Formation and antibacterial action of Pt and Pd nanoparticles sputtered into liquid

Marek Staszek[1], Jakub Siegel[1], Kateřina Kolářová[1], Silvie Rimpelová[2], Václav Švorčík[1]

[1]*Department of Solid State Engineering, Institute of Chemical Technology, 166 28 Prague, Czech Republic*
[2]*Department of Biochemistry and Microbiology, Institute of Chemical Technology, 166 28 Prague, Czech Republic*
E-mail: marek.staszek@vscht.cz

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A report is presented on the antibacterial activity of platinum and palladium nanoparticles (NPs) prepared by direct sputtering into glycerol. Prepared platinum faceted NPs ($n = 500$) with the diameter of (1.7 ± 0.3) nm and palladium faceted NPs ($n = 500$) with the diameter of (2.4 ± 0.4) nm were investigated. The size and shape of NPs were studied by transmission electron microscopy. Moreover, the size and its distribution were studied by dynamic light scattering. The concentration of metal NPs in prepared solutions was determined by atomic absorption spectroscopy. The optical properties of aqueous NP solutions were studied by UV–vis absorption spectroscopy. Antibacterial properties were tested against two common pollutants (*E. coli* DBM 3138, a Gram-negative bacteria and *S. epidermidis* DBM 3179, a Gram-positive bacteria). Owing to the increasing resistance of bacteria strains to common antibiotics, this study may provide an alternative way to fight these pollutants.

1. Introduction: Over the past decades, noble metal nanoparticles (NPs) have been widely studied because of their unique optical, catalytic, electromagnetic and bactericidal properties, which are strongly influenced by their shape and size. Platinum NPs (PtNPs) constitute a versatile material with a unique combination of thermal, chemical and optical plasmonic properties [1]. Besides other interesting applications, noble metal NPs (e.g. Au, Ag, Pd and Pt) are catalysts for a wide range of chemical reactions, such as oxidation, hydrogenation and carbon–carbon coupling reactions [2]. Furthermore, these NPs have been intensely studied because of their unique properties and potential applications in the development of fuel cells [3], solar cells [4], hydrogen storage materials [5] and (bio) molecular sensors [6].

Palladium (Pd) has very similar properties to platinum (Pt), and Pd is at least 50 times more abundant on earth than Pt, also the cost of Pd is lower than that of Pt [7]. In the case of Pd NPs (PdNPs), the main subject of research has focused on nanocatalyst reactions, such as carbon–carbon and carbon–heteroatom cross-coupling reactions (the Suzuki-Miyaura reaction, Heck reaction, Kumada reaction, Sonogashira reaction, Negishi reaction, Stille reaction, Buchwald-Hartwig reaction and hydrogenation reactions) [8], (bio) chemical sensors, switches and hydrogen storage media, and for the degradation of harmful automobile pollutants [9].

Unfortunately, there are only a few research studies addressing the biological applications of pure Pt and Pd NPs [9] and their antibacterial properties are almost unknown. When studying the interaction of metal NPs with living tissues, crucial attention must be paid to the preparation process of NPs itself. To determine antibacterial properties correctly, the preparation process must produce uniform NPs with narrow size-distribution. The preparation technique is also of essential interest since it must not include the toxic solvents or reduction agents. Considering this, direct metal deposition into glycerol seems to be a promising technique compared to classical wet-based methods.

Recently, our group demonstrated a unique approach for noble metal NP synthesis [10]. This Letter shows that the proposed approach may be used even in the case of Pt and Pd NPs. The average size and size-distribution of the NPs were measured by transmission electron microscopy (TEM), dynamic light scattering (DLS) and UV–vis absorption spectroscopy. The inhibition potency of NPs was investigated towards two common bacterial strains of *E. coli* (Gram-negative bacteria) and *S. epidermidis* (Gram-positive bacteria), naturally occurring on the skin and mucous membranes of humans.

2. Experimental

2.1. Materials, apparatus and procedures: Metal sputtering was performed in a sputtering device, SputterCoater SCD 050 (BAL-TEC), using 99.999% pure Pt and Pd targets. The sputtering was accomplished at room temperature (20°C), with a deposition time of 300 s, current of 40 mA, voltage of 420–430 V, total argon pressure of 4–6 Pa (gas purity 99.99%) and an electrode distance of 5 cm. A more detailed description of the deposition procedure can be found in [10].

