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PREPARATION OF NOBLE NANOPARTICLES BY SPUTTERING – THEIR CHARACTERIZATION

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In this work we present gold (AuNPs) and platinum (PtNPs) nanoparticles prepared by direct sputtering into liquid medium polyethylene glycol (PEG) with molecular weight of 600 g Mol^{-1}. PEG was chosen for its properties and ability to stabilize NPs. The metal/PEG dispersions were mixed with water for their stabilization. This approach for nanoparticles preparation can be realized without harmful reducing agents or additional stabilizers and resulted in preparation of spherical AuNPs and rod PtNPs of the size below 10 nm. The $D_{0.9}$ diameter of AuNPs was 6.3 nm. On contrary, the $D_{0.9}$ diameter of PtNPs was 3.9 nm. The nanoparticles were characterized by transmission electron microscopy, atomic absorption spectroscopy, ultraviolet-visible spectroscopy and by dynamic light scattering. *In vitro* tests of cytotoxicity were carried out with prepared AuNPs and PtNPs and human osteoblastic cells and more cytotoxic effect was observed for AuNPs in comparison with PtNPs of similar concentrations.

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1. Introduction

Nowadays nanoparticles represent one of the most studied topic. They have an important role in many areas such as catalytic reactions [1], medicine [2-4], food industry [5], etc. So this research topic is studied by scientists from different fields of science e.g. physicists, scientists, architects and researcher [6]. In recent years metal nanoparticles were used from industry to fine medical or biochemical utilization [7]. In medicine, metal nanoparticles were used as drug delivery systems, e.g. functionalized AuNPs or contrast agents. For example, magnetic resonance imaging (MRI) supported with Pt or PdNPs on Al_2O_3 can provide high sensitivity during magnetic resonance imaging. Other well-known metal nanoparticles used in medicine are AgNPs. They are used as antimicrobial wound dressings [8]. The development in the field of nanotechnology focused on nanoparticle preparation is influenced by several requirements including disease diagnosis and therapy, energy and environmental protection. There are several approaches for gold nanoparticle preparation. The solutions based on polyethylenglycol, glycerol and several other liquids were proposed as liquid media for gold nanoparticle preparation and consequent testing as potentially suitable for antibacterial applications [9-11] or in tissue engineering for analysis of cell adhesion [12-14].

Many of chemical and physical methods have been used to prepare nanoparticles. Chemical approaches are based on reduction of metal compound to form colloidal solutions. These

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solutions contain stabilizers to prevent aggregation of nanoparticles. There is problem with by-product and it is necessary to perform purifying steps after synthesis. Also almost all of these methods require the stabilizers, that means other chemical substances. Physical methods of preparation nanoparticles include sputtering, evaporation, laser ablation, ion ejection and electron-beam lithography. The sputtering is one of the cheapest physical methods how to prepare pure materials and nanomaterials. Nanoparticles prepared by the direct sputtering into liquid medium usually contains fewer impurities than those which were prepared by chemical synthesis. And these nanoparticles are often also very stable for a long time [15].

Nowadays there is also an effort to prepare nanoparticles without any harmful reducing agents, chemical stabilizers and to reduce generated waste, which can be achieved by direct sputtering of metals into liquid medium e.g polyethylene glycol (PEG) or glycerol. Sputtering is a well-established method, which is environmentally friendly [16, 17]. In all applications of nanotechnology, the size and shape of the nanoparticles play one of the most important role [13]. Nanoparticles are studied because they have unique physical and chemical properties that are different from "bulk" materials [7]. Concentration and particle size of nanoparticles can be regulated (influenced) by the target-substrate distance, the chamber pressure, the substrate temperature and the sputtering time [15]. Hatakeyama et al. [18] prepared solution of AuNPs in pure polyethylene glycol with particle size less than 50 nm. On the other hand Siegel et al. [10] prepared solutions of AgNPs and AuNPs by sputtering into glycerol/water mixture with particle size of 3.5 ± 1.4 and 3.5 ± 2.4 nm, respectively. Sputtering allows preparation of the spherical nanoparticles. Cha et al. [19] used liquid substrate which contains carbon and polyethylene glycol for sputtering of PtNPs. The particle size of these NPs was about 2 nm.

