

Risk assessment for silver nanoparticles in environment

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Abstract

Nanotechnology, the science which offers great opportunities, involves manipulation and application of engineered particles or systems that have at least one dimension being less than 100 nm in length. Products of nanotechnology, e.g. silver nanoparticles are widely used in many industries due to their unusual properties, which are dependent on the nanometric sizes. Despite the fact that materials containing nanosilver are widely available, their possible impact on the human health and the environment is still not fully understood. Detailed information about the risk assessment for nanoparticles is required, especially data on the potential toxicity, routes of exposure and environmental effects, including application, transformation and bio-accumulation. The main aim of this paper is to present the possible negative interaction of nanosilver on the environment and toxicity to organism, as well as to draw attention to a new type of the waste – nanowaste and present probable ways to eliminate a destructive impact of nanoparticles.

Key words: nanotechnology, silver nanoparticles, risk assessment, toxicity, nanowaste, prevention

Introduction

Nanotechnology as a science about producing structures, devices and systems with one dimension between 1 and 100 nm is described as one of the key technologies of the present century. According to the National Nanotechnology Initiative of the U. S. government the main aim of this science is the research and development to understand and work with a matter at the atomic, molecular and supramolecular levels (Scott, 2007). In the other words the nanotechnology is focused on preparing the miniature products determined by the size of their elements. Nanoproducts e.g. nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology, if compared with the larger particles of the bulk material they are made of (Song and Kim, 2009). Using these unique features will allow to control many processes at the level of atoms and also offers opportunities to create new devices with unprecedented functionality (Świdorski and Waszkiewicz-Robak, 2006).

Recently, the attention has been focused around the metallic nanoparticles which are widely used in the different areas of life due to their unusual properties (Panyala et al., 2008). One of the most important advantages of them is a large surface to the volume ratio with decreasing size of nanoparticles leading to increasing the chemical activity. Highly developed surface area has a significant effect on the adsorption properties, reactivity of nanomaterials, optical features and the other properties including antimicrobial activity (Song and Kim, 2009; Panighari et al., 2004).

Risks associated with nanotechnology

Though the research in nanotechnology continues apace, relatively little is known about the subsequent health effects of the exposure. Despite the fact that materials containing nanoparticles are widely available, one of the side effects of nanotechnologies is the release of nanomaterials to the environment. It may have a hazardous effects on the environment and living organisms, therefore the risk assessment of used nanomaterials is needed.

As mentioned, the nanomaterials show quite different features in comparison with the bulk materials and their toxicity may be different from that of the same chemical substance in the form of larger particles. There are examples where nanoparticles can produce toxic effects even if the bulk substance is nonpoisonous (Dowling, 2004). For example there is demonstrated that the ultrafine TiO₂ particles (20 nm diameter) elicited a persistently high inflammatory reaction in the lungs of the rats compared to the larger-sized particles (250 nm diameter) (Oberdörster et al., 1994). Also the research studies of Chae et al. (2009) suggest that two different silver forms (silver ions and silver nanoparticles) have various impact on the fish *Oryzia lapites*. The silver nanoparticles appear to be more toxic than the ionic form of silver which leads to cellular damage.

Due to smaller size, nanoparticles easier penetrate cells which is not always desirable, moreover they have increased surface so they are more reactive. Therefore more research on the toxicity of nanoparticles is needed, because besides the many advantages they can be also

a threat. It should be noted that the potential risks of available nanoproducts are currently the subject of many different studies and toxicological effects are poorly understood despite rigorous risk control assessment (Hallock et al., 2009). To illustrate the problem, as an example there could be a new anti-bacterial material with the silver nanoparticles for the meat packing. The main advantage is the longer product shelf life and disadvantages include the uncertainty whether the silver nanoparticles might migrate from the packaging material into the meat. Another use of nanoscience with unexplained effects on the health of living organisms is a nanomaterial with the beta-carotene closed in small particles which better dissolves in the water, but experts still do not know the side effects of the nanoproduct (Siegrist et al., 2007). It is obvious that not all nanomaterials have a negative impact on the surrounding nature. Toxicity experiments of American researchers demonstrated that colloidal spherical silver nanoparticles and silver nanoprisms of 30 nm sizes are not cyto-, photo- or genotoxic to human skin HaCaT keratinocyte (Lu et al., 2010). However the risk assessment needs to protect the environment from the unwanted release.

