ACUTE AQUATIC TOXICITY OF GOLD NANOPARTICLES TO FRESHWATER GREEN ALGAE

Kateřina DĚDKOVÁ[a], Zdeněk BUREŠ[b], Jiří PALARČÍK[c], Miroslav VLČEK[b], Jana KUKUTSCHOVÁ[a]

[a] *Nanotechnology Centre, VSB - Technical University of Ostrava, Ostrava, Czech Republic, EU,* katerina.dedkova@vsb.cz

[b] *Department of General and Inorganic Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic, EU*

[c] *Institute of Environmental and Chemical Engineering, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic, EU*

Abstract

Gold nanoparticles are currently being extensively prepared and studied due to their unique physicochemical properties. Gold nanoparticles are used in biomedical fields, optical engineering, catalysis and other fields of human interest. It is widely known that these materials may have adverse effects to various organisms including green algae. Nano-gold may be released from commercially available products and pose an environmental risk to freshwater organisms e.g. *Desmodesmus subspicatus*. The paper describes the study of acute aquatic toxicity of gold nanoparticles prepared via reduction with ascorbic acid to freshwater green algae. These nanoparticles were observed to be capable of interacting with freshwater green algae and thus have potential adverse effects to freshwater phytoplankton.

Keywords: Acute aquatic toxicity, gold nanoparticles, *Desmodesmus subpsicatus, Raphidocelis subcapitata*

1. INTRODUCTION

The increased applications of engineered nanomaterials (ENPs) in new materials developed in industry require increased attention given to the respect of their ecotoxicity, therefore identification of environmental risks is needed. Aquatic systems contain ecologically significant organisms which can serve as s detection organisms for evaluation and determination of toxicity of chemical solutions or different substances [1].

The toxic effect of engineered nanomaterials is being influenced by several key factors e.g. their intrinsic nature and capacity to form larger aggregations (in size and shape), the apparent route of exposure, dose response, exposure time, the response of the receptor organisms to the lack of biocompatibility of ENPs, the way of preparation of ENPs and the interactions in the mechanisms involved in the physiological process of uptake. Additionally, their transport in the water column (suspensions, colloids, sediments) and some important environmental factors e.g. salinity, temperature and dissolved oxygen need to be discussed inside the overall biological complexity of the water media [1].

Studies dealing with aquatic toxicity of gold nanoparticles (Au-NPs) with different size and coatings to freshwater algae, *Daphnias* or zebra fish embryos were already published, however authors resolved contrarily Au-NPs toxicity, where some authors identified that Au-NPs are toxic to certain organisms [2,3], others concluded that Au-NPs are non-toxic to the same detection organisms [4,5].

The objective of this study was to evaluate acute aquatic toxicity of Au-NPs prepared via new laboratory method to freshwater green algae *Desmodesmus subspicatus* and *Raphidocelis subcapitata*.

2. MATERIALS AND METHODS

2.1 Preparation of gold nanoparticles

$HAuCl_4$ was used as an initial solution for preparation of gold nanoparticles. 25 ml of 1mM $HAuCl_4.3H_2O$ (Carl Roth GmbH + Co. KG) was added into 50 ml of redistilled water, vigorously stirred and subsequently

reduced by gradual addition of 25 ml ascorbic acid solution (0,06% wt, Penta) in redistilled water. Final concentration of the prepared colloid suspension was 41 mg/L. The prepared gold nanoparticles were stabilized using polyviniylpyrrolidone (PVP K 25, mol wt ~24000, Carl Roth GmbH + Co. KG), where 1.5 ml of 0.3% (wt) PVP was added into 100 ml of gold nanoparticles colloid suspension.

2.2 Characterization of gold nanoparticles

The size of colloidal gold particles was determined using Malvern Zetasizer Nano ZS. The average hydrodynamic size of the particles was determined in redistilled water by dynamic light scattering (DLS) operating at 633 nm (He-Ne laser, with a light output of 4 mW). Measurent was performed at 25 °C in a polystyrene measuring cell. The value of hydrodynamic diameter was statistically evaluated from 12 parallel measurements (arithmetic mean and relative standard deviation).

