Synthesis of magnetic nanoparticles by laser ablation in a liquid and verification of their antibacterial properties

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Abstract. Nickel nanoparticles were obtained by nanosecond laser ablation in deionized water and in air in different laser regimes. The nanoparticles were characterized by scanning electron microscopy, method of dynamic light scattering, and optical spectroscopy. Solutions with the highest concentration of nanoparticles were tested for antibacterial activity. The antibacterial properties of nickel nanoparticles were demonstrated on gram-positive (S. aureus) and gram-negative (P. aeruginosa) bacteria using the LIVE/DEAD method.

1. Introduction

Pathogens are a common cause of many diseases. Nowadays, the treatment of various types of bacteria is complicated due to the developed resistance of these organisms to antibiotics [1-2]. There are high risks that shortly (about 10-20 years), the problem of bacterial resistance to antibiotics may become the main cause of patient mortality [3-5]. Many science groups all over the world work hard to resolve this problem [6-9]. One of the promising and easy-to-implement methods of bacteria fight is the use of nanoparticles (NPs), including magnetic, produced by laser ablation in different medium [10-12].

We choose nickel as starting material and obtained its nanoparticles by laser ablation in deionized water [13-14, 17] and in the air [14-17]. NPs from both solutions were characterized by scanning electron microscopy, dynamic light scattering, and optical spectroscopy. We study the antibacterial properties of the obtained colloidal solutions. Both colloids were tested on gram-positive (S. aureus) and gram-negative (P. aeruginosa) bacteria by using Live/Dead cell viability assays kit [18-20].

2. Materials and methods.

To obtain colloidal solutions of nickel in deionized water, the bulk nickel target was placed in a glass beaker. HTF Mark Bulat nanosecond laser was used as a radiation source. The fixed laser parameters are as follows: wavelength $\lambda = 1050$ nm, pulse width FWHM $\tau = 120$ ns, frequency $\nu = 20$ kHz, ablation time $t = 7$ min, ablation area on the target – $7\times 7$ mm$^2$. Among the variable parameters, the laser energy ($E = 0.2, 0.4, 0.6$ mJ) and the speed of the laser beam passing through the target ($v = 20, 100, 650, 1500$ mm/sec) were selected. Among the 12 modes listed, the maximum concentration of the solution was
obtained at $E = 0.6 \text{ mJ}$ and $v = 650 \text{ mm/sec}$. The nickel concentration, in this case, was 0.966 mg/ml. Visualization and sizes distribution of the obtained NPs are shown at Figure 1.

The same target was used to produce nickel nanoparticles in the air. The experimental setup was constructed so that a slide was fixed above the target at a distance of 2 mm. In this case, the modes were chosen as follows: laser energy ($E = 0.4, 0.6 \text{ mJ}$) and the speed of the laser beam passing through the target ($v = 200, 500, 1500 \text{ mm/sec}$), and instead of 7 minutes of ablation, the laser made 10 passes through the specified area on the target. Among the 6 listed modes, the mode with the maximum yield of nickel nanoparticles ($E = 0.6 \text{ mJ}, v = 500 \text{ mm/sec}$) was also selected. The resulting particles were dissolved in 6 ml of deionized water. The final concentration of nickel in the solution was 0.978 mg/ml. The characteristics of the obtained NPs are shown at Figure 2.

We used a Nikon H600L microscope (Eclipse Ni, Japan) with a fluorescent lens to take the pictures of experimental samples.

The resulting bacteria were stained with a dye solution Live/Dead Biofilm Viability Kit (ThermoFisher, USA) according to the instructions.

3. Results and discussions

3.1. Characterization Ni NP

The nanoparticles obtained in deionized water has a shape close to spherical and were not covered with an oxide film. The size of the nanoparticles varied from tens to hundreds of nanometers. The nanoparticles obtained in air has the form of ellipsoids fused with each other, and were also covered with an oxide film. The sizes ranged from tens of nm to several microns.

3.2. Antibacterial properties

The results of studies showed high efficiency of nickel nanoparticles obtained in both deionized water and air. Bacterial cultures of S. aureus and P. aeruginosa (200 ml drops) were applied to the surface of the test and control samples and incubated for 30 minutes in a wet chamber. The samples were then stained with propidium iodide and CITO 9 fluorescent paints, following the Protocol of the Live/Dead staining method. Live bacteria fluoresce green, while dead bacteria fluoresce red. The results of microscopy are shown at the Figure 3.

The exact mechanism of action of nickel NPs has not yet been studied. We assume that the antibacterial properties of the NPs are that they violate the integrity of the bacterial membrane, which leads to their death.

Figure 1. SEM image, size analysis and elemental composition of Ni NP, obtained in water

Figure 2. SEM image, size analysis and elemental composition of Ni NP, obtained in the air
**Figure 3.** The results of Live/Dead + RGB analysis (a – control sample-glass slide; b, c – *P. aeruginosa* with Ni NPs obtained in water and air respectively; d, e – *S. aureus* with Ni NPs obtained in water and air respectively). Green color means live bacteria and red – dead

**Conclusion**
In this article, the antibacterial properties of Ni NPs obtained by laser nanosecond ablation in deionized water and air were investigated. The resulting NPs were characterized by SEM, dynamic light scattering, and optical spectroscopy. *S. aureus* and *P. aeruginosa* were selected as experimental pathogens. As a result, it was found that NPs of both formations showed good antibacterial properties for both these types of bacteria.

**Acknowledgments**
This work is funded by Russian Science Foundation (grant #18-15-00220). The presentation and publication of these results was supported by the conference RFBR grant № 20-02-22038.

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