As a capturing media for the preparation of Pt and Pd NPs we used anhydrous glycerol (propane-1,2,3-triol, Penta, $M_w = 92.1$ g mol^{-1} and purity 99.8%). The Petri dish with an inner diameter of 4 cm was filled with 5 ml of glycerol. After the metal sputtering, the glycerol with metal NPs was transferred into 40 ml vials and mixed with distilled water in the volume ration 1:3 (glycerol:water).

2.2. Analytical methods: The prepared solutions of colloidal PtNPs and PdNPs were analysed by TEM (TEM, HRTEM), DLS, atomic absorption spectrometry (AAS) and UV–vis analysis, and were tested for their bactericidal activity.

Samples for TEM 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. The excess of the solvent was removed. Samples were dried and kept under vacuum in a desiccator before putting them in a specimen holder. TEM characterisation of the sample was performed on a JEOL JEM-1010 (JEOL Ltd, Japan) operated at 400 kV. Particle size was measured from the TEM micrographs and calculated by taking at least 100 particles. HRTEM characterisation was carried out on a JEOL JEM-2200FS (JEOL Ltd, Japan) operated at 220 kV.

The particle size was also determined by a Zetasizer ZS90 (Malvern Instruments Ltd, UK) in the DLS regime for particle size-distribution equipped with an avalanche photodiode for detecting the signal. As a light source, a DPSS laser (50 mW, 532 nm) was used. The measurement was performed in polystyrene cuvettes and at room temperature.

The UV–vis absorption spectra of the NP solutions were recorded on a Varian Cary 50 UV–vis spectrophotometer (Varian Inc., USA), equipped with deuterium and halogen lamp light sources. The spectra were collected at room temperature (recording

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rate 240 nm min^{-1}, collection interval 1 nm). All spectra were normalised to the 1 cm path length of the cuvette. All measurements were baselined on a blank sample of corresponding glycerol–water solution.

The concentration of Pt and Pd NPs was determined by means of AAS on a VarianAA880 device (Varian Inc., USA) using a flame-atomisator at a 242.8 nm wavelength. The typical uncertainty of the concentration determined by this method is less than 0.5%.

2.3. Antimicrobial test: The antibacterial potency of PdNPs and PtNPs was assessed by the drip method using two environmental bacterial strains, Gram-negative *Escherichia coli* (*E. coli*, DBM 3138) and Gram-positive *Staphylococcus epidermidis* (*S. epidermidis*, DBM 3179). The experiments were performed in a similar way to that in [11]. The desired bacterial strains were cultured in nutrient broths (LB broth for *E. coli* and PCA broth for *S. epidermidis*) at 37°C overnight under continuous agitation. The optical densities of the overnight cultures were measured at 600 nm. The starter inocula were prepared by serial dilutions of the cultures in a fresh sterile physiological saline solution (0.9%w/v of NaCl). We examined the antimicrobial effects of these metal NPs regarding dependence on bacterial concentration; *E. coli* was inoculated in densities of $1{\cdot}10^3$, $1{\cdot}10^4$, $1{\cdot}10^5$ and $1{\cdot}10^6$ cells ml^{-1}, and *S. epidermidis* in densities of $1{\cdot}10^4$, $1{\cdot}10^5$, $1{\cdot}10^6$ and $1{\cdot}10^7$ cells ml^{-1}. Two microlitres of freshly prepared PdNPs (11.8 mg ml^{-1}) and PtNPs (16.6 mg ml^{-1}) were used per 1 ml of physiological saline solution. In parallel, *E. coli* and *S. epidermidis* incubated solely in a physiological saline solution were used as controls. All samples were incubated statically at 24°C for 4 h, afterwards the samples were vortexed and 25 µl aliquots of each sample were dripped onto pre-dried LB (*E. coli*) and PCA agar (*S. epidermidis*) plates and incubated for 24 h at 24 and 37°C for *E. coli* and *S. epidermidis*, respectively. The growth of both bacterial strains was evaluated by direct counting of the colony forming units (CFU). Samples were done in triplicates (plus 15 drops of each sample). The experiments were accomplished under sterile conditions.

3. Results and discussion: Generally, two major aspects determine whether the growth of NPs during metal sputtering into liquids will take place rather than the formation of a thin film on the liquid surface [12]. Firstly, the kinetic energy of sputtered atoms or clusters must be sufficient to penetrate the surface of the capturing media. Secondly, the first condition may be fulfilled only in the limited range of media viscosity, thus the liquid media cannot be too viscous.

When these conditions are met, the formation of NPs occurs. Thereafter, their size can be varied by changing the temperature of the capturing media since it has been demonstrated that NP growth is governed by diffusion from the bulk of colloidal dispersion to the solution/particle interface [13].

