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Review

Nanosilver — does it have only one face?

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Silver nanoparticles (NPs) have at least one dimension of a particle smaller than 100 nm and contain 20-15,000 silver atoms. Due to its antibacterial activity nanosilver (NS) is used for medical purposes. NS particles can be obtained by various methods. Potentially, the best method of the NS synthesis for medical purposes is based on a brief flow of electric current between two silver electrodes placed in deionized water. It is accepted that the major antibacterial effect of silver is its partial oxidation and releasing silver ions, which interact with thiol groups of peptidoglicans of bacterial cell wall, and proteins of the cell membrane causing cell lysis. Silver ions can also bind to bacterial DNA preventing its replication and stopping synthesis of bacterial proteins. The rise in exposure to silver NPs has spurred interest into their toxicology. NS undergoes a set of biochemical transformations including accelerated oxidative dissolution in gastric acid, binding to thiol groups of serum and tissue proteins, exchange between thiol groups, sulfides and selenides, binding to selenoproroteins and photoreduction in skin to zerovalent metallic silver. Animal studies have shown that exposure to NS may lead to liver and spleen damage. NS can also stimulate an increased secretion of proinflammatory cytokines by monocytes. As a spectrum of NS applications is still growing, the complex evaluation of a safety of its use becomes an important task. This requires an elucidation of not only the influence of NS on human cells and organism, but also its biotransformation in organism and in environment.

Key words: silver nanoparticles, antibacterial activity, toxicology

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INTRODUCTION

The story began in 1959 at the meeting of American Physical Society, when a Nobel Prize winner, Richard A. Frey, gave a lecture "There is plenty room at the bottom". He is considered as a father of the nanotechnology idea (Feynman, 1992). According to National Nanotechnology Institute, this field of science comprises research, and development aimed at noticing, comprehension, measuring, and manipulating the matter at a level of atoms and molecules (Scott, 2005). A word "nano" originates from Greek and means "dwarf". Reducing the overall dimensions of a single particle to nanoscale changes its properties and gives it unique physical, chemical and biological features. 100 nm is a threshold dimension. Beneath this value the relation between a surface of a particle to its mass is big enough to alter the properties of such particle. Ultrasmall particle size leads to ultralarge area per mass, where large population of atoms are in immediate contact with ambience and readily available for reaction. At the nanoscale particles exhibit different physical, optical and chemical properties owing to the dominant of quantum mechanics (Martinez-Gutrierrez *et al.*, 2010; Lok *et al.,* 2007; Martinez-Gutrierrez *et al.,* 2012; Chen & Schluesener, 2008).

Antibacterial properties of silver have been known since ancient times. In ancient Egypt silver bars were put into water, which was drunk as a medicine for ulcers. Food and wine were stored in silver vessels in order to prevent them from getting spoilt. Soldiers in Roman legions used to put silver coins on their wounds to accelerate their healing. In Mead Ages reach people were in the habit of giving their children silver spoons to suck as a protection against various diseases. Furthermore, a silver powder was administered orally as a medicine (Russell & Hugo, 1994).

In 1884 German obstetrician, C.S.F. Crede administered 1% silver nitrate solution to prevent Gonococcal conjunctivitis in neonates. This was probably the first scientifically documented usage of silver in medicine (Russell & Hugo, 1994). Chemical compounds containing silver were the main weapon against infections during the World War 2. Irreversible pigmentation of skin and eyes resulting from the deposition of silver compounds led to their withdrawal as antibacterial agents (Russell & Hugo, 1994; Spencer *et al.,* 1980).

Silver particles having at least one dimension, which is less than 100 nm containing 20–15000 silver atoms, are termed nanosilver (NS) or silver nanoparticles (NPs). They should not be confused with nanocrystals, nanosferes, or colloidal silver. The most important difference between NPs and nanocrystals is that nanocrystal is a crystalline nanoparticle or any singlecrystalline nanoparticle with at least one dimension not larger than 100 nm. Nanocrystal can be also definied as a nanoparticle with any kind of crystalline structure. By contrast, NPs do not have to posses any crystalline structure (Chen & Schluesener, 2008; Sun & Xia, 2002; Xu *et al.* 2008; Burt *et al.* 2005). Nanocrystals, nanosferes or colloidal silver is not discussed in this paper.