2.3 Toxicological testing

Acute aquatic toxicity bioassay was carried out according to ČSN EN ISO 8692 Standard [6] and OECD Guideline 201 [7]. The aim of the test was to determine the median inhibition concentration EC50, i.e. the concentration of a toxicant which causes 50% inhibition of algal cells growth in comparison with control [8]. As detection organisms for the evaluation of toxicity, *Desmodesmus subspicatus* and *Raphidocelis subcapitata*, freshwater green algae, were used. Toxicity bioassay was performed with 3 days old algae culture, which was counted before the test using light microscope CX31 (Olympus) and counting Bürker chamber. It enabled to calculate the volume of culture to be added at the beginning of the test to suspension of nutrient medium and tested gold nanoparticles. The initial colloid suspension was diluted to concentrations 0.082, 0.041, 0.0205, 0.01025 and 0.005125 $mg \cdot ml^{-1}$ respectively. The samples prepared for toxicity tests were placed in the air-conditioned box at temperature (23±2) °C, with exposure to 24 hours daylight on a shaker (125 ± 25) rpm for 72 hours. At the end of the test, growth inhibition was calculated using light microscope with counting Bürker chamber [6, 7].

3. RESULTS AND DISCUSSION

3.1 Characterization of gold nanoparticles

From the distribution curve (**Fig. 1**) obtained by DLS the fact that colloid gold suspension is a polydisperse system with two significant maxima about 5 nm and 58 nm can be deduced. The average hydrodynamic diameter of gold nanoparticles is about 43.67 ± 0.85 nm.

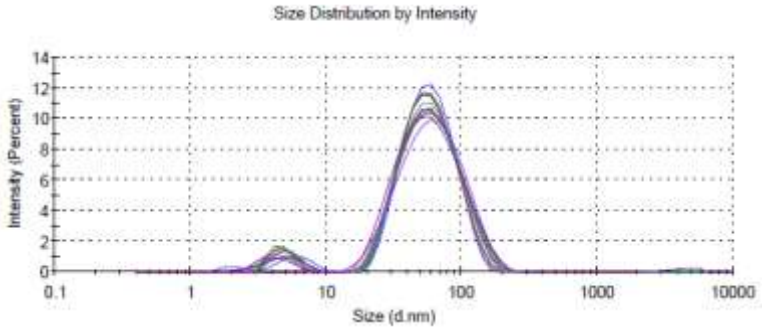

Fig. 1 Size distribution of prepared gold nanoparticles

3.2 Toxicological testing

Effective concentration of toxicity (EC_{50}) was determined from the acquired experimental data (numbers of cells). EC_{50} was subtracted from the biomass growth curve inhibition in % to a concentration of the sample in

mg · ml^{-1} (**Fig. 2**) for gold nanoparticles without the addition of PVP. The parameter EC_{50} was determined to be for the concentration of 0.028 mg · ml^{-1} to *D.subspicatus* and 0.014 mg · ml^{-1} to *R.subcapitata*, respectively.

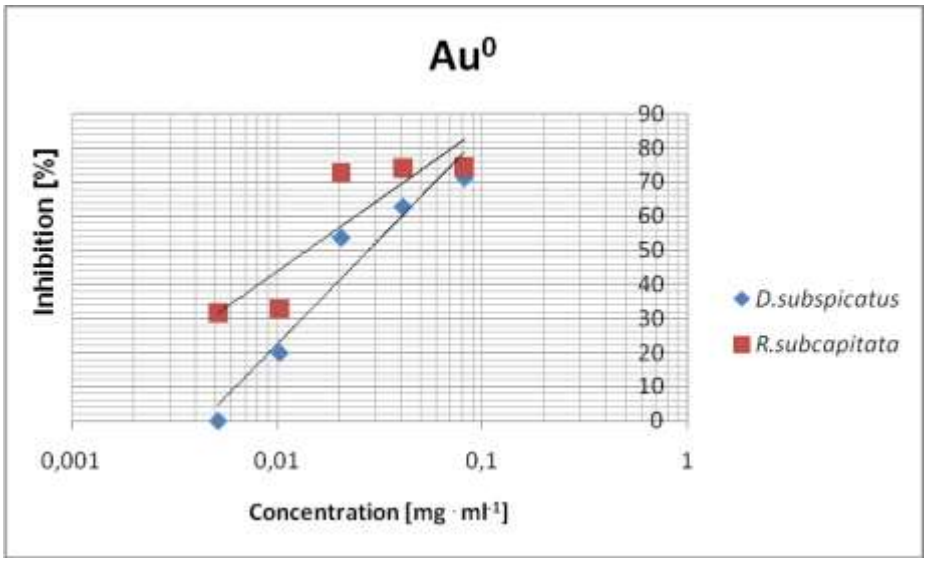

Fig. 2 Biomass growth inhibition curves for single algal strains exposed to gold nanoparticles

