

Physical and Biological Properties of Layers with Nanoparticles Based on Metal Chalcogenides and Titanium Synthesized by Femtosecond Laser Ablation and Fragmentation in Liquid

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Abstract. In this paper, we present the physical properties and toxicological assessment of layers with nanoparticles based on metal chalcogenides and titanium on human fibroblast cells. Nanoparticles layers based on metal chalcogenides (MoS₂, WS₂, ZnS) and titanium were applied onto substrate by spray deposition method. Nanoparticles and flakes were synthesized by laser ablation and fragmentation in liquid by femtosecond pulses. We investigated the size and morphology of the synthesized nanoparticles: WS₂-based flakes have a polygonal shape with dimensions up to 600 nm, other types of nanoparticles have a shape closer to spherical with sizes from 50 to 150 nm. Interaction of ultrafast laser radiation with materials in liquid is accompanied by the dissociation of water molecules leads to formation of hydrogen sulfide and oxides. To assess the biocompatibility of layers with synthesized nanoparticles, the MTT assay was performed with fibroblast cells. According to *in vitro* studies, Ti-based nanoparticles have the largest biocompatibility, and WS₂-based flakes have the smallest ones. Thus, synthesized Ti-based nanoparticles can be used in biomedical applications to support tissue regeneration without additional modification. Due to their properties, metal sulfides-based nanoparticles can be used in the photodynamic therapy of oncological diseases to destroy cancer cells. © 2023 Journal of Biomedical Photonics & Engineering.

Keywords: metal chalcogenides; pulsed laser ablation in liquid; pulsed laser fragmentation; nanoparticles; toxicity; theranostics; targeted drug delivery; biological markers.

Paper #3587 received 21 Jan 2023; revised manuscript received 24 Mar 2023; accepted for publication 10 Apr 2023; published online 3 May 2023. [doi: 10.18287/JBPE23.09.020301](https://doi.org/10.18287/JBPE23.09.020301).

1 Introduction

Currently, various nanoparticles based on metals and their compounds for biomedical applications are widely used due to new synthesis methods development and the need to find innovative solutions for the therapy of socially significant diseases [1–2]. Immobilization on the surface or nanocapsulation of medicinal substances and receptor molecules in such nanoparticles, their high thermal and electrical conductivity, which facilitates the detection of nanoparticles, will allow combining therapy and diagnostics within the concept framework of theranostics [3–5].

For each new synthesis method of nanoparticles, deeper physicochemical studies to assess their biocompatibility depending on their size, shape, and surface features are required [6–8].

Laser ablation in a liquid medium is a complex and multifactorial process. The main criteria that determine the performance of this operation is the energy expended and the yield of the target product. The use of laser systems with subpicosecond duration makes it possible to fine-tune the synthesis of nanoparticles with desired properties and dispersion, but clearly loses in terms of the mass yield of the ablated material [9]. The advantages of femto- and picosecond laser ablation processing for micro- and nanotechnologies are also due to non-thermal interaction with solid target materials, which is especially important when processing in liquid media.

According to the review [10], the fundamental difference between laser ablation using femto- and picosecond laser pulses and nanosecond ones lies in the sequence and cumulative interaction of such processes as absorption of laser radiation, emission of nanoparticles, plasma interaction with the medium, a gas bubble formation.

With femtosecond laser ablation in a liquid, it is possible to differentiate these processes by controlling the energy in the pulse and the environmental conditions. It is possible to fine-tune the process of laser ablation and, as a result, obtain predictable results. Femto- and picosecond laser processing is also accompanied by the phenomenon of multiphoton ionization, which improves the efficiency of laser beam energy transfer to the ablation process; the effects of the dependence of the absorption spectrum of the processed material on the wavelength of the acting radiation are not dominant.

The defining fact in liquid media processing is the achievement of conditions that facilitate precise processing of the material with a minimum thermal load [11]. The mechanism of production of nanoparticles and the sequence of processes of interaction of laser radiation with matter is presented as follows: within a few femtoseconds, multiphoton and tunneling ionization takes place; after about 50 fs, avalanche ionization will occur; and after 100 fs, the thermalization of the electrons

of the metal target will occur; after 1 ps, the transfer of electron energy to the lattice is observed; then, after 10 ps, some thermodynamic processes are possible, such as thermal diffusion, synthesis of nanoparticles, and explosion [12]. Photochemical processes and phase transformations take place in time intervals from 1 ns [13]. The question arises about the effective difference between femto- and picosecond laser systems, since during ablation the phenomena occurring in the impact zone in the energy and time intervals are comparable. In Ref. [14], the authors note a 20% performance advantage under the ablation effect of femtosecond laser pulses.

The size distribution and formation rate of laser-generated nanoparticles depend on the power density of laser radiation, the overlap of zones of influence of laser pulses, and the properties of the medium [15–16]. Therefore, the irradiated area at a certain pulse energy and the ratio of scan rate to repetition rate must be kept constant in order to obtain predictable and reproducible size distribution results. The use of F-Theta scanning system allows to control these parameters in a wide range of speeds. The linear mechanical scanning systems are often limited to beam travel speeds of up to 20 mm/s. If the task is to obtain nanopowders of a given dispersion, stoichiometric composition, and particle shape, the validity of the low cost of nanosecond systems and the complexity of using F-theta scanners are not taken into account. It is also noted that the size and dispersion of nanoparticles can be controlled by changing the wavelength [12, 17].