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Abbreviations: $[Ag(NH_3)_2]^+$, diamminesilver ion; Ag, silver; APTT, activated partial thromboplastin time; BMP-2, bone morphogenetic protein-2; EC20, the highest tested concentration causing less than 20% reduction in weight; HIV-1, human immunodeficiency virus-1; IL-10, interleukin-10; IL-6, interleukin-6; MMPs, matrix metalloproteinases; NPs, nanoparticles; NS, nanosilver; OmpA, outer membrane protein A; OmpC, outer membrane protein C; OmpF, outer membrane protein F; PLGA, poly(lactic-*co*glycolic) acid; PT, prothrombin time; PVP, poly(N-vinyl)-2-pyrolidone; TNF-α, tumor necrosis factor α; TNF-β, tumor necrosis factor β; UV, ultraviolet

Table 1. Methods of NS synthesis.

Methods of NS synthesis
Reduction of nitrate by a reducing agent e.g. sodium borohydrate
Photoreduction of nitrate by UV light
Synthesis using microorganisms
Reduction of nitrate by gamma radiation in a presence of chitosan
Synthesis of peptide-coated NS
Brief flow of electric current between two silver electrodes in deionized water

NANOSILVER SYNTHESIS

There are many methods of NS synthesis, however, not all of them allow to obtain NS particles for biomedical applications (Table 1). The most often used method is a reduction of silver nitrate using either a reducing agent, e.g. sodium borohydride, or a photoreduction via UV light (Sato-Berru *et al.,* 2009; Courrol *et al.,* 2007). During these reactions silver ion (Ag^+) receiving an electron from the reducing agent reverts to its metallic form (Ag^0) which clusters to form NS. Copping agents, such as citrate or starch, are used to prevent aggregation and agglomeration of NPs. Each cluster contains between 100 and 1000 atoms of silver. NS can be also sythetised by the use of various species of bacteria e.g. *Staphylococcus aureus* and fungi (Shahverdi *et al.,* 2007; Shaligram *et al.,* 2009). These microorganisms are the source of enzymes and they reduce such compounds like hydrochinons. Other method uses chitosan obtained from microorganisms. Silver nitrate is added dropwise to chitosan dissolved in acetic acid. Silver ions in this solution are reduced by gamma radiation and stabilized by chitosan (Reicha *et al.*, 2012). Another method is based on photoreduction of aqueous solution of $[Ag(NH_3)_2]^+$ by UV light in the presence of poly(N-vinyl)-2-pyrolidone (PVP). This method allows to obtain very small NPs of 4–6 nm, which may be too small for medical purposes (Vigneshwaran *et al.,* 2006).

The methods mentioned so far have some disadvantages. They do not allow for the precise control of NPs size resulting in a wide range of size of obtained NPs. The use of chitosan may alter the properties of NS. Graf *et al.* (2009) proposed a method of synthesis of peptide-coated silver NPs. This method was aimed at reducing the toxicity of NS, however peptide coated NS can aggregate in response to changes of pH. Acidic pH, which may occur at a place of pathology in human organism, e.g. inflammation, may cause agglomeration of peptide-coated NS, and can lead to occlusion of capillary blood vessels. This method of NS synthesis has another disadvantage. Peptide sequences can be immunogenic and trigger unwanted immune response (Chaloupka *et al.,* 2010).

Potentially, the best method of the synthesis of NS for medical purposes is based on a brief flow of electric current between two silver electrodes placed in deionized water. An application of high voltage causes silver atoms to evaporate from the electrodes and condense back into aqueous NS. As no chemicals are used in this method, NS does not contain toxic residues or contaminations (Xu *et al.,* 2008).

MECHANISMS OF NANOSILVER'S ANTIBACTERIAL ACTION

NPs possessing "altered" physical, chemical or optic properties may present a wide spectrum of their action (Fig. 1). Medicine knows the influence of NS on bacterial cells, fungi and viruses. NS can be useful against hundreds of bacterial species and theoretically, there is no problem of bacterial resistance like in case of antibiotic therapy. However, some researchers point out that in case of long term usage of NS there is a possibility of generation NS-resistant species of bacteria (Silver, 2003; Radzig *et al.,* 2013; Lok *et al.,* 2008).