The application of nanomaterials has gained an increasing attention in medicine. Several kinds of metal-based NPs were established for biomedical application, such as gold (AuNPs), silver (AgNPs) and other metal nanoparticles [20, 21]. One of the most important applications of metal nanoparticles (NPs) are in drug delivery systems and in disease diagnosis and treatment of human beings as imaging probes. AuNPs have been tested as targeted delivery agents because of their high chemical stability and surface plasmon properties [22].

It was demonstrated that sole modulation of the surface area would make it possible to use AuNPs for therapeutic purposes [22]. Interactions between nanoparticles (NPs) and biomembranes depend on the physicochemical properties of the NPs, such as size, shape and surface charge. For example hydrophobic gold core can embed into the hydrophobic membrane interior and thus influence the cytotoxicity of NPs [21]. The influence of the cultivation medium was also studied with the aim to estimate nanotoxicity. It was observed that AuNPs can undergo an oxidation process in the supernatants and only a small amount of AuNPs and dissolved Au^{3+} was associated with cells. It was showed that 10 nm AuNPs exhibit a slight toxic effect [23]. The internalization of nanoparticles by cells (and more broadly the nanoparticle/cell interaction) is a crucial issue both for biomedical applications (for the design of nanocarriers with enhanced cellular uptake to reach their intracellular therapeutic targets, while many parameters can influence the nanoparticle/cell interaction, among them, the nanoparticle physico-chemical features) [24]. AuNPs showed interesting properties compared with natural materials and traditional polymer based materials with wide potential of antitumor activity [25] or as a carrier for in vivo gene activation in tissue regeneration [26], suggesting its potential as a multifunctional system with both gene delivery and antibacterial abilities in clinic [27]. Different sizes of PtNPs were employed for photothermal treatment of Neuro 2A cell lines [28].

We would like follow the published study which was focused on stabilization of gold and silver nanoparticle in PEG/water colloid solutions [9]. In this work we present a simple, reproducible and environmentally friendly approach of Au and PtNPs preparation by direct sputtering of Au or Pt into liquid polyethylene glycol (PEG). We studied properties of prepared inert Au and PtNPs dispersions. We characterized concentration, particle size and shape of Au and PtNPs (with AAS, TEM, DLS). We studied also their cytotoxicity at different concentrations with potential application as anti-bacterial agents [29, 30] or cell markers [31]. We chose these metal NPs because their cytotoxicity is relatively poorly studied.

2. Experimental

2.1 Materials, apparatus and procedures

Au and Pt targets (Safina s.r.o., purity of 99.9999%) were carried out at room temperature in a sputter coater device SCD 050 (Baltec, argon pressure 8 Pa), with the current of 30 mA and the distance of electrode about 50 mm. Polyethylene glycol with molecular weight 600 g Mol^{-1} (PEG, Sigma Aldrich) was used as a medium for nanoparticles sputtering. The PEG volume for the Au or Pt deposition was 2 mL. The mixtures were diluted in distilled water at ratio 1:9 (PEG/water) [9, 32]. The schema of NPs preparation by direct sputtering of Au into pure PEG is presented in Fig. 1.

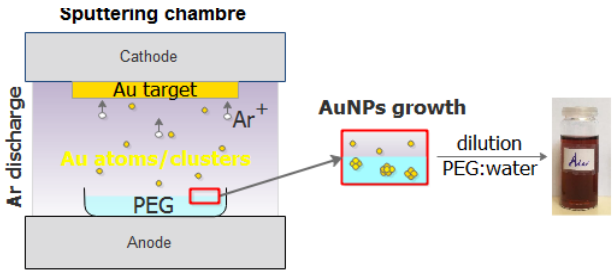

Fig. 1. Principle of AuNPs solution preparation by direct sputtering into liquid (PEG). The description corresponds to AuNPs.

2.2 Analytical methods

2.2.1 Atomic absorption spectrometry (AAS)

Au and Pt concentrations were determined by AAS (AAS spectrometer Varian AA 880 with flame automatization to determine the total amount of elements, experimental error ±5%).

2.2.2 Transmission electron microscopy (TEM)

Size and shape of AuNPs and PtNPs were examined by TEM (JEOL JEM-1010 (Japan), their images were taken by Megaview III digital camera (Soft Imaging Systems-Olympus, acceleration voltage of 80 kV) and analyzed by AnalySIS 2.0 software. Au and PtNPs in dispersions were analyzed also by HRTEM. HRTEM characterization was carried out on JEOL JEM-2200FS (JEOL Ltd., Japan) operand at 220 kV. Samples for transmission electron microscopy (TEM, HRTEM) were prepared by putting a drop of the colloidal solution on a copper grid coated with a thin amorphous carbon film placed on filter paper. Excess of solvent was removed. Samples were dried and kept under vacuum in a desiccator before putting them in a specimen holder [26]. Particle size was measured from the TEM micrographs and calculated by taking into account at least 400 particles.