High fluence densities make it possible to create conditions for rapid heating of the material structure and rapid fixation of the crystalline structure. These properties are achieved due to local action and rapid cooling of the flow of ablated particles in the volume of the liquid medium. With femto- and picosecond laser ablation, a higher temperature and pressure in the local volume can be achieved. These conditions favor the synthesis of materials corresponding to the high-temperature phases of the crystal lattice; smaller nanocrystals with a narrow size distribution can be obtained [18–19]. Such results are unattainable for laser processing using long pulses (longer than ns duration) in view of the possible relaxation of structural changes during prolonged exposure to temperature. The absence of volumetric heating of the surface in the environment allows ablation even in temperature-sensitive liquids such as organic solvents or biomolecular solutions [20–21]. In addition, nanoparticles after ultrashort pulsed laser ablation retain the stoichiometric composition of the target material better than nanoparticles after nanosecond pulsed ablation, which is confirmed by the results of EDX analysis of nanoparticles [22]. Nevertheless, laser ablation with

femto- and picosecond laser pulses also has a negative side associated with propagation through liquid media and the development of a number of nonlinear effects. These effects lead to the development of a laser-induced plasma channel in the focal plane and lead, among other things, to beam filamentation [23–24]. Volumetric boiling up of the liquid and difficulty in the propagation of laser radiation through a layer of microbubbles in the caustic zone can be observed, which is less pronounced with nanosecond processing, but can be eliminated with ablation with high-speed beam scanning over the surface. Under the action of nanosecond laser pulses, the processes leading to the ablation of nanoparticles occur almost simultaneously, which makes it difficult to fine-tune the properties of the resulting nanomaterials (of course, if it is necessary to obtain compositions consisting of target materials – a liquid medium, when the liquid medium is used as a reaction medium). The nanosecond ablation effect is characterized by the transition of the ablated material through the melting stage. The generation of large nanoparticles in a liquid medium at a laser pulse duration of nanoseconds or more is due to the significant melting of the target material and the interaction of the laser pulse, including with cavitation bubbles, which are then formed in the liquid medium [25]. Nanosecond laser ablation ejects a stream of ablated particles, gas bubbles, intense hydrodynamic flows, which creates a kind of screen that prevents the energy of the laser beam from reaching the treated surface. Chemical reactions of the material, cumulative changes in the surface texture and morphology of the target material will occur due to surface melting and a combination of ablation and thermal activation processes [26]. A further increase in the pulse duration, on time scales from microseconds to milliseconds, triggers the mechanisms for the production of nanoparticles through plasma plume condensation [27]. Processing with longer pulses does not lead to productive synthesis due to the significant influence of hydrodynamic processes in a liquid medium. Intense boiling prevents effective transfer of laser radiation energy to the surface of the material being processed.

Thus, features of femtosecond laser ablation and fragmentation in liquid are the possibility of synthesizing a wide range of nanomaterials, including both the source material and new compounds formed during laser irradiation and depend on the environment [28–30].

For example, laser processing in water leads to the dissociation of water molecules, the formation of oxygen, and further oxidation of metal sulfides [31].

One of the nanoparticles groups can be successfully used in biomedicine are nanoparticles based on metal chalcogenides [32]. They are used, for example, in bioimaging, biosensing, drug delivery, photothermal therapy due to their excellent characteristics, antibacterial activity and high biocompatibility [33–34]. WS_2 nanoparticles have high antitumor activity, actively absorbing near-infrared radiation and converting it into thermal energy during photothermal therapy [35]. WS_2 nanoparticles accession with various molecules, it

becomes possible to use the obtained complexes for visualization and destruction of tumor lesions *in vivo* [36].

MoS_2 nanoparticles are also of great interest for basic research and applications in biomedicine, such as the development of functional nanoagents for biosensors, drug delivery, bioimaging, cancer therapy, and other biomedical applications [37–39]. There are a number of studies claiming that MoS_2 nanostructures, especially those with appropriate surface functionalization, do not have noticeable toxicity *in vitro* and *in vivo* [40]. Complexes with MoS_2 nanoparticles are used for tissue repair and antibacterial therapy [41].

ZnS nanoparticles are promising materials in the therapy of oncological diseases, since their introduction into tumor cells leads to the generation of reactive oxygen species that trigger cell destruction [42]. Complexes with ZnS nanoparticles can be used in magnetic hyperthermia [43], *in vivo* imaging [44], and skin regeneration [45].

ZnO is a semiconductor material with high biological stability and good exciton response, these properties make it a popular material for biosensors. Doping of ZnO with metals makes it possible to significantly change its sensor and catalytic properties [46–48]. ZnO nanoparticles production by laser ablation and subsequent fragmentation is a flexible method for tune the properties of described properties of zinc oxide.