It is supposed, that in aqueous solution NS releases silver ions, which are responsible for its antibacterial properties. However, a comparative study of few silver salts (nitrate, citrate and chloride) revealed, that NS particles have higher antibacterial potency than free silver ions (Morones *et al.*, 2005; Shrivastawa *et al.*, 2007; Yamanaka *et al.,* 2005).

Cystein is a compound of the bacterial wall. This aminoacid posses reactive thiol groups –SH. Silver ions interact with cysteine residues leading to protein inactivation. Apart from sulfur, silver has a high affinity to phosphorus as well. Forming complexes with compounds containing these elements in cell wall, silver can alter their activity (Gordon *et. al.,* 2010). Deposition of silver NPs in the bacterial cell surface can affect cell membrane permeability. NS can destroy both bacterial cell

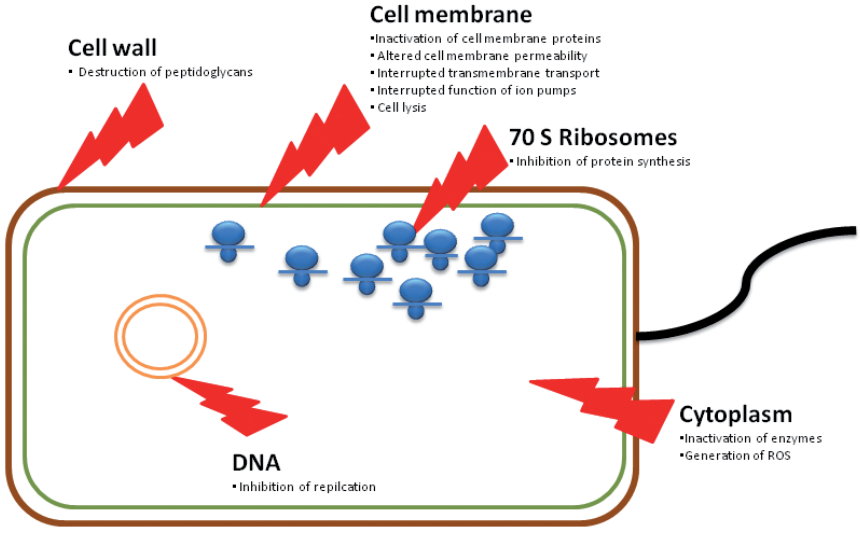

Figure 1. Mechanisms of the antibacterial activity of nanosilver (ROS, reactive oxygen species; 70 S, 70 Svedberg (sedimentation) units).
Detailed explanation can be found in the text.

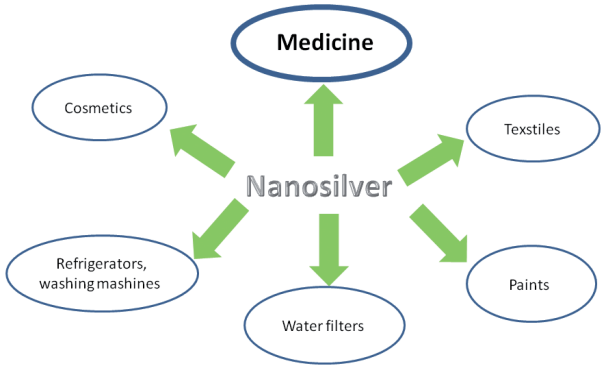

Figure 2. Various applications of nanosilver.

wall and cell membrane as well. It may lead to disturbances in transporting of ions and other substances between bacterial cell and its surrounding. The diminished activity of cell membrane sodium/potassium pumps results in water flux into bacteria cell and an enlargement of the cell volume (Semeykina & Skulachev, 1990). NS can destruct a cell membrane and lead to bacterial cell lysis. Silver ions can penetrate into a bacterial cell causing a damage of its intracellular structures. A denaturation of ribosomes leads to the inhibition of protein synthesis (Jung *et al.,* 2008; Bury & Wood, 1999; Solioz & Odermatt, 1995 & Morones *et al*., 2005).