Fig. 1 shows TEM and HRTEM images of diluted aqueous solutions of Pt and PdNPs. Image analysis of more than 500 particles from 10 different areas of TEM pictures proved that the prepared PtNPs and PdNPs have an average diameter of (1.7 ± 0.3) nm and (2.4 ± 0.4) nm, respectively. The apparent aggregation of particles is because of the preparation method of samples for TEM (HRTEM) analysis. The observed discrepancy in particle size is probably because of the different sputtering yields of both metals (Pt ~1.27 and Pd ~2.09) [14], which in accordance with the supposed growth mechanism causes considerably slower growth of Pt particles (lower concentration gradient).

In addition to TEM analysis, DLS measurement was accomplished to determine the average size and size-distribution of prepared NPs (see Fig. 2). The DLS measurements indicate that both particles are about 7% bigger compared with the TEM-based analysis, which is in good agreement with the published results [11], since the DLS technique provides a hydrodynamic diameter. Moreover, the observed size discrepancy is inherently caused by

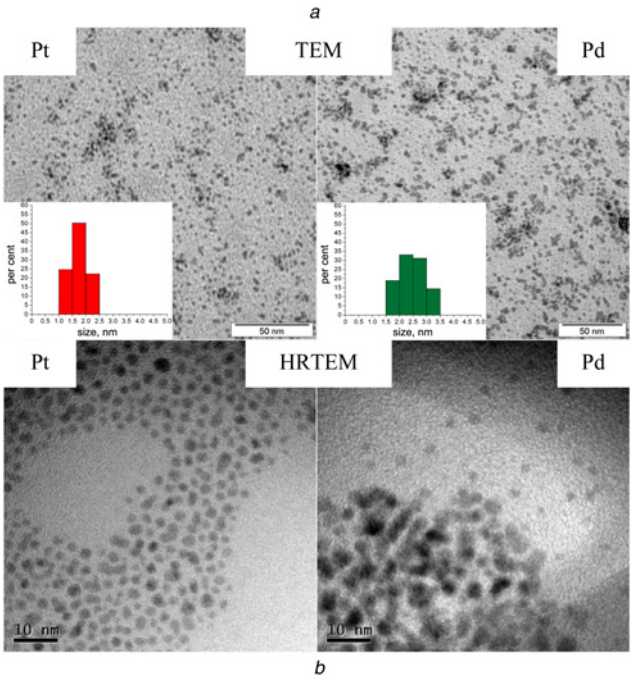

Figure 1 *TEM and HRTEM images of diluted aqueous solutions of Pt and PdNPs*
a TEM images
b HRTEM images
Insets: Histogram of corresponding particle size and distribution

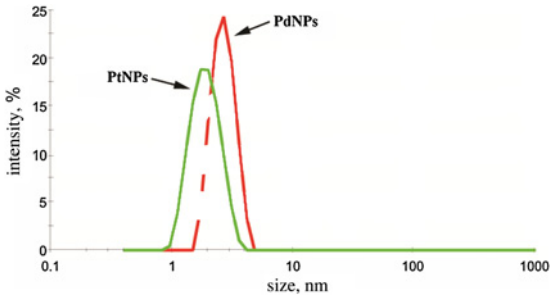

Figure 2 *Characterisation of aqueous solutions with metal NPs by dynamic light scattering*
Green line refers to PtNPs and red one to PdNPs

the intensity weighted mean particle diameter in the case of DLS, contrary to the number weighted diameter obtained by TEM analysis [15]. More importantly, the DLS proves that prepared NPs are not agglomerated, which is of crucial importance for evaluation of their bactericidal effects.

The formation and stability of the metal NPs in an aqueous colloidal solution were confirmed by UV–vis spectroscopy. Fig. 3 shows the UV–vis spectra recorded from the Pd and Pt NPs immediately after NPs preparation (solid lines) and after three months storage at laboratory conditions (dashed lines). It can be seen that apart from a distinct surface plasmon resonance (SPR) characteristic for other noble metal NPs (Au and Ag) [13], no distinctive SPR peaks occur in the case of both Pd and Pt NPs, which is in accordance with earlier reports [16, 17]. The studied metal NPs exhibit increasing absorption towards a shorter wavelength [17–20]. The course of dependence in the case of both NPs indicates the presence of zerovalent metals in the solution [19]. The slight shift of the curves to the lower absorbances in the red region of the visible spectrum (dashed lines) refers to the mild agglomeration of individual particles in the solution after three months of storage. The bactericidal potency of various metal NPs has been previously reported [8, 21–23]. It is known that their antimicrobial potency is governed