Titanium and its alloys have a unique combination of strength and biocompatibility, which allows them to be used for medical purposes and explains their widespread use as materials for implants [49]. Compounds with TiO nanoparticles are also used in biomedicine to suppress tumor growth and have an antibacterial effect [50].

TiO_2 nanoparticles are widely used in the phototherapy of cancer cells, bacteria and are potential photosensitizing agents for photodynamic therapy due to their high stability and unique phototoxic effects upon irradiation [51–52].

All these nanoparticles can be obtained using method of laser ablation and fragmentation. Its advantages are simplicity, versatility, high purity of nanoparticles and absence of toxic products during synthesis [53]. Femtosecond regime gives an opportunity to control precisely nanoparticle structure [54].

In this work, we have considered the possibilities of using various dichalcogenides as starting materials for the formation of nanoparticles using the femtosecond laser ablation and fragmentation in water. The article considers the physicochemical characteristics and biocompatibility of nanoparticles synthesized by laser ablation and fragmentation in deionized water with femtosecond pulses of WS_2 , MoS_2 , ZnS and Ti targets. The sizes and morphology of nanoparticles were analyzed by dynamic light scattering (DLS) and scanning electron microscopy (SEM), and the features of the elemental composition were analyzed by energy dispersive spectroscopy (EDS). *In vitro* biocompatibility studies indicate the most suitable application for each of the types of synthesized nanoparticles.

2 Materials and Methods

2.1 Synthesis of Nanoparticles

For the synthesis of nanoparticles, the techniques of laser ablation and fragmentation in liquid with femtosecond pulses were used. Schemes, as well as images of the processes are presented in Fig. 1.

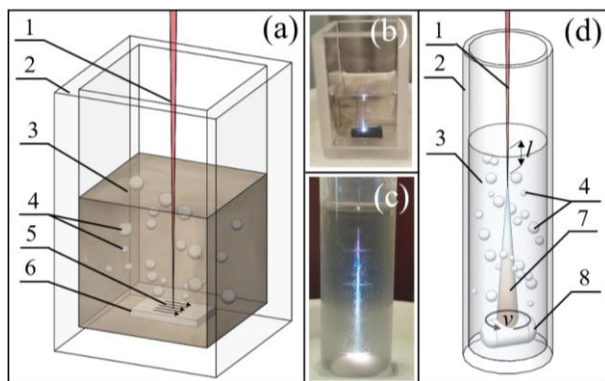


Fig. 1 Schemes and images of the processes of laser ablation of the target (a, b) and fragmentation (c, d) in liquid.

In our experiments, we used a Yb:KGW femtosecond laser system (Avesta Ltd., Russia) generating 280 fs pulses at 1030 nm wavelength of with 10 kHz repetition rate and maximum pulse energy of 150 μ J. In Fig. 1 the following positions are indicated: 1 – laser radiation; 2 – cuvette/tube; 3 – liquid medium; 4 – nanoparticles; 5 – trajectory of laser beam scanning; 6 – target; 7 – supercontinuum; 8 – magnetic anchor.

In the process of laser ablation and fragmentation, scanning with laser beam over the target surface, as well as scanning in the volume of the liquid, was carried out using a two-coordinate X-Y scanner system Raylase RLA-1504, equipped with a F-Theta lens with a working distance of 200 mm. Focused beam diameter according to the intensity level $1/e^2$ was 50 μ m. Deionized water was used as the liquid medium. In laser ablation, the targets were a synthetically grown molybdenum disulfide (MoS_2) crystal, a bulk sample of polycrystalline CVD zinc sulfide (ZnS MS), and high-purity titanium.

In experiments on laser fragmentation in liquid, in addition to colloidal solutions prepared by laser ablation in liquid, suspensions of monolayer tungsten disulfide (WS_2) of various concentrations were also used. The starting material consisted of flakes with lateral sizes of 0.1–4 μ m and a thickness of ~ 1 nm. Suspensions of monolayer tungsten disulfide were prepared by adding WS_2 powder to test tubes with deionized water and then placed in an ultrasonic bath. Suspensions of WS_2 were prepared at concentrations of 0.2 and 1 mg/ml then irradiated with femtosecond laser pulses.

During the laser ablation synthesis of MoS_2 nanoparticles, the crystal was located at the bottom of a cuvette filled with 5 ml of deionized water, the processing area was a square with a side of 5 mm, the scanning density was 20 lines/mm, the scanning speed

was 100 mm/s, the number of processing iterations was 60. This regime made it possible to achieve the required concentration of nanoparticles (0.2 mg/ml). Synthesis of ZnS nanoparticles was carried out at the same parameters. In the case of laser ablation of a titanium plate, the processing area was a square with a side of 1.5 mm with scanning density was 20 lines/mm and scanning speed was 100 mm/s, time of laser ablation was 10 min (total number of pulses $6 \cdot 10^6$, energy per pulse 100 μ J).