Silver ions can bind to the bases constructing DNA. This condensation with DNA leads to its inability to replicate preventing the bacterial reproduction. NS particles binding to bacterial wall create a coat disrupting moves of bacterial flagella. This multifaceted antibacterial activity of silver and NS is a key to low bacterial resistance rates. The antibacterial action of silver ions and NS starts with the binding to peptidoglicans of bacterial cell wall. Mammalian cells do not poses cell wall covering cell membrane. However, other mechanisms of NS action can affect both bacterial and human cells. The next mode of NS action uses its catalytic properties of generating free protons. They interact with disulfur bonds breaking them and leading to the disfunction of integral proteins of outer cell membrane, such as OmpA, OmpC, OmpF, which are responsible for interactions between bacteria and their environment, stability of bacterial cells, or binding various substrates (Lok *et al*., 2008; Ratsig *et al.,* 2009).

Silver blocks some metabolic reactions taking place in cells. Silver combines with thiol residues of enzymes inactivating them. NS induces production of reactive oxygen species, which also takes part in a destruction of bacterial cell (Lok *et al*., 2007). NS also influences the cell wall of fungi, and interacts with proteins of proteinolipid core of viruses (e.g. HIV-1) (Panácek *et al*., 2009; Lara *et al.,* 2010; Elechiguera *et al.,* 2005).

Table 2. Toxicity of NS.

Toxicity of NS
Argyria
Oxidation of NS and ion release in digestive tract and uptake of these ions into blood
Binding of silver ions distributed by circulating blood to thiol groups of enzymes
Binding of silver to sulphides
Skin inflammatory response
Possible liver and spleen damage
Possible induction of proinflammatory response
Influence on coagulation
Possible cytotoxic effect to monocytes
Accumulation in brain

MEDICAL APPLICATIONS OF NANOSILVER

Due to its strong antibacterial properties, NS has a wide spectrum of medical and paramedical applications as well (Fig. 2 and Table 3). NS is used as an addition to various products, which should poses antibacterial properties. NS is a component of paints used to cover walls of hospital wards and operating rooms. It is also used to impregnate clothes. NS used in production of socks was to reduce odors (Benn & Westerhoff, 2008). NS was also added to a variety of cosmetic products for everyday hygiene, like soap, shampoos, deodorants, gels, and creams (Drake & Hazelwood, 2005; Lee *et al.,* 2007; Vigneshwaran *et al.,* 2007; Walser *et al.,* 2011).

Medical products have given a wide spectrum for the use of NS. It is used as an addition to protective clothes, mattresses, bed clothes, gloves, syringes, masks, and respirator tubes. The efficacy of these uses of NS is estimated even as 99%. A next field where NS has been introduced is a protection of infections of wounds, burnings, ulcers and pemphigus. Creams with solutions of silver nitrate have been used to accelerate healing of burns for a long time (Singh & Singh, 2012; Cho *et al.,* 2002; Lee *et al.,* 2002; Madhumati *et al.,* 2010). Dressings or bandages contain polyethylene nets with NS particles with dimensions of 10–15 nm. The application of such dressings can accelerate the process of wound healing of three days. Some dressings contain chitosan to prevent an absorbtion of NS from the dressing and its accumulation in patient's organism. Anti-inflammatory properties of NS were evaluated in pigs with experimental dermatitis. Treatment with dressings containing NS resulted in a reduction of serum levels of proinflammatory molecules, tumor necrosis factor α and β (TNF-α and TNF-β). There are three possible mechanisms of anti-inflammatory action of NS. A reduction of a release of proinflammatory cytokines, a reduction of a number of lymphocytes and mast cells, and the third one is the induction of apoptosis of the inflammatory cells. Matrix metalloproteinases (MMPs) play an important role in protracted ulcerations. Problems with healing of such ulcerations are connected with an overexpression of MMPs. Dressings containing NS can reduce production of MMPs. NS is also used in dentist seals and dentist dressings. These components contain NS connected with calcium phosphate (Huang *et al*., 2007; Lu *et al.,* 2008; Yang *et al.,* 2007; Wright *et al.,* 2002).

