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# Ecotoxicity of different-shaped silver nanoparticles: Case of zebrafish embryos

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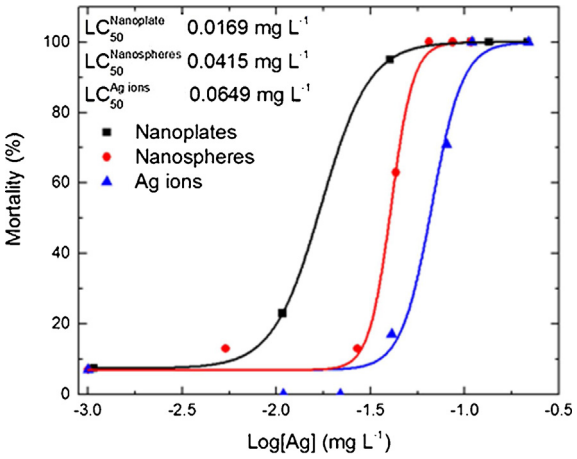
## HIGHLIGHTS

- Toxic effect of flat and spherical silver nanoparticles on zebrafish embryos was evaluated.
- Both types of silver nanoparticles appeared toxic to *Danio rerio* embryos.
- Silver nanoplates induced higher toxicity than nanospheres and $Ag^+$ ions for embryos.

## GRAPHICAL ABSTRACT

Ecotoxicity order (zebrafish emryos): Ag nanoplates > Ag nanospheres > $Ag^+$ ions.

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## ABSTRACT

As the worldwide application of silver nanomaterials in commercial products increases every year, and concern about the environmental risks of such nanoparticles also grows. A clear understanding of how different characteristics of nanoparticles contribute in their toxic behavior to organisms are imperative for predicting and control nanotoxicity. Within our research, we investigated the toxic effect of two types of silver nanoparticles (spherical and flat Ag nanoparticles) on zebrafish (*Danio rerio*) embryos. Particular interest was paid to proper characterization of Ag nanoparticles initially and during the experiment. A proper test medium was found and used for ecotoxicity evaluation. The behavior of flat silver nanoparticles with respect to embryos of zebrafish was analyzed and compared to the ecotoxicity of silver ionic form ($AgNO_3$). Both types of nanoparticles showed a more pronounced toxic effect to *Danio rerio* embryos than silver ions ($AgNO_3$), while silver nanoplates were more harmful than Ag nanospheres.

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While previous investigations showed that toxicity of Ag nanoparticles can be explained by the presence of $Ag^+$ in solution of silver nanoparticles, our results demonstrate that the harmful effects of nanosilver may be associated with silver nanoparticles themselves than with ionic silver released into solution.


## 1. Introduction

The field of nanomaterials has experienced unprecedented growth during the last few years. Due to the unique properties of nanoparticles (NPs), they found wide commercial applications [1,2]. According to Vance et al. [2], the number of the products with the prefix ïanoïn 2012 exceeded 1500 and in 2014 this number came close to 2000 units, while the silver nanoparticles (Ag NPs) represent 24% of the total production in nanotechnology industry. Subsequently, this NPs had received a great attention from the public and scientific communities. Silver NPs have been widely investigated as toxic materials for numerous organisms and cell lines [3–5]. Toxicity of Ag NPs is usually correlated with silver ions emission from the surface of NPs [6–9]. On the other hand, Ag NPs might cause themselves a negative effect on life system due to specific properties of Ag NPs or a combined mechanism of toxicity due to the presence of both $Ag^+$ ions and Ag NPs may take place [10,11].

Due to a small size and high surface area to volume ratio of NPs, they can strongly interact with life cells and demonstrate high toxicity to cells and tested animals [12]. Size-dependent toxicity of Ag NPs was shown in a large number of publications [13–15], they usually postulate that the NPs with smaller sizes cause more harmful effects to tested organisms and cell lines compared with larger particles. The role of other physical and chemical parameters is often not taken into consideration. However, European Centre for Ecotoxicity and Toxicity Of Chemicals (ECETOC) and the Organization for Economic Cooperation and Development (OECD) recommend to consider also other physicochemical characteristics in case of toxicity of NPs [16–18]. The shape (or morphology) of NPs, their surface area, size distribution and other parameters of NPs are the most prominent key factors that may determine the activity of NPs.

In this paper, we evaluated the toxicity of flat and spherical Ag NPs using zebrafish (*Danio rerio*) embryos as test organisms. The influence of NPs on the survival, hatching rate and morphogenesis of zebrafish embryos was analyzed. Toxicity of NPs was compared with the data for $Ag^+$ ions and toxic effect of a solution with supernatant and stabilizers.