A test tube with a prepared suspension/colloidal solution, including those containing large particles in the form of shatter of ablated materials, was subjected to laser fragmentation. The solution was continuously stirred during laser fragmentation. The mixing of the solution during laser processing was carried out as follows: test tube was filled with a solution of 5 ml in volume; at the bottom, a magnetic anchor was located, and a torque was transmitted to the magnetic anchor by means of a magnetic stirrer. The waist region of the laser beam was located 10 mm below the air/liquid interface (in Fig. 1d distance is marked with l), while the rotation speed of the magnetic anchor was 400 rpm. The speed was chosen to ensure efficient mixing and to prevent the formation of a funnel on the solution surface. The beam scanning trajectory during the laser fragmentation of the solution was a 4 mm diameter circle with a linear filling density of 20 lines/mm and a scanning speed of 100 mm/s, while the scanning direction was changed by 90° , so the processing area was a grid.

Laser fragmentation of ZnS and MoS_2 colloidal solutions obtained by laser ablation in liquid was for 20 min (total number of pulses $12 \cdot 10^6$), pulse energy 100 μ J, energy fluence 10.2 J/cm².

During laser fragmentation of WS_2 suspension with concentration of 0.2 mg/ml, the pulse energy was set at the level of 30 μ J, energy fluence was 3 J/cm², and the laser irradiation duration was 2 min. In the case of WS_2 suspension with a concentration of 1 mg/ml, the pulse energy was 100 μ J, energy fluence was 10.2 J/cm², and the duration of the laser fragmentation process was 20 min.

2.2 DLS

To determine the sizes of the obtained nanoparticles, DLS measurements were performed using a Photocor setup (Photocor Ltd., Russia). The measurements were carried out at a temperature of 23 $^\circ\text{C}$. The autocorrelation function was processed with the DynaLS software. Based on the measurement results, the hydrodynamic diameter of nanoparticles was calculated.

2.3 Layers Deposition on Substrates

Each of the solutions was applied by spray deposition onto sterile clean 1×1 cm silicon substrates. The substrates were fixed in a special holder and placed in an E2V dispensing system for deposition (Nordson EFD, Westlake, OH, USA). The setup was a deposition system that can be positioned relative to three axes with high

accuracy. The diameter of the nozzle was 0.5 mm, the solutions were supplied under a pressure of 0.05 bar at air pressure 20 bar. To form a layer, 1.5 ml of solution was applied to each of the substrates. During the deposition process, the substrates were heated using a heating table to ensure faster drying of the solution and formation of a layer. For physical studies, WS₂ nanoparticles with lower concentration were deposited on the substrate for the convenience of analyzing individual nanoparticles. In biological studies, for the most complete coverage of the substrate and the maximum consideration of the effect of nanoparticles on cells, WS₂ nanoparticles with higher concentration were applied.

2.4 Microscopy

The surface features of the deposited layers of nanoparticles on silicon were studied using an FEI Helios NanoLab 650 scanning electron microscope (FEI Ltd., Hillsboro, OR, USA) equipped with an attachment for energy-dispersive X-ray spectroscopy (EDS). The samples were attached to the holder with carbon tape. Accelerating voltage of the electron beam was 5 kV, electron probe current was 21 pA. Images were obtained both in frontal projection and with the substrates tilted by 52°.

2.5 Layer Studies In Vitro

For a comparative assessment of the cytotoxicity of the synthesized nanoparticles, the surfaces of the silicon substrates were completely covered with a layer of each type of nanoparticles according to the method described in Section 2.3. Thus, the layer on the substrate was a sample with the highest possible concentration of nanoparticles that affect the vital activity of cells. This method makes it possible to make a choice of the type of nanoparticles for further research, depending on the specific application. After fabrication, the samples were sterilized with ultraviolet for 20 min. Cellular experiments were carried out with FH-T cell line – human fibroblasts, which was acquired at the National Research Center for Epidemiology and Microbiology of the Ministry of Health of the Russian Federation. Protocol for incubation of nanoparticles with cells was as follows. The samples with layers of nanoparticles were placed to the bottom of the wells of a 12-well culture plate (the area of the well bottom was 3.8 cm²) and filled with a cell suspension in a DMEM culture medium supplemented with 10% calf serum in an amount of 2×10^5 cells/ml. 1 ml of cells was added to each well. The plates were placed in a CO₂ thermostat and kept there for 48 h.

To quantify the cells on the samples, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay was performed according to the standard method [55]. At the end of the incubation period, the culture medium was removed. 100 µl of pure culture medium was added to each well with 20 µl of MTT at a concentration of 5 mg/ml. MTT is a tetrazole-based dye that interacts with the mitochondria of living cells to form

formazan crystals. MTT remained in the wells for 2 h, after which the culture medium was removed. Further, dimethyl sulfoxide was added to dissolve the formazan crystals and obtain a purple color of the solution, the cell pellet was resuspended for 10 min. Staining intensity is proportional to the number of living cells, a microplate photometer Immunochem 2100 (High Technology Inc., North Attleboro, MA, USA) was used to quantify staining intensity.

3 Results and Discussion

3.2 Nanoparticles Fabrication and Determining Their Size in a Colloidal Solution

Nanoparticles solutions and samples obtained after solutions deposition to the substrate are presented in Fig. 2. The average hydrodynamic diameters of nanoparticles in a colloidal solution after synthesis were obtained by the DLS method. The calculated nanoparticle sizes are given in Table 1.