NS is also used to cover surgical threads and tools (Saxena *et al.,* 2011). Catheters introduced into veins to administer drugs and obtain blood samples, or monitor blood pressure in specific regions of circulatory system, are a potential gateway of infection. These catheters are covered with NS. This antibacterial protection is non toxic and can inhibit a growth of bacteria for at least 72 hours (Samuel &

Table 3. Medical applications of nanosilver.

Medical applications of NS
Wound dressings
Surgical threads
Various implants
Catheters
Bone cement
Materials for bone regeneration
Dentist seals and dressings
Syringes
Gloves
Bed clothes and mattresses
Respirator tubes
Wall paints
Protective gloves

Guggenbichler, 2004; Davenas *et al.,* 2002; de Mel *et al.,* 2012). Patients suffering from hydrocephalus often have implanted valve system containing catheters, which evacuate an excess of cerebrospinal fluid from brain chamber system to abdominal cavity. These catheters are a plausible gateway of bacterial infection spreading to central nervous system. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are dangerous bacterial species, which may cause meningitis. The use of NS to cover the catheters introduced into brain chambers reduces this risk (Roe *et al.,* 2008).

Orthopedics is a next branch of medicine where infections are dangerous and very difficult to treat. NS is used to cover various orthopedical implants as well as materials used for bone regeneration (Pishbin *et al.,* 2013). Some researchers used complexes of bone morphogenetic protein-2 (BMP-2) and NS particles with dimensions between 20 and 40 nm, which were placed on poly(lactic-*co*glycolic) acid (PLGA). They presented strong antibacterial properties. NS in these complexes did not present cytotoxic properties and did not inhibit an osteoinductive influence of BMP-2 on bones (Zheng *et al.,* 2010). NS is also used as a component of bone cement to prevent development of bacterial infections (Alt *et al.,* 2004). Complexes of connective tissue proteins, collagen, laminin or fibronectine and NS have been used to treat an experimental 10 nm break in rat ischiac nerve giving promising results (Ding *et al.,* 2011).

Antibacterial properties of NS are used in production of drug and food packings (Tankhiwale *et al*., 2009).

IS NANOSILVER TOXIC?

Silver NPs have become increasingly prevalent in various consumer products as antibacterial agents. The number of products containing NS has grown more than 10 times between 2006 and 2011 (Stensberg *et al.,* 2011). It is estimated that in 2015 more than 1000 ton of NS particles will be produced for use in commercial or industrial products (Stensberg *et al*., 2011). It is important to consider safety issues of the use of NS (Table 2). There are three fields where humans may be exposed to NS. Commercial products used in everyday life, such as water filters and water purificants, soap, deodorants, laundry detergents, room sprays, clothing, underwear, socks. The next field comprises various paramedical and medical products. The third aspect, often underestimated, is the environment pollution with NS (Liang *et al.,* 2010; Kiser *et al*., 2012; Wang *et al.,* 2012; Benn *et al*., 2010; Benn & Westerhoff 2008; Blaser *et al.,* 2008; Kim *et al.,* 2010).

For a long time NS has been considered as a quite safe antibacterial agent, and the only side effect of overdosage was an irreversible pigmentation of skin and/or eyes called argyria or argyrosis. Engineered nanomaterials can undergo profound transformation between the time of their synthesis, and reaching various tissues or intracellular structures. These changes may involve adsorbtion, chemical reactions, dissolution and aggregation influencing bioavailability, transport, accumulation and toxicity (Liu *et al.,* 2012).