2.2.3 Dynamic light scattering (DLS)

Electrokinetic analyses (zeta potential determination) and size and distribution determination of the samples were performed with a Zetasizer Ver. 6.32 device, and Malvern software was used for data evaluation. As a light source, a laser with 366 nm wavelength was used. All samples were analyzed in 24 h from the preparation, at constant pH and room temperature [9, 33].

2.2.4 Ultraviolet-visible spectroscopy (UV-Vis)

UV-Vis spectroscopy was used to characterize optical properties of Au and PtNPs in dispersions. Absorbance was measured in a 10-mm cell (Hellma Analytics cell, Quartz SUPRASIL, Type No. 100-QS) using a Perkin-Elmer Lambda 25 spectrophotometer (USA). Spectra were acquired in the range of 300-800 nm for AuNPs and for PtNPs in the range of 200-800 nm.

2.2.5 Cells and culture conditions

The human osteoblast-like cell line SAOS-2 was obtained from DSMZ, Germany. SAOS-2 cells were cultivated in McCoy's 5A medium without phenol red (PromoCell, Germany) and supplemented with 15% heat-inactivated FBS (PAA, Austria), penicillin (20 U mL^{-1}, Sigma-Aldrich, USA) and streptomycin (20 µg mL^{-1}, Sigma-Aldrich, USA) at 37 °C and in a 5% CO_2 atmosphere. Cells were collected at 60 – 90% confluence using trypsin and seeded onto 96-well plate (TPP, Switzerland) at a density of $1x10^4$ cells cm^{-2} and cultivated in this medium for 24 h. Then different concentration of AuNPs and PtNPs were added and cells were incubated for 24 h when the images were taken and metabolic activity was determined (see later).

2.2.6 Cell imaging

Phase contrast images of the cells were acquired using an Eclipse Ti-S microscope (Nikon, Japan) with a Plan Fluor 10x (N.A. 0.30) objective and DS-U2 digital camera (Nikon, Japan).

2.2.7 Determination of Cell Metabolic Activity

The cell metabolic activity test (Cell Titer 96 AQueous One Solution Cell Proliferation Assay, MTS, Promega, USA) was performed according to the standard protocol. Absorbance was determined using a multi-detection micro-plate reader (Synergy™ 2, BioTek, USA). The results were normalized (in percentage) with respect to the control cells with no NPs added.

All the data presented was derived from three independent experiments performed in triplicate. The results are presented in the form of mean values with error bars indicating standard deviations. The nonparametric Wilcoxon matched pairs test was used in order to determine significant differences between the datasets of the untreated control and the rest of the variables. An ANOVA was used to compare differing concentrations of Au or Pt with each other. P values of less than 0.05 were considered statistically significant. Extreme values were excluded from the analysis. Statistical analysis was performed using STATISTICA (StatSoft, Inc.) software.

3. Results and Discussion

3.1 Concentration and morphology of NPs

The concentration of AuNPs and PtNPs was determined by atomic absorption spectroscopy (AAS). Au/PEG solution contained 56 mg L^{-1} of Au and the Pt/PEG solution contained 60 mg L^{-1} of Pt. Particle size and shape of prepared NPs were studied with TEM (see Fig. 2) and HRTEM (see Fig. 3). From Fig. 2 it is apparent that AuNPs are smaller than 10 nm and they had spherical shape. Particle size distribution of AuNPs is presented in histogram ($D_{0.9}$ = 6.3 nm). On contrary, PtNPs are even smaller and they embodied the rod shape of two different sizes. Particle size distribution of PtNPs is presented in histogram ($D_{0.9}$ = 3.9 nm). It is known that gold (Au) and platinum (Pt) grows in a square fcc crystal structure. Fig. 3 shows prepared NPs studied with HRTEM.