## 2. Experimental

### 2.1. Chemicals

Silver perchlorate hydrate ($AgClO_4 \cdot H_2O$, 99%), silver nitrate ($AgNO_3$, 99%), sodium borohydride ($NaBH_4$, 98%), sodium citrate tribasic dihydrate ($Na_3Cit$, 99.5%), sodium polyphosphate (PPNa, 96%), and polyvinylpyrrolidone (PVP, Mw. 360000) were purchased from Sigma-Aldrich. Sodium formate (HCOONa, 99%) was purchased from Merck, and hydrogen peroxide ($H_2O_2$, 30% aqueous solution) was obtained from ZAO «Baza №1 Chimreactiv».

### 2.2. Characterization

The absorption spectra of silver colloidal solutions were measured using a Cary 100 Scan UV–vis spectrophotometer (Varian Inc., USA) equipped with a thermostated cuvette compartment at 20 °C (wavelength range 190–900 nm, resolution 2 nm, integration time 0.1 s, data interval 1 nm, scan speed 600 nm/min$^{-1}$). Nanoparticle sizes as well as values of the ζ-potential in the colloidal solution were determined by a dynamic light scattering (DLS) technique. The measurements were performed with a Delsa™ Nano C particle analyzer (Beckman Coulter, USA) at a wavelength of 658 nm. Prior to the onset of the measurements, the examined solution was thermostated at 20 °C.

The sizes and shapes of the silver NPs were analyzed in a Leo-912 AB Omega (Carl Zeiss, Germany) and JEM-2100 (JEOL, Japan) transmission electron microscopes. For this purpose, a droplet of a silver colloidal solution was kept on a formvar-coated copper grid for 30 s. The histograms were computed using ImageJ (NIH) on a minimum of 100 randomly selected NPs.

### 2.3. Synthesis of silver nanoplates

Triangular silver nanoplates with a different size were synthesized according to Mirkin's method with some modifications [19]. Nanoplates with other forms were obtained by varying the time of heating the solutions of triangular nanoplates and by varying the reagents concentrations. The most of the NPs were synthesized by the similar procedure to minimize the differences in the chemical composition of suspensions.

#### 2.3.1. Different time of heating

Aqueous solutions of $AgClO_4$ (60 ml, 1 mmol L$^{-1}$), trisodium citrate (108 ml, 0.1 mmol L$^{-1}$), PVP (42 ml, 0.1 mmol L$^{-1}$ on the basis of the monomer) and $H_2O_2$ (1.44 ml, 30% by mass) were combined with $NaBH_4$ (48 ml, 0.1 mmol L$^{-1}$). The obtained solution (Ag_1.1) was divided into three parts. Samples Ag_1.2 and Ag_1.3 were prepared by heating 200 ml of the primary solution at 95 °C during 3.5 and 10 min, respectively.

#### 2.3.2. Different concentration of a reducing agent ($NaBH_4$)

Silver nanoplates were obtained by varying the sodium borohydride concentration: 0.4, 0.6, 0.7, and 0.8 mmol L$^{-1}$. Deionized water (72 ml), a 1 mmol L$^{-1}$ solution of $AgClO_4$ (20 ml), a 10 mmol L$^{-1}$ solution of sodium citrate (36 ml), a 10 mmol L$^{-1}$ solution of PVP (14 ml), and a 98.4 mmol L$^{-1}$ solution of hydrogen peroxide (42 ml) were mixed together under thorough stirring. Then a 5, 7.5, 8.75, or 10 mmol L$^{-1}$ solution of sodium borohydride (16 ml) was rapidly added to the resulting mixture. The samples obtained were denoted as Ag_2.1, Ag_2.2, Ag_2.3, and Ag_2.4. The solution changed color after 0.5 h from initially pale-yellow to blue.

### 2.4. Synthesis of silver nanospheres

Spherical silver NPs were synthesized by two methods: (1) chemical reduction (Ag_sphere) as described above with the exception of adding peroxide into the reaction mixture and (2) reduction of $Ag^+$ by γ-irradiation (Ag_gamma) in aqueous solutions [20].

The solutions containing 0.1 mmol L$^{-1}$ $AgClO_4$, 1 mmol L$^{-1}$ HCOONa, and 0.5 mmol L$^{-1}$ sodium polyphosphate were prepared and deaerated by deep evacuation before irradiation. The solutions in special glass vessels equipped with a quartz cell for optical measurements were irradiated by using a $^{60}Co$ source.