The ways of exposure to NS include ingestion, inhalation, dermal contact, wound surface application, and insertion or implantation of medical devices. NS is unstable to oxidation and releases ions through gradual reaction with dioxygen and protons. Biological fluids have a wide range of pH. Liu *et al.* (2012) estimated the influence of pH on silver NPs dissolution. Acidic pH accelerated this process. In humans, this process takes place in stomach, where ingested NS is exposed to hydrochloric acid secreted there. Silver ions generated in digestive tract can be brought into blood stream through ion or nutrient uptake channels. However, the ability of silver particles to cross the gut epithelium is limited, so ion uptake seems to be the main route of silver absorbtion from gastrointestinal tract (Liu *et al.,* 2012; Johnston *et al.,* 2010). It is supposed, that silver ions may be transported by mechanism responsible for transport of sodium or copper ions (Bury & Wood, 1999; Solioz & Odermatt, 1995). In patients with argyria silver deposits in the connective tissue were found. It was also found that this deposits were collocated with sulphur and selenium. Silver deposits in patients with argyria are often placed in skin regions exposed to light. Majority of silver in circulation is predicted to be bound to thiol groups of proteins. Although silver has high binding affinity to these groups, it is easily exchangeable giving silver significant biomolecular mobility. Sulphides and selenides have higher binding affinities for silver, but their concentrations in biological fluids are lower. When silver complexes with thiol groups reach skin or near-skin region, it can be easily reduced by visible or UV light to metallic NS particles. This process results in an immobilization of silver as metallic NS. In this form silver has low particle diffusity and cannot undergo chemical thiol exchange reactions (Liu *et al.,* 2012). These findings put a new light on a pathogenesis of an old side effect of a treatment with silver compounds, argyria, and explain why skin regions exposed to light are the favorite sites of pigmentation in argyria.

Although silver NPs have been for a long time considered as non toxic to mammals, recent years have given new evidence making us look more cautiously at NS. Korani *et al.* (2011) performed on genuine pigs a study of acute and chronic dermal toxicity of colloidal NS. Skin inflammatory response was detected in all experimental animals. Despite the fact that NS was applied only topically, these exposures led also to slight liver and spleen damage detected in histopathologic examinations. This experiment proves that NS can be absorbed by skin and distributed through the organism. Other researchers (Martinez-Gutrierez *et al.,* 2012) evaluated an influence of NS on cultured human monocytes. NS induced secretion of proinflammatory cytokines, interleukin-6 and 10 (IL-6 and IL-10). These effects were observed

at low concentrations of NS (10 μg/mL). In this experiment, small NS particles were used, majority of them did not exceed 30 nm. The choice of small NPs, which are known to be more toxic, could have an important influence on observed results. As monocytes and macrophages constitute one of the main mediators of the immune response, these findings should be taken into consideration while estimating the safety of long term human exposure to NS. The same authors studied an influence of NS on coagulation of normal human plasma. NS did not influence the extrinsic pathway followed by prothrombin time (PT), but inhibited the extrinsic pathway of coagulation measured by activated partial thromboplastin time (APTT). In a previous study, (Martinez-Gutrierez *et al.,* 2010) they showed that cultured monocytes are sensitive to cytotoxic influence of NS when its concentration is greater than 5 μg/mL. Other experiment showed that mice injected with silver NPs presented a decrease in platelet aggregation (Shrivastava *et al.,* 2009). The influence of NS particles on presented aspects of coagulation system requires further studies and elucidation. Animal studies showed that inhalation of NS particles can lead to alveolar wall thickening and macrophage infiltration. However, silver NPs can be absorbed from lung alveoli and transported in blood to brain. It was confirmed in animal model that silver NPs injected into the blood stream can cross the blood-brain barrier and accumulate in brain. This deposition of NS can cause neuronal degeneration and necrosis (Sung *et al.,* 2008; Takenaka *et al.,* 2001; Tang *et al.,* 2008).