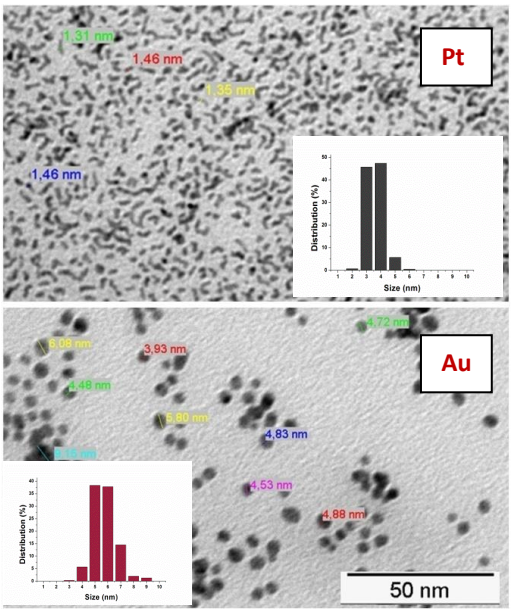

Fig. 2.TEM images of AuNPs and PtNPs sputtered into PEG after deposition diluted with water in ratio 1:9 with inset figure of size distribution histograms.

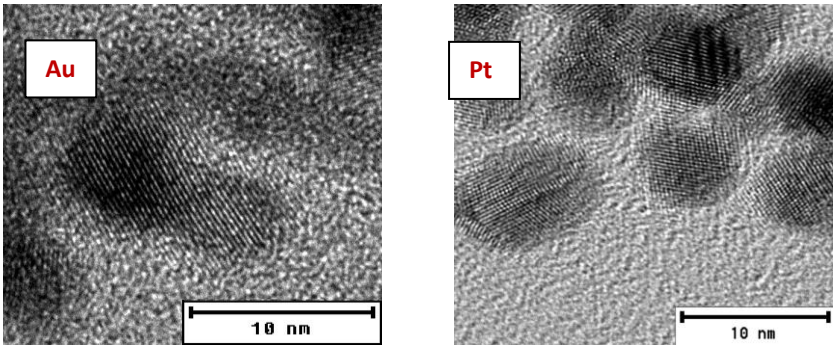

Fig. 3. HRTEM images of AuNPs and PtNPs sputtered into PEG after deposition diluted with water in ratio 1:9.

3.2 Zeta potential and NPs size

Dynamic light scattering (DLS) served for (i) zeta potential determination and for (ii) NPs size and distribution determination. Results are presented in Fig. 4 for freshly prepared NPs (fresh) and NPs diluted in cell cultivation medium (diluted). Zeta potential values of prepared NPs (-66.5 ± 0.9 mV for AuNPs and -64.2 ± 8.7 mV) indicate very good stability of nanoparticles colloids. In a literature the zeta potential about +/- 30 mV indicate stable colloidal samples, zeta potential about +/- 50 mV even the good stability [33, 34]. It is evident the zeta potential both of NPs samples dramatically have changed after dilution in cell cultivation medium (to the values of -7.3 ± 0.8 mV for AuNPs and -4.8 ± 0.3 mV) which indicated a poor stability. It can be explained by the fact the cultivation medium changed dramatically concentration of liquid medium surrounding NPs due the presence of salts of quiet high concentration. This higher concentration of medium resulted in press of electrical double layer on the nanoparticle surface and due to this to the dramatic changes of surface charge. Surface charge plays the important role on nanoparticle size. For zeta potential below 30 mV the stability of nanoparticles decreases and particles agregates. This is visible in Fig. 4 (right) where sizes of nanoparticles are presented. While size for Au NPs (fresh) was determined by DLS as 15.8 ± 0.9 nm, which indicate spherical particles of uni-

dispersed size of very small distribution, size of AuNP diluted in cell cultivation medium was determined as 33.7 ± 17.7 nm which indicates bigger particles of quite wide distribution. DLS study of size of PtNPs resulted in three dimensions for fresh PtNPs (1.5 ± 0.5 nm, 4.2 ± 0.4 nm and 7.9 ± 0.9 nm) which indicated particles of rod shape which had the uniform wide of 1.5 ± 0.5 nm and two different lengths 4.2 ± 0.4 nm or 7.9 ± 0.9 nm, which is visible in Figure 4 right in details. Also after dilution in cell cultivation medium the PtNPs sizes increase to the 8.0 ± 1.5 nm and 37.9 ± 7.6 nm which indicate the rod shape have stayed for PtNPs but their size is bigger and distribution is wider.

Estimated sizes and shapes of NPs correspond well with that obtained by TEM analyses.

