subsequent toxicity of nanoparticles. Thus, in this study, we prepared stock suspensions of silver (Ag) and copper oxide (CuO) nanoparticles using different methods and compared their acute toxicity against *Daphnia magna*. The results showed that the dispersion method, filtration and initial concentration largely affected their toxicity, when the toxicity was expressed as the total concentrations of Ag and Cu. In case of Ag nanoparticles, the toxicity was also influenced by their different particle size. However, negligible differences in 24 h-median effect concentration (EC_{50}) values, which were calculated in terms of their dissolved concentrations, were observed. When expressing toxicity on the basis of dissolved concentrations, 24 h-EC_{50} values of the Ag and CuO nanoparticles were also found to be similar to those of the counterpart ionic species, i.e., Ag (as $AgNO_3$) and Cu (as $CuCl_2 \cdot 2H_2O$). These findings indicate that the dissolved fraction of nanoparticles largely contributes to their acute toxicity. Our results may help in establishing a useful guideline for preparing nanoparticle suspensions with reproducible toxicity.

1. Introduction

Nanotechnology has become a rapidly growing industry with a vast number of potential applications such as cosmetics, electronics, paints, medical devices, food packaging, catalysts, antimicrobial fabrics, water treatment membranes, etc. [1–6]. The use of nanoparticles is expected to dramatically increase at an estimated rate of 58,000 ton year^{-1} in 2011–2020 [7]. The nanoparticles widely used in commercial products include titanium dioxide (TiO_2), zinc oxide (ZnO), silica (SiO_2), silver (Ag), copper oxide (CuO), carbon such as carbon nanotube and fullerene, etc. These engineered nanoparticles are inevitably released into the environment and have created significant concerns regarding their potential to cause adverse effects on the environment and human health [5,6].

Due to the increasing attention to the safety of nanoparticles, many studies have examined the harmful effects of nanoparticles on human health. Donaldson et al. [8] demonstrated that carbon nanotubes could induce pulmonary inflammation through pharyngeal aspiration. Also, Trouiller et al. [9] reported that TiO_2 could cause genotoxicity in vivo in mice through inflammation and/or oxidative stress. Furthermore, Lewinski et al. [10] claimed that exposure to various nanoparticles could be cytotoxic. To date, nanoparticles are believed to be possibly cytotoxic, genotoxic, and carcinogenic to humans, and can cause other undesirable side effects [6].

Over the past several years, a large number of studies have also evaluated the aquatic toxicity of nanoparticles. To address the aquatic toxicity of various types of nanoparticles, microorganisms, algae, invertebrates, and fish have been generally used [11–16]. Sinha et al. [13] have investigated the toxic effects of Ag and ZnO nanoparticles on bacteria, suggesting that their toxicity was largely affected by the nature of membrane (Gram positive and negative bacteria). According to the work of Aruoja et al. [11], the toxicities of TiO_2 and CuO nanoparticles to *Pseudokirchneriella subcapitata* were more toxic than their counterpart bulk chemicals. Recently, it has been also reported that Ag nanoparticles coated with citrate or

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* Corresponding author. Tel.: +82 2 958 5844; fax: +82 958 5839.
E-mail address: swhong@kist.re.kr (S.W. Hong).

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polyvinylpyrrolidone could be taken into gill cells and cause cytotoxic effects on rainbow trout [12]. For the toxicity tests, aqueous suspensions of nanoparticles have been prepared using a few different methods (e.g., stirring, ultrasonication, filtration and solvent addition) because of their unique properties such as insolubility and easy aggregation in water. However, methods used to prepare the nanoparticle suspension could have a considerable impact on the results of the toxicity tests. In fact, for C_{60} fullerenes, the 48h-LC_{50} values for *Daphnia magna* were significantly dependent upon the preparation methods [17–19]. These data suggest that it is essential to elucidate the changes in acute toxicity in response to different preparation methods for stock suspensions of nanoparticles. However, to the best our knowledge, little effort has been directed towards quantifying and estimating the effects of preparation methods on the toxicity of metal nanoparticles.

Of all the metal nanoparticles studied, Ag nanoparticles are now widely used in numerous consumer products including catalysts, antimicrobial materials, paint, textiles, laundry additives and even food storage containers [20]. Also, CuO nanoparticles are widely used in antimicrobial preparations, catalysts, semiconductors and heat transfer fluids [21]. Due to the well-known toxicity of the ionic forms of some metals, a larger number of studies have examined their toxicity to aquatic invertebrates such as daphnids [22,23]. Recently, several studies have shown that Ag and CuO nanoparticles exhibit relatively high toxicity to *D. magna* due to the ions (i.e. Ag^+ and Cu^{2+} ions) released from the nanoparticle surface [24,25]. However, there is still much debate about the causes of the acute toxicity associated with Ag and CuO nanoparticles.