Other aspect of NS toxicity is focused in its influence on neoplasmatic cells. Moaddab *et al.* (2011) observed that NS with very small particles, with average size of 4.5 nm, presented a concentration-dependent toxicity for cultured osteoblast cancer cells. IC_{50} determined to 3.42 μg/mL suggested that these NS particles were more toxic to cancerous cells comparing to other heavy metal ions (Moaddab *et al.,* 2011). NS was also reported as toxic to human glioblastoma cells (Asharani *et al.* 2009). This study also showed a genotoxicity of NS. Ahamed *et al.* (2008) evaluated an influence of two kinds of NS particles on mouse embryonic stem cells and mouse embryonic fibroblasts. Both uncoated NS with dimension of 25 nm and polysaccharide surface coated NS elicited genotoxicity. An increase in expression of p53 protein was detected 4 hours after exposition to NS. Also and upregulation of DNA damage repair protein Rad51 was detected. Both forms of NS induced also apoptosis. Polysaccharide coated NS particles exhibited more toxic influence than uncoated. These differences in severity of genotoxic influence of NS particles of the same dimension were probably caused by the fact, that uncoated particles agglomerated, what limited the surface area availability and access to membrane bound organelles (Ahamed *et al.,* 2008). These results raise a question about consequences of long term, low level exposure to this kind of NS particles or NS at all. Shrivastava *et al.* (2012) evaluated the influence of NS and silver ions at subtoxic doses on selenium metabolism in cultured keratinocytes and human adenocarcinomic alveolar basal epithelium cells. Both NS and silver ions led to a significant decrease in incorporation of selenium into selenoproteins, such as glutathione peroxidase, thioredoxin reductase, or methionine sulfide reductase. These enzymes play vital role in the defence against oxidants, such as superoxide or peroxides. They contain selenocysteine at their active sites. This decrease in synthesis of selenoproteins is like to have significant implications in the defence against oxidative stress. Thioredoxin reductase plays also a crucial role in production of a reduced thioredixin for the ribonucleotide reductase, and thus DNA synthesis. The high affinity of silver for selenium leads not only to silver immobilization causing argyria, but also can lead to disturbances in DNA synthesis, increased oxidative stress in cell and damages of cell structures caused by reactive oxygen species (Shrivastava *et al.,* 2012).

In 1939 Hill and Pillsbury evaluated the exposure limit above which the development of argyria could be expected. The threshold value was found to be the intake of 0.9 g of silver over the whole lifetime (Hill & Pilsbury, 1939). Although this value was calculated more than 70 years ago, American modern drinking water standard for silver concentration (less than 100 μg/L) is based on this value. Discussing the toxicology of silver one should consider a distinction between bulk metallic silver, NS and silver ions. American Conference of Governmental Industrial Hygienists has established separate thresholds limits values for metallic silver (0.1 mg/m^3), and soluble compounds of silver (0.01 mg/m^3) (Nowack *et al.,* 2011). In 2010 it was estimated that about 320 tons of NS were produced and used worldwide each year (Nowack *et al.,* 2011). Silver and NS released from various materials, especially from silver algicides and disinfectants used in swimming pools is discharged into sewer system, wastewater treatment plants and natural waters. Does it cause an increased risk of long time exposure to humans? Many of the aquatic species are several orders of magnitude more sensitive to silver than mammals and humans. For some of those organisms lethal concentration is only 1–5 μg/L (Nowack *et al.,* 2011). *Daphnia magna*, an aquatic invertebra, has focused the researchers' interest. It has been observed that this organism accumulates NS from aquous, as well as a foodborne exposure (Zhao & Wang, 2011). Hoheisel *et al.* (2012) observed an increased toxicity of NS with decreasing particle size. However, both 96 hours and 7 days sublethal 20% effective concentrations (EC20) were not significantly different for NS and silver ions. Interesting results were obtained by Shi *et al.* (2012). Comparison of toxicity of NS particles and silver ions to *Tetrahymena pyriformis* gave various results depending on conditions of the experiment. The toxicity of NS was higher than silver ions in the dark environment without light, but under the light condition the toxicity of NS decreased greatly. The presence or absence of light did not influence the toxicity of silver ions. The light irradiation could induce the enlargement of NS particles and formation of bulk agglomeration resulting in losing the properties of NPs and slowing the release of silver ions. NS is not only the product of industrial nanotechnology, but also a result of spontaneous formation in environment and biological systems following exposure to traditional forms of silver like silver nitrate. On the other hand, manufactured silver NPs released to the environment from various commercial products can agglomerate and lose the properties characterizing NPs (Liu *et al.,* 2012).

CONCLUDING REMARKS

NS can have a wide spectrum of medical, paramedical and everyday use. Taking into consideration that in various environmental conditions silver can undergo spontaneous transformation leading either to creation of NS particles from silver ions, or agglomeration of NS into greater particles, sometimes it is difficult to demarcate the toxicity of traditional silver compounds and NS. The complete evaluation of safety of products containing NS

requires an elucidation of not only the influence of NS on human cells and organism, but also its biotransformation in the organism and in the environment. Centuries ago Paracellsus said: "everything is a poison and nothing is a poison, it is only a matter of a dose". In case of NS it is a matter of both dose and particle size.

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