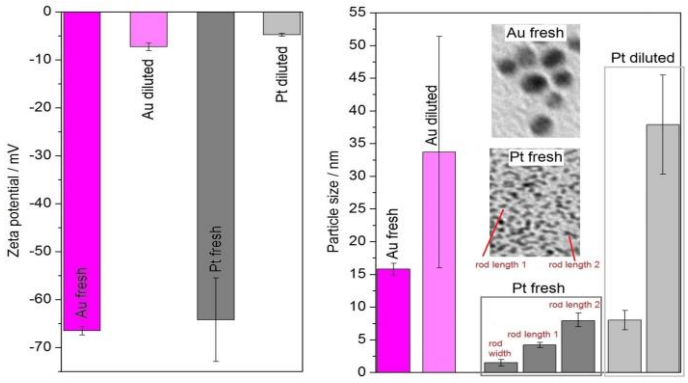

Fig. 4. Zeta potential (left) and nanoparticle size (right) of AuNPs and PtNPs after deposition diluted with water in ratio 1:9 (fresh) and subsequently diluted in cell cultivation medium (diluted).

3.3 Optical properties

Optical properties of Au and PtNPs in dispersions were measured by UV-Vis spectroscopy. Fig. 5 shows UV-Vis spectra of Au and PtNPs. The colloidal dispersion of AuNPs have a signifiant absorption peak maxima. Prepared AuNPs solution has absorption peak maxima at 515 nm and it is in accordance with [9], where the peak maximum of prepared AuNPs was determined at 517 nm. The position of peak maxima depends on particles size, shape and concentration of NPs in solutions [9]. UV-Vis spectrum of PtNPs exhibits an increasing absorption at a part of ultraviolet wavelength. It is in accordance with measurements of pure PtNPs in [35, 36]. At detail of PtNPs spectrum in Fig. 5, there are two small peaks. First peak has absorption maximum at 202 nm and second at 206 nm. These two small peaks can indicate two dimension of PtNPs rods. There is no literature which could be used for comparison of this results and presumption. But, in our cases, we can confirm our observation due to the size and shape of PtNPs were confirmed by transmission electron microscopy (TEM) and dynamic light scattering (DLS) with the similar results. Similar case was published about gold nanorods. Because of different plasmon oscillations, one of peaks corresponding to the tranverse and second to the longitudinal plasmon oscillation [37, 38].

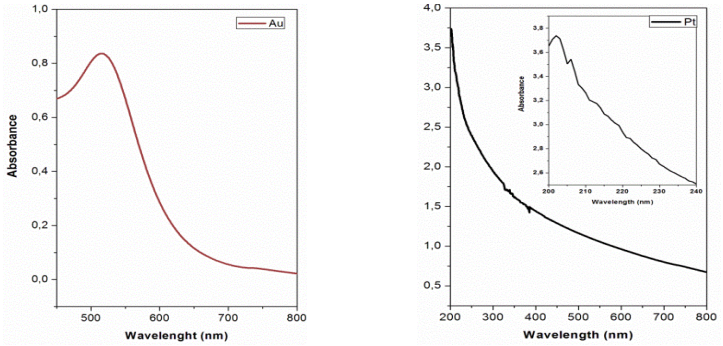

Fig. 5. UV-Vis spectra of AuNPs (left) and PtNPs (right) with inset images of prepared NPs solutions.

3.4 Cytotoxicity

To find out the effect of AuNPs and PtNPs on cells (osteoblasts) were cultivated in a medium supplemented with 15 % FBS with gradually increasing concentrations of AuNPs and PtNPs (0.56, 2.8, 5.6, 8.4, 11.2 and 14.0 mg L^{-1} of AuNPs and 0.6, 3.0, 6.0, 9.0, 12.0 and 15.0 mg L^{-1} of PtNPs, respectively) which was followed by the determination of their metabolic activity after 24 hours (Fig. 6). It is apparent that both particles in dependence on their concentration had cytotoxic effect on these cells. Despite similar concentrations of AuNPs and PtNPs used in the experiment more cytotoxic effect was observed in case of cells treated with AuNPs. Significant reduction of cell metabolic activity with cytotoxic effect (25 % of reduction) [39] was observed in cells treated with AuNPs of 11.2 mg L^{-1}, whereas the same was observed with PtNPs only after using 15.0 mg L^{-1}). The dying cells treated with the highest concentration of AuNPs (14.0 mg L^{-1}) are presented in Fig. 7, where also unaffected cells treated with lower concentrations of AuNPs and low and high concentration of PtNPs are presented. According to results presented in Fig. 4, despite the fact that freshly prepared AuNPs and PtNPs differ in size, shape and zeta potential, after their addition to the cell cultivation medium they equalize in these parameters. Thus their different cytotoxic effect is most probably caused by other properties, which can be different surface chemistry, reactivity or catalytic properties. To conclude, both prepared AuNPs and PtNPs can be applied as delivery vehicles to cells, however, their concentration used should be well controlled.