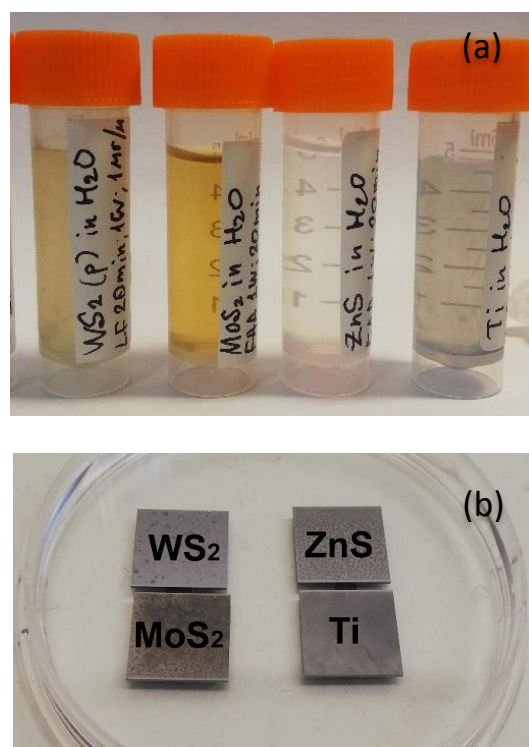


Fig. 2 Appearance of colloidal solutions of synthesized nanoparticles (a) and layers of nanoparticles applied to silicon (b).

Despite the possibility of studying nanoparticles directly in a liquid medium, the DLS method is inferior in accuracy to microscopic methods in determining the size of nonspherical particles, as well as nanoparticles with a large size spread in one solution [56]. In addition, various surface non-homogeneities, such as porosity or the presence of smaller particles on the surface, also cannot be assessed by the DLS method [57].

Table 1 Results of DLS measurements.

Types of nanoparticles from starting materials	Average hydrodynamic diameter, nm
WS ₂	226
MoS ₂	92
ZnS	106
Ti	166

3.3 Morphology and Composition of Layers

The surface features of the deposited layers were analyzed by SEM (Fig. 3). According to microscopy data, WS₂-based nanoparticles are polygonal sheets 120 to 600 nm in size (Fig. 3 a, b). In addition, accumulation of agglomerates of nanoparticles is found in some areas, which indicates the requirements for their longer processing to break the agglomerates before application. MoS₂-based nanoparticles are more uniformly distributed over the silicon surface, have a shape close to spherical, and sizes from 50 to 100 nm (Fig. 3 c, d).

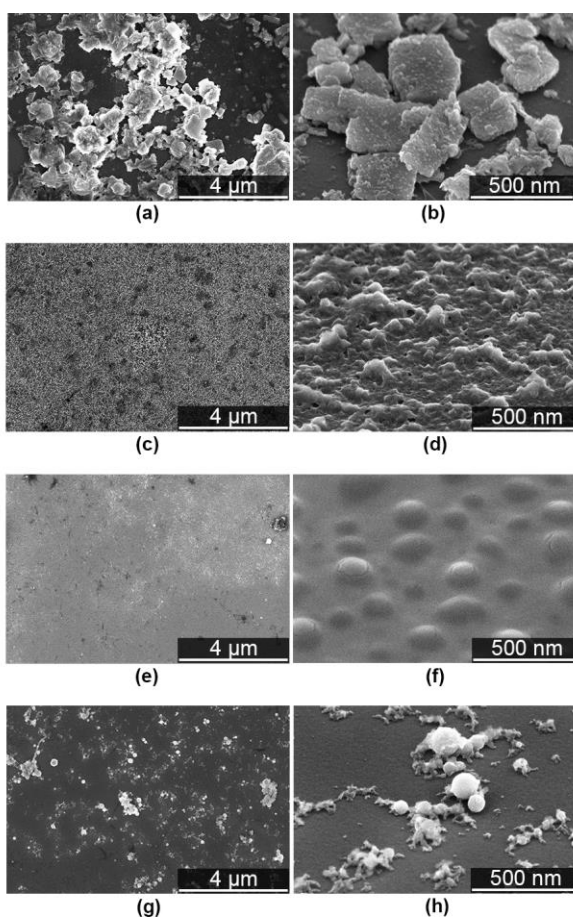


Fig. 3 SEM images of the surface of layers of deposited WS₂-based (a, b), MoS₂-based (c, d), ZnS-based (e, f), and Ti-based (g, h) nanoparticles obtained by passing an electron beam at a right angle to the surface (a, c, e, g) and at a surface tilt of 52° (b, d, f, h).

ZnS-based nanoparticles have a spherical shape and sizes from 60 to 130 nm, distributed mostly uniformly, occasionally there are agglomerates of 2–3 connected particles (Fig. 3 e, f). Ti-based nanoparticles are individual spheres with sizes from 60 to 140 nm (Fig. 3 g, h), distributed over the surface less uniformly compared to ZnS-based nanoparticles. The sizes of nanoparticles measured by the SEM method approximately correspond to the DLS estimate, except for WS₂-based nanoparticles, which have a nonspherical shape and a large size spread.