Applications of silver nanoparticles

In recent years the nanostructured silver particles have been the subject of much intensive research. Already in 2007 at Woodrow Wilson International Center of Scholar there have been tested more than 500 commercial nanoproducts and 20 % of them contained silver nanoparticles (Lu et al., 2010).

At the beginning, silver was used for making ornament, but after the discovery of the extraordinary antimicrobial properties silver was utilized for preservation of food, water and milk and for the treatment of wounds and infections (Tien et al., 2008). Actually chemicals based on nanosilver are applied in electronic, optic, chemical and textile industries, as well as in pharmacy, cosmetology, medicine, food production and packing where they play a significant role as substrates for synthesis, catalytic materials, sensors, conductors, detergents or antimicrobial coatings (Xu et al., 2006; Siegrist et al., 2007; Chen et al., 2009; Lu et al., 2010). It is also possible to modify materials and raw materials by the deposition of the silver nanoparticles on the carrier or covering their different surfaces, whereby they shown deodorize, antistatic and impregnation properties (Zhang et al., 2007).

Influence of the silver nanoparticles on living organisms

Particles with the dimension below 100 nm are synthesized not only in industrial processes, but also the nature enriches the environment by the different nanosized particles. They are generated by natural events such as volcanic eruptions, forest fires and they are components of fumes formed during the automobile exhaust or other industrial combustion reactions (Navarro et al., 2008; Hallock et al., 2009; Bystrzejewska-Piotrowska et al.,

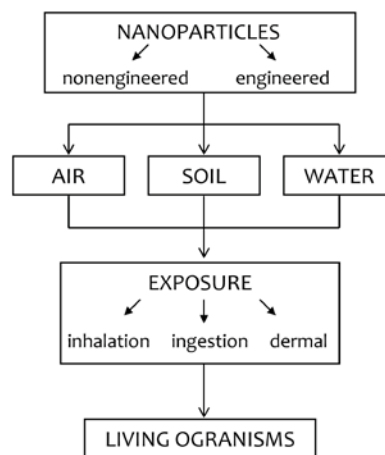


Fig. 1. Pathways of nanoparticles in the environment.

2009). In other words, people have always had contact with the nanoparticles of various origin. Rapid growing production of nanomaterials caused the increase of amount of nanomaterials in the environment, what which may have adverse effects on organisms.

Due to the multiplicity of routes of penetration into the body of organisms, many studies on the toxicity of nanoparticles on living organisms have been conducted. However, they do not give an unequivocal answer whether nanosilver harms, because some reports demonstrate the toxic effects on the health and another show that silver nanoparticles do not induce any negative consequences. There exist three main groups of exposures – inhalation, ingestion and skin absorption (Friedrichs and Schulte, 2007; Hoyt and Mason, 2008; Hallock et al., 2009). For the silver nanoparticles the most frequent is inhalation and skin absorption routes due to size, mass and shape of nanoparticles (Hyun et al., 2008; Larese et al., 2009).

It is found that silver metal and silver dressing has no negative effects (or even favourable) when is used in reasonable amounts, but many scientific reports prove that silver in various forms, also, nanosized silver is extremely toxic to some plants, fungi, bacteria, fish and mammalian cells (Panyala et al., 2008). It is shown that silver nanoparticles disintegrated cell membranes of bacteria e.g. *Escherichia coli* and *Staphylococcus aureus* and kill them (Martínez-Castañón et al., 2008). Moreover, as already mentioned silver especially silver nanoparticles causes changes in the expression of the stress-related genes of fish leading to disruption of metabolism and DNA damage (Chae et al., 2009). Other research showed that silver nanoparticles induced embryo-cytotoxicity in the mouse blastocysts and decrease their viability increasing apoptosis (Li et al., 2010). Cytotoxic effect of nanosilver was also demonstrated using the in vitro rat liver derived cell line (Hussain et al., 2005). It was noted that the presence of the silver and other nanoparticles leads to oxidative stress and interferes with mitochondrial function. Braydich-Stolle et al. (2005) estimated that silver nanoparticles already at 5 to 10 µg/ml concentration caused changes like necrosis and apoptosis of cells and drastically reduces mitochondrial

activity. Further research suggests that nanoparticles can have a severe toxic effect on the male reproductive system by the capability to cross the blood-testes barrier (Panyala et al., 2008). Nanosized silver also easily pass through the blood-brain and skin barriers in the model animals (Borm and Kreyling, 2004).