In the case of gold nanoparticles stabilized by PVP, the parameter EC_{50} was determined in the same way only to *D.subspicatus* (**Fig. 3**) as a detection organism. On contrary to the previous experiments, gold nanoparticles stabilized by PVP exhibited growth stimulation in the range of 2-18% according to selected concentrations to *R.subcapitata*; therefore EC_{50} cannot be subtracted from the biomass growth inhibition curve. The parameter EC_{50} was determined for the concentration of 0.4823 mg · ml^{-1} to *D.subspicatus.*

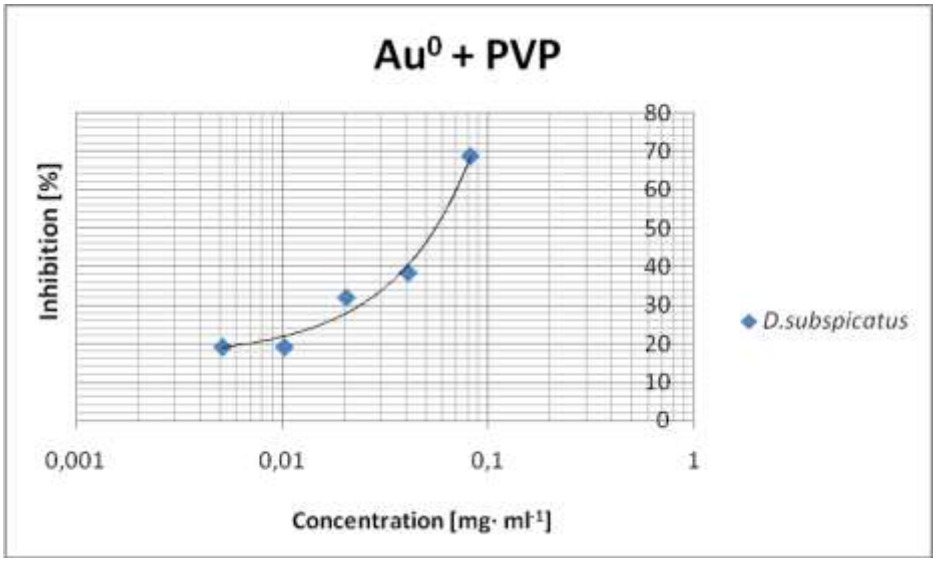

Fig. 3 Biomass growth inhibition curve for gold nanoparticles stabilized by PVP.

One potential description for the toxicity of AuNPs is that its toxicity may be related to the generation of ROS, which may be linked to the properties of AuNPs as a catalyst. Hence, the activation of molecular oxygen by AuNPs is possible with or without the presence of water [8]. Co-adsorbed water and O_2 generate atomic

oxygen and hydroperoxy intermediates; hydroperoxy is considered a precursor to the creation of atomically-adsorbed oxygen and hydroxyl, which activate the production of molecular oxygen and ROS. It has also been revealed that Au ions – Au^{3+} or possibly $AuCl_4^-$ (where the precursor is $HAuCl_4$,), depending on the pH, are associated with significantly higher ROS levels [9]. Each living organism has different needs to life conditions; therefore the toxicity effect of AuNPs is different to each one. PVP coating of AuNPs may affect the metabolism of *R.subcapitata*, mechanically or physico-chemically touch the life conditions of it and thus cause growth stimulation. Mattson and Calabrese [10] presented a definition of the hormesis phenomenon: "Hormesis describes any process in which a cell, organism, or group of organisms exhibits a biphasic response to exposure to increasing amounts of a substance or condition (e.g., chemical, sensory stimulus, or metabolic stress); typically, low dose exposures elicit a stimulation or beneficial response, whereas high doses cause inhibition or toxicity." This theory may also explain growth stimulation of *R.subcapitata* caused by PVP-coated AuNPs, when low concentrations of PVP-coated AuNPs were used. Further experiments are planned for better understanding of this problem.