Thus, according to the DLS and microscopy data, among the synthesized nanoparticles, MoS₂-based nanoparticles have the smallest sizes, while WS₂-based nanoparticles are the largest sizes. Large flat nanoparticles can be efficiently absorbed by cells and accumulate in tumors, which is an advantage in cancer therapy [58]. Non-spherical nanoparticles also have a large surface area for attaching different molecules. WS₂-based and Ti-based nanoparticles are deposited on substrates less uniformly and tend to aggregate; therefore, when using them *in vivo*, special attention should be paid to the methods of their separation in order to avoid an uneven and hardly predictable distribution in the tissue volume.

3.4 EDS Measurements

During laser fragmentation of chalcogenides (ZnS, MoS₂, WS₂), hydrogen sulfide evolution was observed. The formation of hydrogen sulfide indicates the dissociation of water and processed chalcogenides. The conditions of irradiation during laser fragmentation promote the formation of new chemical bonds, and a partial replacement of sulfur by oxygen is observed. Laser processing of Ti in water results in the formation of oxides. The identification of the formed chemical compounds required an energy dispersive analysis of the formed nanoparticles. The results of EDS analysis are presented below in Figs. 4–7.

The oxygen content in the resulting nanoparticles can be effectively controlled during fragmentation, just as the size dispersion of nanoparticles. Energy dispersive analysis is a convenient tool for diagnosing the elemental composition of micro- and nanostructures.

During laser fragmentation of the WS₂ suspension, the presence of oxygen was established (Fig. 4). In Ref. [59], the results of the assessment of the toxicity of tungsten oxide nanoparticles are presented, it is noted that it can be controlled by changing the concentration of nanoparticles, as well as by the optical emission spectrum in view of the high photocatalytic activity of tungsten oxide, especially in the short-wavelength part of the spectrum. The pronounced dependence of toxicity on the concentration of nanoparticles was considered in Ref. [60]. There is a high sorption capacity of nanoparticles relative to microparticles by the tissues of the body of rats. In Ref. [61], the stabilization of tungsten oxide nanoparticles is noted when polyvinylpyrrolidone (PVP) is used as a coating agent. As a result of laser fragmentation, the WS₂ suspension is less susceptible to oxidation than ZnS and MoS₂ nanoparticles.

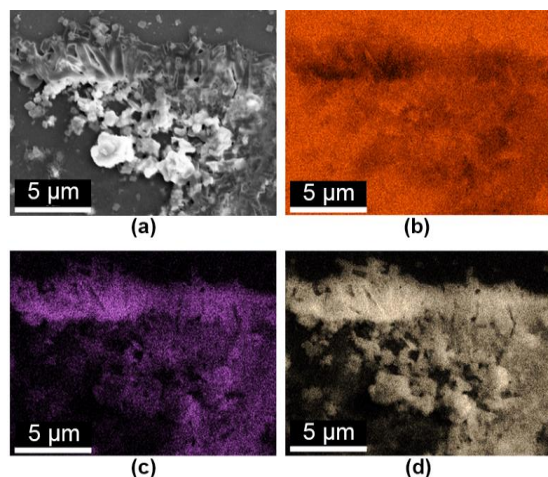


Fig. 4 SEM image of the surface of WS₂-based nanoparticles (a): distribution of tungsten (brightness + 50%) (b), sulfur (c), and oxygen (d).

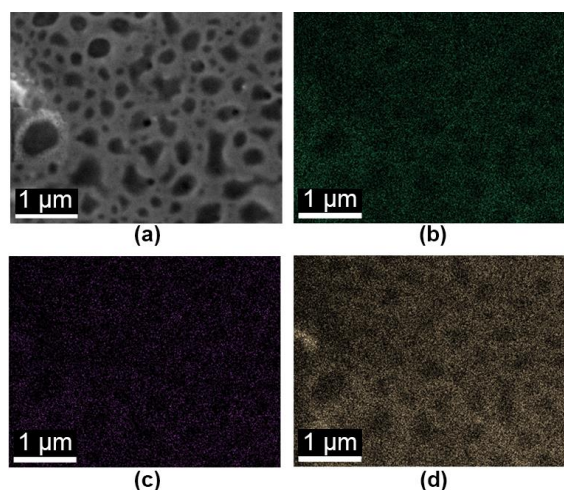


Fig. 5 SEM image of the surface of Zn-based nanoparticles (a): distribution of Zn (brightness + 50%) (b), sulfur (c), and oxygen (d).

Slight changes in the shape of the nanoparticles obtained after fragmentation may indicate an insignificant value of the laser effect, or a high stability of the tungsten oxide suspension for this type of processing. A sufficiently large size of the obtained particles may indicate the need to increase the fragmentation time in order to reduce the size.

During laser fragmentation of ablated zinc sulfide nanoparticles in water, ZnS can be destroyed and both oxygen and hydroxyl bonds can form. It is possible to violate stoichiometry, especially on the surface of nanoparticles, and, as a result, oxygen sorption from air during sample preparation. SEM image of a continuous film-like coating on the surface of the obtained samples with pronounced local elevations, while maintaining the signal of sulfur and oxygen, is shown in Fig. 5.