On the other hand, there are reports of non-toxic effect of silver nanoparticles on living organisms like mentioned earlier tests results of nanosilver influence on the human skin HaCaT keratinocyte. Another example are studies on the nasal septum respiratory mucosa of rats. The animals exposure on the silver nanoparticles by inhalation exhibited the changes in the properties of the goblet cells in the respiratory system, but without toxicological significance (Hyun et al., 2008). Similar results were observed during experiments on mice, which shown lack of negative changes in the functionality of organism. Two different concentrations of the specially prepared silver nanoparticles were injected intra-peritoneally. Despite of penetration of the nanosilver across the blood brain barrier it was not observed toxicity. Additionally, silver nanoparticles apparently can be an alternative to conventional chemotherapy by increased neurotransmission and activity of the brain (Daniel et al., 2010).

It is true that organism's response to the presence of nanoparticles may be different and in the vitro models there is a simplification of natural conditions, nevertheless each result can be a basis for the further assessing of the potential risk of exposure. The amount of potential applications of silver nanoparticles shows that more research is needed to improve nanomaterials and release them of any toxicity.

Influence of silver nanoparticles on the environment

It is believed that the development of nanotechnology may help to protect the environment by improving the water and food quality and reducing the energy consumption. Among the numerous applications of nanotechnology, there are already many that involve the environment. Using the iron nanoparticles for remediating of contaminated land and waters (Owen and Depledge, 2005; Tratnyek and Johnson, 2006), europium oxide nanoparticles for detecting the antrazine pesticide in the water (Matecka, 2007) or silver nanoparticles in the water treatment applications as antibacterial water filter (Lv et al., 2009) are only some examples.

However, there are also alarming reports concerning the uncontrolled release of nanomaterials (nanowastes) to the atmosphere, the soil and to the aquatic systems. Fig. 1 presents a schema of the nanoparticles pathways in the environment.

This problem also includes the silver nanoparticles. Based on several studies, it can be concluded that nanosilver appears to be a threat for natural ecosystems and thus indirectly to living organisms. Silver and silver nanoparticles exhibit toxic effect on human-friendly microbes like heterotrophic and chemolithotrophic bacteria in the soil, which play a major role in the nitrogen fixation

and decomposition of inorganic matters (Panyala et al., 2008). The model for nanoparticles toxicity in aquatic system is in vitro culture of rainbow trout hepatocytes. There was investigated the influence of the different forms of silver on trout hepatocytes and the results showed that the nanosilver was highly cytotoxic and even at low concentrations nanoparticles reduced of metabolic activity and the membrane integrity (Farkaš et al., 2010). Moreover, researchers observed that silver nanoparticles were slightly higher toxic than silver ions. It is found that the silver nanoparticles toxicity was mediated by the silver ions – nanoparticles were interacting with the cells and thereby releasing a high number of ions directly inside the cells. Another example proving accumulations of nanosilver in the aquatic environments are experiments on zebrafish embryo. Silver nanoparticles increased apoptosis in nanoparticles-treated embryos and caused brain, heart, eyes injury and other serious damages (Navarro et al., 2008). Other reports demonstrated that, silver nanoparticles also have the negative influence on the phytoplankton. In the study of Miao et al. (2009) silver nanoparticles cause the inhibition to the cell growth of coastal marine diatom *Thalassiosira weissflogii*.

It should be paid an attention that some living organisms like fungi or plants also introduced silver nanoparticles into the soil environment. Extracellular biosynthesis has been shown e.g. in *Fusarium solani* (Ingle et al., 2009) and in the different plants as *Magnolia kobus* or *Ginkgo boloba* (Song and Kim, 2009).

Because the nanotechnology is a relatively young branch of science, the release of nanomaterials to the environment is still not fully understood. It is not known yet what concentration, size and shape of nanoparticles are toxic in different environments, what are mechanisms of the trophic transport and exposure routes, therefore the further research are absolutely needed.

