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Influence of stabilizers on the antimicrobial properties of silver nanoparticles introduced into natural water

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ABSTRACT

Physical, chemical and biochemical properties of silver nanoparticles (AgNPs) depend to a great extent on their size, shape, size distribution, and stabilizers located on their surface. This study focused on two typical stabilizers, namely citrates (cit), low molecular ions protecting nanoparticles by electrostatic repulsion, and polyvinylpyrrolidone (PVP), a hydrophilic, neutral, high molecular polymer protecting nanoparticles by steric stabilization. Natural bacterioplankton was collected from a eutrophic, downtown lake and exposed to five concentrations (0.1–5 mg/L) of AgNPs-PVP and AgNPs-cit. Responses were monitored after 1, 3, 5 and 7 days of exposure, by evaluating the survival rate of bacteria, their respiratory activity, and the general activity of extracellular esterases. A significantly better (greater) survival rate of bacterioplankton was observed in water with an addition of AgNPs-cit. The inhibition of extracellular esterases was observed only in samples containing AgNPs-PVP. The inhibitory effect increased proportionally to the concentration of AgNPs-PVP applied. Within the studied concentration range, there was no statistically significant inhibition of bacterioplankton respiratory activity by AgNPs-PVP and AgNPs-cit.

Introduction

The last two decades saw a rapid development of nanotechnology. The structure, properties, and methods for obtaining nanoparticles (NPs) have been widely discussed in scientific literature (Kahru and Dubourguier, 2010). Nanoparticles have been successfully applied in electronics, tissue engineering, biomedicine, nanocomposite manufacturing, and everyday objects and substances such as paints, cosmetics, and underwear (Nanowerk Nanomaterial Database Inventory, 2012).

At the same time information on potential risks connected with NPs is 10–20 times less abundant that the information on their obtaining and applying. Recent research indicates that NPs are not as indifferent to human health and natural ecosystems as previously believed. Their large active surface area, biological reactivity, size, shape, durability, and hydrophobicity may facilitate their movement in the air, soil, and water (Colvin, 2003; Lecoanet et al., 2004; Biswas and Wu, 2005; Wiesner et al., 2006; Holbrook et al., 2008). Moreover, NPs can also act as carriers for dangerous contaminants dispersed on their surface, and thus promote their translocation in the environment (Kleiner and Hogan, 2003; Shelley, 2005; Maynard and Aitken, 2007). Although toxicological data on NPs are easily available (for different biological levels, from *in vitro* cell cultures to *in vivo* research in rodents), they cannot be directly related to environmental conditions. Although not yet extensively developed, the research on ecotoxicological properties of NPs seems to be of great importance (Nowack and Bucheli, 2007; Kahru and Dubourguier, 2010).

Silver nanoparticles (AgNPs) are currently one of the most common metal nanoparticles found in consumer

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products. From a practical point of view bactericidal and fungicidal properties of AgNPs, undeniably their most valuable features, guarantee a variety of medical applications: silver-based dressings as well as surgical clothes and medical instruments coated with AgNPs have gained immense popularity (Furno et al., 2004; Li et al., 2006; Duran et al., 2007; Rai et al., 2009; Hamouda, 2012). Owing to their bactericidal properties, materials containing AgNPs are widely applied in everyday products as well – they are added to detergents, food packaging, textiles including socks, underwear, and bed linen (Morones et al., 2005; Maynard and Michelson, 2006). This widespread use inevitably leads to the release of AgNPs to natural water bodies (Benn and Westerhoff, 2008; Hassellv and Kaegi, 2009; Kim et al., 2010); the results of numerous studies indicate that AgNPs have a negative impact on the crustaceans, algae and fish, and can be considered highly toxic because L(E)C50 < 0.1 mg/L (Kahru and Dubourguier, 2010).