On the basis of the results of this work and published results [40, 41], it can be assumed that the NPs thus prepared could find the application in tissue engineering and in medicine such as biosensors [42], drug carriers [19], or for example contrast agents for imaging (e.g. MRI) [8]. It is very important to know the particles' cytocompatibility for all these applications.

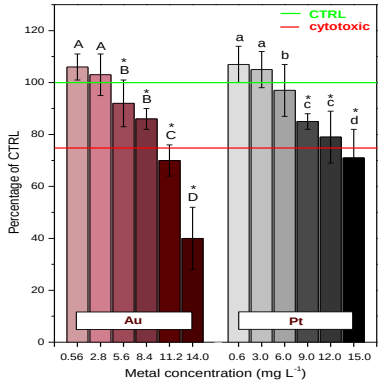

Fig. 6. Metabolic activity of osteoblasts incubated with different concentrations of Au and PtNPs for 24 h. Relative values are expressed as a percentage of untreated cells (CTRL). (Wilcoxon matched-pairs test, $p < 0.05$). Different upper case letters express significant inter-group differences for the group of Au concentrations and lower case letters for the group of Pt concentrations (ANOVA, LSD post hoc test, $p < 0.05$).

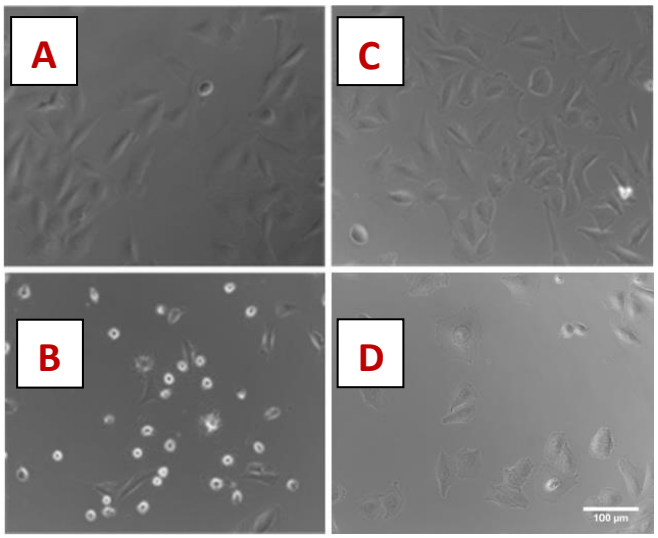

Fig. 7. Microscopic images of osteoblasts incubated with different concentrations of Au (A,B) and PtNPs (C,D) for 24 h: A–osteoblasts incubated with 0.56 mg L^{-1} AuNPs, B–osteoblasts incubated with 14.0 mg L^{-1}AuNPs, C–osteoblasts incubated with 0.6 mg L^{-1} PtNPs, D–osteoblasts incubated with 15.0 mg L^{-1} PtNPs.

4. Conclusions

We prepared Au and PtNPs by direct sputtering into liquid polyethylene glycol with molecular weight of 600 g Mol^{-1}. The metal/PEG solutions were mixed with water. It is a simple and environmentally friendly method for preparing metal nanoparticles without any harmful reducing agents and chemical stabilizers. Sputtering is other way how to prepare nanoparticles by the so called green chemistry. The particle size of AuNPs and PtNPs were smaller than 10 nm. AuNPs had spherical shape of unique size.

The $D_{0.9}$ diameter of AuNPs was 6.3 nm. PtNPs had rod shape of two lengths and the $D_{0.9}$ diameter of PtNPs was 3.9 nm. UV-Vis spectra both of NPs colloidal samples were measured. AuNPs has significant absorption peak maxima at 515 nm. On the other hand colloid of PtNPs has two small peaks in wavelength about 200 nm. These two small peaks can indicate two dimensions of PtNPs rods. Their cytotoxicity was tested and despite the comparable properties of AuNPs and PtNPs in cultivation medium, AuNPs were significantly more toxic.

Acknowledgements

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