Nanowaste – possible ways to eliminate it

New, rapidly developing science – nanotechnology leads to the formation of a new type of the waste – nanowaste. It contains collections of nano-objects like engineered particles, fibres or plates in the nanoscale. Due to the diversity of nanoobjects synthesized by the nature or researchers their potential threats to environment and the composition of the nano-waste is a very different. Depending on the amount, types and composition of the nano-objects the different quantities of nanowaste are produced (Allan et al., 2009). There exist four main types of nanowastes, which can act as an ecotoxicological hazard through the biodegradation or bioaccumulation in the food chain:

1. Pure materials,
2. Items contaminated with nanoparticles,
3. Liquid suspensions containing nanomaterials,
4. Solid matrices with nanomaterials (Hallock et al., 2009).

It is necessary to find how nanomaterials will be treated and disposed of after the end of their use, therefore the

examination of the harmfulness must include: identification and quantification of the sources, determination of the environmental release pattern, establishment of concentrations in the environment, and examination of the potential bioaccumulation (Bystrzejewska-Piotrowska et al., 2009).

It is obvious that all the problems concerning the hazard evaluation of nanotechnology cannot be rapidly solved, but so far has been proposed many ideas for possible ways to eliminate them. Firstly, due to nanoparticles are 100 % respirable it could be considered to change of form. For example the reducing the airborne concentrations may be obtained by the receiving or handling nanomaterials only in wet form (Hoyt and Mason, 2008). An interesting idea about the removal of nanoparticles from the environment encompass a method developed by Iwashita et al. (2007). They elaborated a device for trapping nanoparticles by collision and attaching them by their surface. The trapping efficiency of the device for nanoparticles above 2 nm in size is more than 99.8 % at a low pressure. Another promising way to decrease of toxicity is the surface modifications by adding different functional groups or encapsulation. Interesting results of experiments conducted with the use of carbon nanotubes. Test consisted from the adding various chemical groups to an aqueous solution of the carbon nanotubes, which changed the surface structure of nanotubes. As a result, there was observed the reduction of mortality of the human skin cells from 50 % to more than a dozen (<http://www.nanotec.org.uk>). For the identification and observation (morphology, composition or interparticles relations, distributions of nanomaterials in the organisms) there is possible to use the fluorescent detection, surface plasmon resonance, photocatalytic detection and another analytical techniques (Silbergeld et al., 2010; Zhang and Fang, 2010).

Attention is also drawn to a nanowaste recycling, emphasizing segregation, nanowaste storage, recovery and reuse. Other ideas are based on the possibility of bioaccumulation of nanowastes (mainly nanoparticles) by plants, fungi and microorganisms as is the case with the heavy metals. It is believed that bioutilization may help to remediate the environment and protect fauna and flora (Bystrzejewska-Piotrowska et al., 2009).

Conclusions

New types of risks to the environment resulting from the release of nanomaterials on the environment should be considered. Therefore the potential risks of the silver nanoparticles and another nanomaterials are currently the subject of hundreds conducted studies. Numerous institutions are collecting data about ongoing research projects and the test results – e.g. the International Council of Nanotechnology – ICON, the Woodrow Wilson International Centre for Scholars, National Nanotechnology Initiative – NNI and other (Friedrichs and Schulte, 2007; Hoyt and Mason, 2008).

Considering the nanotechnology it is needed to look not only what damaged it causes, but what opportunities

it offers. Phillipus Aureolus Bombastus von Hohenheim also referred to as Paracelsus created the classic toxicology maxim: "All things are poison and nothing is without poison; only the dose makes a thing not a poison." Currently, still is little known about the effects of the implementation of nanotechnology, however, it is estimated that in the future the world will develop, owing to nanotechnology, free of any threat.