Considering both the importance of microorganisms in the aquatic environment and the bactericidal properties of nanosilver, the impact of AgNPs on microorganisms in the natural ecosystem should be carefully examined. This study involved investigating bacterioplankton from a natural water body and assessing several indicators of microbial activity, including respiration and enzymatic activity of microorganisms.

Physical, chemical, and biochemical properties of AgNPs depend crucially on their size and shape (Morones et al., 2005; Pal et al., 2007), as well as on their surface functionalization (Wang et al., 2005). Stabilizers are used to control the process of obtaining AgNPs and then to prevent their aggregation. What happens to AgNPs in the environment (aggregation, sedimentation, oxidation) is directly related to their stability guaranteed by appropriate surface modification (Lok et al., 2007; Kittler et al., 2010). The study is based on two typical stabilizers, i.e. citrates: low-molecular ions protecting nanoparticles by electrostatic repulsion and polyvinylpyrrolidones (PVP), hydrophilic, neutral, high-molecular polymer protecting nanoparticles by steric stabilization. In previous studies of surface-dependent toxicity of AgNPs were used only bacterial strains (El Badawy et al., 2011; Xiu et al., 2012; Sadeghi et al., 2012) or model organisms (Yang et al., 2012; Tejamaya et al., 2012). In this study, we compared the antimicrobial activity of AgNPs introduced into natural water, because the research on influence of AgNPs on environmental microorganisms seems to be not yet extensively developed.

1 Materials and methods

1.1 Materials preparation

Silver nanoparticles were prepared in water solution by a chemical reaction of silver nitrate ($AgNO_3$) with sodium borohydride ($NaBH_4$) in the presence of one of the stabilizers: polyvinylpyrrolidone (AgNPs-PVP) or sodium citrate (AgNPs-cit). Reference solutions of the same composition except for silver nanoparticles were prepared using HNO_3 instead of $AgNO_3$. All solutions were aged for a few days, then pH was set at 7, and the solutions were diluted to obtain the concentration of Ag equal to 100 mg/L.

Typical UV-Vis spectrum of AgNPs obtained by reduction of $AgNO_3$ with $NaBH_4$ possesses one maximum at 390–410 nm and FWHM (full width at half maximum) equal 50–70 nm. TEM enables to determine their shape, size and size distribution, silver nanoparticles obtained in this way and characterized by such UV-Vis spectrum are spherical, their size is in the range 5–20 nm with the maximum frequency equal 12–14 nm (Solomon et al., 2007).

UV-Vis spectrum of our AgNPs with maximum at 408 and 397 nm and FWHM equal 57.4 and 66 nm for AgNPs-PVP and AgNPs-cit respectively, enables to assume that our AgNPs are typical, similar to those obtained and characterized in other articles.

1.2 Natural bacterioplankton

Natural bacterioplankton was collected from a eutrophic, downtown lake Martówka in Toruń, Poland (**Table 1**). Natural lake water (1 L) in glass flasks was mixed with AgNPs-PVP and AgNPs-cit to achieve their final concentrations of 0.1, 0.5, 1.0, 2.0, and 5.0 mg/L. Responses were monitored after 1, 3, 5 and 7 days of exposure at 20°C. Control samples contained natural lake water. Reference samples (water with the solutions of the two stabilizers, i.e., citrates and PVP, the concentration of 5 mg/L) were prepared in order to exclude the influence of the stabilizers on bacterioplankton.

Table 1 Morphometric and trophic characteristics of “Martówka”

Characteristic	Value
Latitude	53°00′N
Longitude	18°34′E
Area	2.8 ha
Maximum depth	2.5 m
Maximum length	640 m
Maximum width	61 m
pH	8.3–8.5
Electrolytic conductivity	668–955 µS/cm
Water transparency	0.8-1.2 m
Total organic carbon	8.5–19.7 mg/dm^3
Chlorophyll *a*	1.44–16.88 µg/dm^3

The data source was from Dembowska, Nicolaus Copernicus University, Department of Hydrobiology, unpublished data.