We hypothesized that different methods of preparing the stock suspensions of nanoparticles would result in different responses to the acute toxicity tests since it is believed that the dissolved concentration released from the nanoparticle suspensions can vary with respect to the preparation methods and act as a crucial determinant of the acute toxicity. In the present study, changes in toxicity of nanoparticles prepared using different preparation methods were evaluated using *D. magna* as a model organism. Ag and CuO nanoparticles were selected as model nanomaterials because they are the most toxic metals towards *D. magna*. We exposed *D. magna* to nanoparticle suspensions prepared under different conditions, for example, by changing the dispersion method, the initial concentration of the stock suspensions, their particle size, and the filtration conditions. The changes in their toxicity were then compared in terms of total and dissolved concentrations.

2. Materials and methods

2.1. Preparation of Ag and CuO nanoparticle suspensions

All chemicals, including Ag (<100 nm, cat. 576832 and <150 nm, cat. 484059) and CuO (<50 nm, cat. 544868) nanoparticles, used in this study were purchased from Sigma Aldrich (USA). The stock suspensions of nanoparticles were prepared by adding the appropriate amounts of each nanoparticle in moderately hard reconstituted water consisting of 96 mg L^{-1} $NaHCO_3$, 60 mg L^{-1} $CaSO_4{\cdot}2H_2O$, 60 mg L^{-1} $MgSO_4$ and 4 mg L^{-1} KCl at pH 7.6 [26]. By preparing 0.1 g L^{-1} Ag and 0.5 g L^{-1} CuO nanoparticle suspensions with either stirring for 24 h or sonicating for 1 and 2 h [11,27], the effect of different dispersion methods on toxicity was investigated. Stirring was performed at 150 rpm with a magnetic stirrer, and sonication was conducted with an ultrasonicator (Power Sonic 420, Seoul, Korea) at a frequency of 40 kHz. To investigate the influence of filtering on the toxicity of nanoparticles [15], 0.1 g L^{-1} Ag and 0.5 g L^{-1} CuO nanoparticle suspensions sonicated for 2 h were filtrated through 0.05, 0.1, and 0.45 μm membrane filters (Whatman GmbH, Germany). For the experiment to determine the relationship between initial concentrations of stock suspensions and toxicity, the stock suspensions having 0.1, 0.5 and 1.0 g L^{-1} of Ag and CuO nanoparticles were prepared, followed by sonication for 2 h. The comparative toxicity of the different particle sizes was assessed using two stock suspensions of Ag nanoparticles having particle sizes of less than 100 and 150 nm [15]. The stock suspensions were sonicated for 2 h. Each stock suspension was used in the toxicity tests immediately after preparation.

2.2. Size and zeta potential measurements

The hydrodynamic size and zeta potential of nanoparticle suspensions were analyzed using Zetasizer Nano ZS (Malvern, UK). For the size measurements, 2 mL of the sample was delivered into a disposal polystyrene cuvette, and then the measurement was performed with at least 20 runs at a duration of 10 s (632.8 nm laser source and 173° backscatter). Also, the zeta potential was measured with 10 runs by injecting approximately 1 mL of sample into a clear disposable zeta cell.

2.3. Toxicity testing

According to the USEPA protocol [26], all acute toxicity tests were performed on *D. magna* obtained from National Institute of Environmental Research in Korea. Briefly, each toxicity test consisted of 6–9 concentrations of test solution and one control with four replicates. The nominal exposure concentrations of Ag and CuO nanoparticles were determined on the basis of initial toxicity results. The highest exposure concentrations of Ag and CuO nanoparticles ranged from 1 to 32 mg L^{-1} and 50 to 800 mg L^{-1} with a serial dilution factor of 2, respectively. Moderately hard reconstituted water was used as the dilution water and control. Five neonates less than 24 h old were placed in 50 mL of vessel containing 30 mL of test solution. After 24 h of exposure to the test solutions at 20 °C with a photoperiod of 16 h light and 8 h darkness, immobilized neonates were counted. Neonates that were not able to swim within 15 s were considered to be immobilized after gentle agitation of the test vessel. To create dose-response curves and calculate the median effect concentration (EC_{50}) values, a two-parameter logistic model was fitted to the immobility of *D. magna* [15]. The curves were generated using the self-defined regression equation in SigmaPlot 10.0 (Systat software, CA, USA). Also, the variability was expressed using a 95% confidence interval.

2.4. Metal analysis

When measuring the total concentrations of Ag and Cu in Ag and CuO nanoparticle suspensions, all stock suspensions prepared under conditions were acidified with high purity nitric acid (Sigma Aldrich, USA) and digested according to EPA Method 3050 [28]. To obtain dissolved fractions of Ag and CuO nanoparticle suspensions, 10 mL of sample was transferred to Amicon Ultra-15 centrifugal filter unit with a nominal molecular weight limit of 10 kDa (Millipore, USA), followed by centrifugation at 5000 × *g* for 20 min [29]. The Ag and Cu concentrations were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, Varian Vista PRO, CA, USA) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, Varian 820-MS, CA, USA). The detection limits of ICP-OES and ICP-MS for both metals were 5 μg L^{-1} and 1 ng L^{-1}, respectively.

3. Results and discussion

3.1. Hydrodynamic sizes and zeta potentials

The size and zeta potential of the model nanoparticles dispersed in moderately hard reconstituted water are summarized