References

- ALLAN, J., REED, S., BARTLETT, J. & CAPRA, M., 2009: Comparison of methods used to treat nanowaste from research and manufacturing facilities. Australian Institute of Occupational Hygienists Annual Conference, Canberra, Australia, 5 – 9 December 2009.
- BORM, P. J. & KREYLING, W. J., 2004: Toxicological hazards of inhaled nanoparticles: Potential implications for drug delivery. *J. Nanosci. Nanotechnol.*, 4, 521 – 551.
- BRAYDICH-STOLLE, L., HUSSAIN, S., SCHLAGER, J. J. & HOFMANN, M. C., 2005: In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. *Toxicol. Sci.*, 88, 412 – 419.
- BYSTRZEJEWSKA-PIOTROWSKA, G., GOLIMOWSKI, J. & URBAN, P. L., 2009: Nanoparticles: Their potential toxicity, waste and environmental management. *Waste Manage.*, 29, 2 587 – 2 595.
- CHAE, Y. J., PHAM, C. H., LEE, J., BAE, E., YI, J. & GU, M. B., 2009: Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*). *Aquat. Toxicol.*, 94, 320 – 327.
- CHEN, D., QIAO, X., QIU, X. & CHEN, J., 2009: Synthesis and electrical properties of uniform silver nanoparticles for electronic applications. *J. Mater. Sci.*, 44, 1 076 – 1 081.
- DANIEL, S. C. G. K., THARMARAJ, V., SIRONMANI, T. A. & PITCHUMANI, K., 2010: Toxicity and immunological activity of silver nanoparticles. *Appl. Clay Sci.*, 48, 547 – 551.
- DOWLING, A. P., 2004: Development of nanotechnologies. *Nano Today*, 12, 30 – 35.
- FARKAS, J., CHRISTIAN, P., URREA, J. A. G., ROOS, N., HASSELLÖV, M., TOLLEFSEN, K. E. & THOMAS, K. V., 2010: Effects of silver and gold nanoparticles on rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.*, 96, 44 – 52.
- FRIEDRICH, S. & SCHULTE, J., 2007: Environmental, health and safety aspects of nanotechnology – implications for the R&D in (small) companies. *Sci. Technol. Adv. Mater.*, 8, 12 – 18.
- HALLOCK, M. F., GREENLEY, P., DIBERARDINIS, L. & KALLIN, D., 2009: Potential risks of nanomaterials and how safety handle materials of uncertain toxicity. *J. Chem. Health Saf.*, 16, 16 – 23.
- HOYT, V. W. & MASON, E., 2008: Nanotechnology. Emerging health issues. *J. Chem. Health Saf.*, 15, 10 – 15.
- HUSSAIN, S. M., HESS, K. L., GEARHART, J. M., GEISS, K. T. & SCHLAGER, J. J., 2005: In vitro toxicity of nanoparticles in BRL 3A rat liver cells. *Toxicol. In Vitro*, 19, 975 – 983.
- HYUN, J.-S., LEE, B. S., RYU, H. Y., SUNG, J. H., CHUNG, K. Y. & YU, I. J., 2008: Effects of repeated silver nanoparticles exposure on the histological structure and mucins of nasal respiratory mucosa in rats. *Toxicol. Lett.*, 182, 24 – 28.
- INGLE, A., RAI, M., GADE, A. & BAWASKAR, M., 2009: *Fusarium solani*: A novel biological agent for the extracellular synthesis of silver nanoparticles. *J. Nanopart. Res.*, 11, 2 079 – 2 085.
- IWASHITA, S., KOGA, K. & SHIRATANI, M., 2007: A device for trapping nano-particles formed in processing plasmas for reduction of nano-waste. *Surf. Coat. Technol.*, 201, 5 701 – 5 704.
- LARESE, F. F., D'AGOSTIN, F., CROSERA, M., ADAMI, G., RENZI, N., BOVENZI, M. & MAINA, G., 2009: Human skin penetration of silver nanoparticles through intact and damaged skin. *Toxicology*, 255, 33 – 37.