The prevalence of the oxygen response in fragmented ZnS nanoparticles may indicate a significant content of zinc oxide. In the process of laser ablation of ZnS in an

aqueous medium, the formation of nanoparticles and flocculent deposit of the clusters are often observed [62], during the drying of which they form a continuous coating of the surface. This occurs when the concentration of particles in the solution is high.

The results obtained (Fig. 6) show the oxidative capacity of MoS₂ during laser fragmentation in an aqueous medium. This activity is due to the high catalytic capacity of water vapor for the oxidation of molybdenum [63]. During laser fragmentation, hydrogen sulfide evolution is observed. The formation of hydrogen sulfide is possible during the ionization/dissociation of water or water vapor as a result of the laser irradiation process. Laser fragmentation leads to a violation of the initial stoichiometry of molybdenum disulfide with the formation of many derivative compositions [64–66].

The results of energy dispersive analysis of nanoparticles obtained by laser ablation of titanium in deionized water showed the presence of a significant amount of oxygen (Fig. 7).

The absence of a change in the shape of particles relative to the spherical shape indicates the presence of oxygen on the surface due to some of its deficiency. An excess oxygen content leads to significant changes in the shape of nanoparticles, including intergrow and form of a single common surface. In addition, the formation of core-shell particles is possible. The presence of oxygen on the surface of titanium nanoparticles can significantly expand the area of their application in biosensors. In Ref. [67], high biocompatibility and high stability in both acidic and alkaline media are noted.

The instability of titanium oxide can be compensated for by its deficiency in the nanoparticle. Titanium oxide in different phase states (rutile, anatase) has an ambiguous effect on biological objects. In Ref. [68], the results of the toxic effect of titanium oxide nanoparticles of various sizes and phase composition on cell cultures are presented.

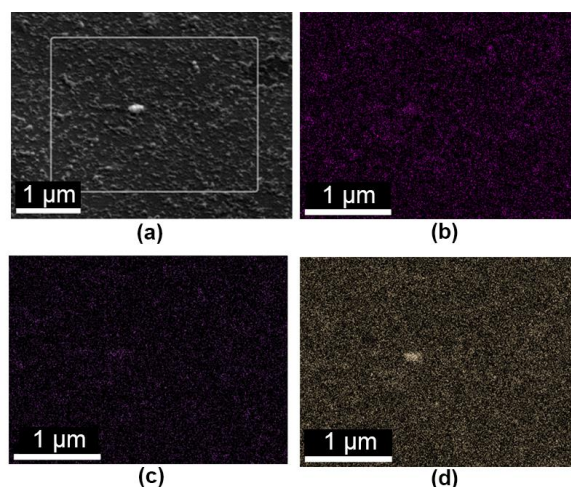


Fig. 6 SEM image of the surface of MoS₂-based nanoparticles (a): distribution of Mo (brightness + 50%) (b), sulfur (c), and oxygen (d).

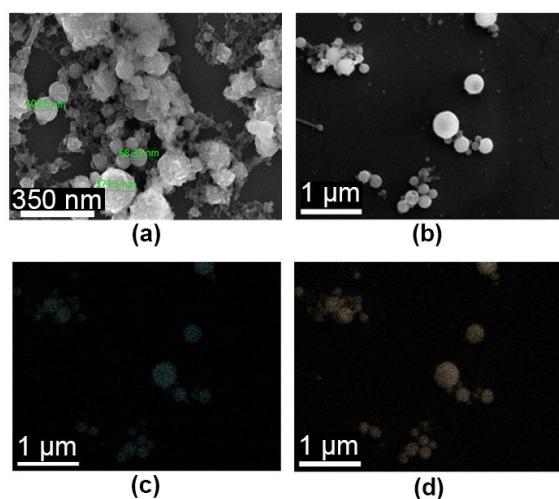


Fig. 7 SEM images of the surface of Ti-based nanoparticles (a, b): distribution of Ti (c), oxygen (d).

Localized areas of titanium oxide on the surface of the obtained titanium nanoparticles are limited by the volume of the material, which hinders its polymorphic transformations when external conditions change. These factors can be used in the production of stable sensor systems based on titanium oxide.

The results obtained allow us to conclude that water is highly reactive in the process of laser ablation and fragmentation, since significant changes in the chemistry of the obtained materials relative to the initial ones are observed. The observed changes, among other things, can be caused both by the nature of the water itself, and by significant changes in the properties of this medium in the region of the caustic of the transmitted laser radiation. The ionization energy of water is from 9.2 eV, pronounced continuum generation in the visible part of the spectrum is observed at energies of 10.5–12.5 eV [69–70] respectively, under our processing conditions, in particular fragmentation, the formation of a continuum during the laser radiation passing through water corresponds to the state ionization of the environment. Particles in a medium corresponding to the state of ionization are capable of forming chemical bonds, which was observed in Refs. [71–74].