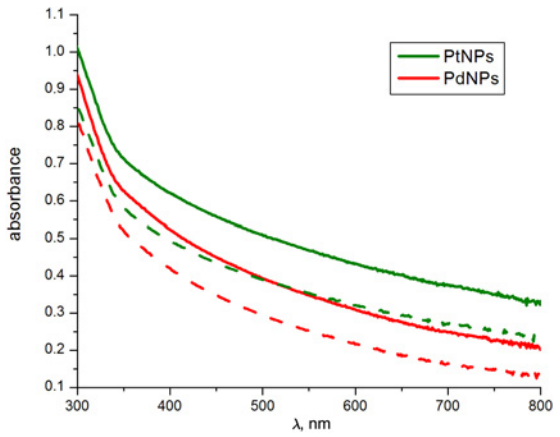

Figure 3 *UV–vis absorption spectra of Pt and Pd aqueous NP solutions*
Dashed lines represent UV–vis spectra of Pt and Pd aqueous NP solutions stored for three months in laboratory conditions

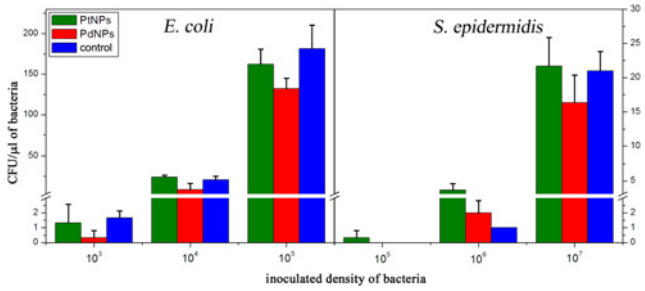

Figure 4 *Inhibition effect of PtNPs and PdNPs towards bacterial strains of E. coli and S. epidermidis with different bacteria concentrations*

by their size, composition, surface area and charge [24, 25]. There has been increased interest in studying the bactericidal properties of noble metals over the past decades; however, only a few studies have been reported on Pt and Pd NPs. The data are rather scarce, thus we decided to decipher their potential in inhibitory action against Gram-positive and Gram-negative bacterial strains.

In this Letter, we report an investigation of the activity of well-defined, small in size 2.4 nm PdNPs and 1.7 nm PtNPs against bacterial growth. To provide comprehensive understanding, two model bacterial strains of *E. coli* and *S. epidermidis* were subjected to drip tests. The inhibitory effect of one NPs concentration and four concentrations of bacteria was examined after 4 h in contact with NPs and 24 h of further post-incubation. From Fig. 4, it is apparent that incubation of PdNPs with *E. coli* had a pronounced effect on its growth up to 1×10^5 CFU per sample when compared to untreated control cells. This remarkable antibacterial activity of Pd was diminished at higher bacterial concentrations ranging from 1×10^6 CFU per sample. The similar potency of PdNPs was observed against *S. epidermidis* (see Fig. 4), even up to 1×10^6 CFU.

As the PtNPs had very similar size to the first examined noble metal NPs, the Pd ones, we expected a comparable antimicrobial potential. Nevertheless, we detected only an insignificant inhibition of bacterial growth induced by PtNPs at a concentration of 1×10^5 CFU of *E. coli* when compared to the control samples. Interestingly, we did not observe any growth inhibition of *S. epidermidis* in the presence of PtNPs. More importantly, we even recorded slight stimulation of their growth.

4. Conclusion: To sum up, we have successfully prepared Pt and Pd NPs by the direct sputtering method into glycerol. The mean average diameter of Pt and Pd NPs prepared by this technique was 1.7 and 2.4 nm, respectively. Such NPs exhibit a rather narrow size-distribution with good potency against agglomeration. We have also demonstrated a significant difference in the biological activities of Pt and PdNPs. More specifically, PdNPs exhibited considerable inhibitory potential against both *E. coli* and *S. epidermidis*, which was in contrast to the ineffective PtNPs. Our results indicate that palladium has a high potential to combat both Gram-positive and Gram-negative bacterial strains. Understanding of NP-bacteria interactions is a key issue in their potential applications in clinics on an everyday basis. The observed discrepancy in bactericidal action between Pt and Pd NPs could be attributed to the different size of individual particles. This aspect seems to play a conical role in antibacterial activity according to numerous studies [26]. Nevertheless, the exact mechanism of bactericidal action of NPs is still unclear, thus opening possibilities for further, more extensive research.

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6 References

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