- LI, P.-W., KUO, T.-H., CHANG, J.-H., YEH, J.-M. & CHAN, W.-H., 2010: Induction of cytotoxicity and apoptosis in mouse blastocysts by silver nanoparticles. *Toxicol. Lett.* (in press).
- LU, W., SENAPATI, D., WANG, S., TOVMACHENKO, O., SINGH, A. K., YU, H. & RAY, P. C., 2010: Effect of surface coating on the toxicity of silver nanomaterials on human skin keratinocytes. *Chem. Phys. Lett.*, 487, 92 – 96.
- LV, Y., LIU, H., WANG, Z., LIU, S., HAO, L., SANG, Y., LIU, D., WANG, J. & BOUGHTON, R. I., 2009: Silver nanoparticle – decorated porous ceramic composite for water treatment. *J. Membr. Sci.*, 331, 50 – 56.
- MALECKA, B., 2007: Nanotechnologie i nanoproducty. *Wszechświat*, 108, 112 – 115.
- MARTÍNEZ-CASTAÑÓN, G. A., NIÑO-MARTÍNEZ, N., MARTÍNEZ-GUTIERREZ, F., MARTÍNEZ-MENDOZA, J. R. & RUIZ, F., 2008: Synthesis and antibacterial activity of silver nanoparticles with different size. *J. Nanopart. Res.*, 10, 1 343 – 1 348.
- MIAO, A.-J., SCHWEHR, K. A., HU, C., ZHANG, S.-J., LUO, Z., QUIGG, A. & SANTSCHI, P. H., 2009: The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. *Environ. Pollut.*, 157, 3 034 – 3 041.
- NAVARRO, E., BAUN, A., BEHRA, R., HARTMANN, N. B., FILSER, J., MIAO, A.-J., QUIGG, A., SANTSCHI, P. H. & SIGG, L., 2008: Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology*, 17, 372 – 386.
- OBERDÖRSTER, G., FERIN, J. & LEHNERT, B. E., 1994: Correlation between particle size, in vivo particle persistence, and lung injury. *Environ. Health Prospect.*, 102 (Suppl. 5), 173 – 179.
- OWEN, R. & DEPLEDGE, M., 2005: Editorial. Nanotechnology and the environment: Risks and rewards. *Mar. Pollut. Bull.*, 50, 609 – 612.
- PANIGRAHI, S., KUNDU, S., GHOSH, S. K., NATH, S. & PAL, T., 2004: General method of synthesis for metal nanoparticles. *J. Nanopart. Res.*, 6, 411 – 414.
- PANYALA, N. R., PEÑA-MÉNDEZ, E. M. & HAVEL, J., 2008: Silver or silver nanoparticles: A hazardous threat to the environment and human health? *J. Appl. Biomed.*, 6, 117 – 129.
- SCOTT, N. R., 2007: Nanoscience in veterinary medicine. *Vet. Res. Comm.*, 31 (Suppl. 1), 139 – 144.
- SIEGRIST, M., COUSIN, M.-E., KASTENHOLZ, H. & WIEK, A., 2007: Research Report, Public acceptance of nanotechnology foods and food packaging: The influence of affect and trust. *Appetite*, 49, 459 – 466.
- SILBERGELD, E., CLARK, K., NYLAND, J. & KATZ, H., 2010: Nanoscale methods for nanotoxicology. Abstracts. *Toxicol. Lett.*, 196, 283.
- SONG, J. Y. & KIM, B. S., 2009: Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioproc. Biosystems Eng.*, 32, 79 – 84.
- ŚWIDERSKI, F. & WASZKIEWICZ-ROBAK, B., 2006: Nanotechnologia – terażniejszość i przyszłość. *Postępy techniki przetwórstwa spożywczego*, 1, 55 – 57.
- TIEN, D.-C., TSENG, K.-H., LIAO, C.-Y., HUANG, J.-C. & TSUNG, T.-T., 2008: Discovery of ionic silver in silver nanoparticle suspension fabricated by arc discharge method. *J. Alloy. Compd.*, 463, 408 – 411.
- TRATNYEK, P. G. & JOHNSON, R. L., 2006: Nanotechnologies for environmental cleanup. *Nano Today*, 1, 44 – 48.
- XU, J., HAN, X., LIU, H. & HU, Y., 2006: Synthesis and optical properties of silver nanoparticles stabilized by gemini surfactant. *Colloids Surf., A*, 237, 1 – 3, 179 – 183.
- ZHANG, L. & FANG, M., 2010: Review. Nanomaterials in pollution trace detection and environment improvement. *Nano Today*, 5, 128 – 142.
- ZHANG, W., QIAO, X. & CHEN, J., 2007: Review. Synthesis of silver nanoparticles – Effects of concerned parameters in water/oil microemulsion. *Mater. Sci. Eng. B*, 142, 1 – 15.
- <http://www.nanotec.org.uk/finalReport.htm> (The Royal Society & The Royal Academy of Engineering Nanoscience and nanotechnologies, July 2004)

Rukopis doručený 22. 6. 2010

Rukopis akceptovaný red. radou 7. 9. 2010

Revidovaná verzia doručená 19. 10. 2010