3.5 Biological Properties of Nanoparticles

Various nanoparticles and nanostructures obtained with laser radiation can be successfully used as components for bioelectronic devices [75] and in biomedical applications, including scaffold materials for tissue engineering [76–78]. Conducting complex studies of the cytotoxicity of nanoparticles is an important step before *in vivo* and clinical studies. This section presents the results of a preliminary assessment of the cytotoxicity of the synthesized nanoparticles in the short term, which makes it possible to outline a further vector of *in vitro* studies. The method of forming layers of nanoparticles on a silicon substrate using a spray application of homogeneous colloidal solutions made it possible to

achieve complete coverage of the substrate surface with a layer of nanoparticles for cell studies in order to take into account their effect on cells to the maximum. This method made it possible to determine the survival of cells on a layer with the maximum concentration of nanoparticles of various types.

One of the most common, simple and reproducible tests for determining the biocompatibility of samples is the MTT assay. The results of the MTT assay of the deposited layers of nanoparticles are presented in Fig. 8.

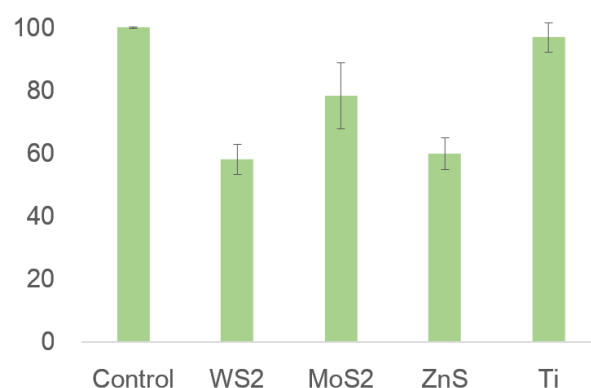


Fig. 8 The number of cells on the surface of the deposited layers of nanoparticles as a percentage of the control sample (clean substrate) according to the MTT assay.

The best biocompatibility is exhibited by layers of Ti-based nanoparticles; the layers of WS₂-based and ZnS-based nanoparticles are the least biocompatible among the studied particles. Layers of MoS₂-based nanoparticles show average biocompatibility values of ~80%. One of the reasons for the decrease in the biocompatibility of the layers may be the ability of metal chalcogenide nanoparticles to form reactive oxygen species, which negatively affects cells [79]. Nanoparticles, however, can be attached to molecules of photosensitizers and used at the studied concentration for an additional destructive effect on cancer cells during photodynamic therapy [80]. The toxicity of these nanoparticles for targeted drug delivery applications can be reduced by covering them with biocompatible coatings [81]. Due to the high biocompatibility, Ti nanoparticles can be used for biomedical applications in drug delivery and tissue regeneration without additional modification. The biocompatibility of synthesized nanoparticles can be affected by the concentration of nanoparticles in solution; additional binding of molecules to nanoparticles can either increase or decrease their biocompatibility.

In the case of using nanoparticles for cancer therapy, due to the characteristics of blood vessels supplying tumor tissues, it is more efficient to use large nonspherical nanoparticles [82]. In this case, the long-term presence of nanoparticles in the tumor volume is more important than their rapid removal from the body.

There is evidence that in the process of additional modification of nanoparticles (for example, addition of a polymer), it is possible to both reduce their size [83] and

change their biological properties in the required direction [84]. Modification of the surface of nanoparticles makes it possible to change the rate of their filtration by the excretory system or to minimize their absorption by the liver [85].

4 Conclusions

In this work, colloidal solutions and layers of nanoparticles of 4 types of starting materials deposited on silicon wafers were studied: WS₂, MoS₂, ZnS, Ti, obtained using the technique of laser ablation and fragmentation in liquid with femtosecond pulses. Among the considered nanoparticles, MoS₂-based nanoparticles have the smallest size, and WS₂-based nanoparticles have the largest size. WS₂-based nanoparticles have a non-spherical shape and, at the studied concentration, have an inhibitory effect on cell growth, which can be used when these nanoparticles are introduced into the tumor zone for its subsequent destruction by photodynamic therapy under the action of laser radiation. ZnS-based nanoparticles have a similar effect. It should be noted that ZnS-based nanoparticles have high antiseptic properties, including those actively used for photocatalytic antibacterial treatment. Our results demonstrate the relatively low toxicity of the structures obtained, which may be due to the presence of a shell of zinc oxide, a significant substitution of zinc sulfide, zinc oxide, which

is characterized by high biological inertness. Ti-based nanoparticles are spherical and highly biocompatible, which makes them promising for targeted drug delivery and tissue regeneration. The content of titanium oxide on the surface of nanoparticles (determined using EDS) requires further research. On the one hand, the presence of titanium oxide on the surface of the obtained nanoparticles can be obtained as a result of laser fragmentation, when the nanoparticles were repeatedly exposed to laser radiation in water, as well as the formation of titanium oxide during the storage of nanoparticles, also, it is impossible to exclude a change in the phase composition of the surface of these particles due to the high chemical activity of these materials. Titanium oxide in different phase states (rutile, anatase) has an ambiguous effect on biological objects.

Disclosures

The authors have no relevant financial interest in this article and no conflict of interest to disclose.

Acknowledgments

This study was funded by the Russian Science Foundation (project no. 22-79-10348, <https://rscf.ru/project/22-79-10348/>).

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