CONCLUSIONS

Gold nanoparticles with the size equal to 43.67 ± 0.85 nm were prepared via simple laboratory method due to reduction of $HAuCl_4.3H_2O$ with ascorbic acid. Prepared Au-NPs were stabilized with addition of PVP. Consequently, Au-NPs prepared by this approach were evaluated for acute aquatic toxicity to freshwater green algae (*Desmodesmus subspicatus, Raphidocelis subcapitata*). It was found that 50% inhibition of algal growth in culture is caused by the non-stabilized Au-NPs in concentrations 0.028 mg/ml to *D.subspicatus* and 0.014 $mg \cdot ml^{-1}$ to *R.subcapitata*, respectively. 0.4823 $mg \cdot ml^{-1}$ of PVP-stabilized Au-NPs caused 50% inhibition of algal culture of *D.subspicatus*, however PVP-stabilized Au-NPs caused growth stimulation of *R.subcapitata*. As a final point, it is important to attempt to form proper methodology for evaluation of the toxicity of nanomaterials, which are not currently adequate in comparison with conventional methods for assessing the toxicity of bulk materials.

ACKNOWLEDGEMENTS

The study was supported by projects Reg.No.SP2014/52, LH12184, P204/11/0832 (Czech Science Foundation), 0021627501 (the Czech Ministry of Education, Youth and Sports) and was elaborated in the framework of the Nanotechnology – the basis for international cooperation project, reg. no. CZ.1.07/2.3.00/20.0074 supported by Operational Programme 'Education for competitiveness' funded by Structural Funds of the European Union and state budget of the Czech Republic.

REFERENCES

[1] LAPRESTA-FERNANÁNDÉZ, A., FERNANDÉZ, A., BLASCO, J., Nanoecotoxicity effects of engineered silver and gold nanoparticles in aquatic organisms. *Trends in Analytical Chemistry*. 2012, vol. 32, issue 1, p 40-59.

[2] HARPER, S. L, CARRIERE, J. L., MILLER, J. M., HUTCHINSONS,J.E., MADDUX,B.L.S., TANGUAY, R.L., Systematic evaluation of nanomaterial toxicity: utility of standardized materials and rapid assays. *ACS Nano.* 2011, vol. 5, issue 6, p. 4688–7697.

[3] BAR-ILLAM, O., ALBRECHT, R.M., FUERGESON, D.Y., Toxicity assessments of multisized gold and silver nanoparticles in zebrafish embryos. *Small*. 2009, vol.5, issue 16, p.1897-1910.

[4] FARKAS, J., CHRISTIAN, P., URREA, J.A.G., ROOS, N., HASSELLOV, M., TOLLEFSEN, K.E., THOMAS, K.V., Effect of silver and gold nanoparticles on rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxicology*. 2010, vol.96, issue 1, p44-52.

[5] LOVERN, S.B., OWEN, H.A., KLAPER, R., Electron microscopy of gold nanoparticles intake in the gut of *Daphnia magna*. *Nanotoxicology*. 2008. vol. 2, p. 43-48.

[6] Český normalizační institut. Jakost vod – zkouška inhibice růstu sladkovodních zelených řas. *Česká technická norma ISO 8692: 2004*, 2005.

[7] Alga, growth inhibition test 201; organization for economic cooperation and development; OECD guidelines for testing chemicals 201, 1984.

[8] CHEN, Z., BERTIN, R., FROLDI, G. EC50 estimation of antioxidant activity in DPPH assay using several statistical programs. *Food Chemistry*. 2013, vol. 138, issue 1, p. 414-420.

[9] GONG, J., MULLINS, C., Surface Science investigations of Oxidative Chemistry on Gold. *Accounts of Chemical Research.* 2009, vol. 42, issue 8, p.1063-1073.

[10] MATTSON, M.P., CALABRESE, E.J., editors. Hormesis: a revolution in biology, toxicology and medicine. New York: Humana Press, 2009.