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CHEMICAL TECHNOLOGY

Elemental Boron Nanoparticles: Production by Ultrasonication in Aqueous Medium and Application in Boron Neutron Capture Therapy

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Abstract—A method for producing elemental boron nanoparticles with a size of less than 100 nm by ultrasonic dispersion in a liquid medium and subsequent cascade fractionation is described. The resulting boron nanoparticles were used as a target for boron neutron capture therapy (BNCT). According to the results of an experimental preclinical study of BNCT with the synthesized boron nanoparticles, neutron irradiation for 1 h of T98G human glioma cells pre-incubated in a medium with boron nanoparticles (10, 20, and 40 ppm in terms of boron-10 isotope) leads to a significant suppression of cell viability.

Keywords: elemental boron, boron nanoparticles, ultrasonic dispersion, cavitation, glioma, cytotoxicity, boron neutron capture therapy, epithermal neutron accelerator

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An applied problem of the cancer therapy is the development of new drugs that would increase the efficiency of the therapy. Elemental boron particles represent a potentially new form of target drug for the boron neutron capture therapy (BNCT) of cancer, owing to high content of boron atoms in one particle. BNCT is a binary radiotherapeutic method based on the capture and fission of neutrons upon irradiation of the stable boron-10 isotope with thermal neutrons, giving high-energy α-particles and lithium nuclei [1–4]. The selective uptake of a boron-10 drug in a necessary therapeutic concentration (10 billion boron-10 atoms per cell) in the damaged area followed by neutron irradiation induces targeted destruction of the specified area owing to the release of nuclear reaction energy within a single cell [4].

In this study, elemental boron nanoparticles of less than 100 nm size were prepared for the first time using ultrasonic dispersion in a liquid medium followed by cascade fractionation. We evaluated for the first time the applicability of boron nanoparticles obtained in this way as a BNCT target drug of a new type, which has no toxicity and ensures sufficient uptake of boron-10 by the test cell line. The viability of boron-containing and intact T98G human glioma cells was estimated after irradiation with epithermal neutrons from the accelerator of the Institute of Nuclear Physics, Siberian Branch, Russian Academy of Sciences (Novosibirsk). It was shown for the first time that irradiation of T98G tumor cells cultured in growth media in the presence of elemental boron nanoparticles present in concentrations of 10, 20, and 40 ppm (in terms of boron-10) reduces the colony forming ability relative to the control (with boron nanoparticle content of 0 ppm).

In industry, elemental boron particles are usually prepared by chemical vapor deposition (CVD) [5]. It is noteworthy that CVD methods afford elemental boron powders in which particles with a wide size distribution (0.5 to 20 μm) prevail. These materials are inapplicable as targets for BNCT, since the boron-10 drugs

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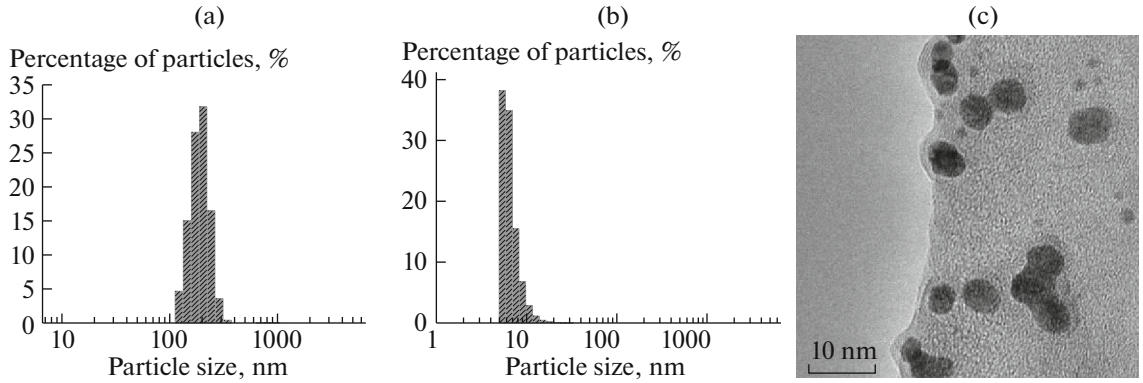

Fig. 1. Size distribution of boron particles after (a) the first and (b) the third stage of ultrasonication. (c) Micrograph of boron particles after the third stage of ultrasonication.

for preclinical studies are used as solutions or colloidal systems [6] with a particle size of not more than 100 nm.

In this study, we demonstrate that modern ultrasonic technologies can be successfully used to advance the industrial CVD process. It was shown that ultrasonication makes it possible to disperse sintered boron powder particles to the nanoscale range. The use of water as the dispersion medium is one of the key advantages for the subsequent manufacture of BNCT agents for preclinical trials.

The nanoparticles of elemental boron were prepared in the following way [7]: A finely dispersed elemental boron powder (0.1 g) (amorphous boron, B99V brand manufactured by AVIOBOR: weight fraction of boron of at least 99%, average particle size of 0.5–4 μm) was placed into a glass beaker, and doubly distilled water (25.0 mL) was added. Long-term ultrasonication (τ_I = 360 min) of the initial micron-size dispersion of boron and cascade fractionation gave a 100–250 nm fraction of particles (stage I; Fig. 1a). Further ultrasonic treatment of the 100–250 nm fraction (τ_{II} = 160 min) yielded nano-sized boron particles with 50–70 nm particle size (stage II). Ultrasonic dispersion of the 50–70 nm fraction for τ_{III} = 180 min resulted in the formation of 5–15 nm nanodispersions (stage III-A). The treatment of 50–70 nm fraction for τ_{III} = 250 min gave a dispersion of spherical nanoparticles with 5–10 nm sizes (stage III-B; Figs. 1b, 1c).

The ultrasonic dispersion was carried out using an Inlab ultrasonic generator (Russia) with an immersion probe made of titanium alloy with an output power of 0.63 kW. After every stage of dispersion, the particle size distribution in boron hydrosols was determined by dynamic light scattering on a Microtrac Zetatrac analyzer. The particle size and shape at the final stage of ultrasonic dispersion were studied using a Hitachi H-800 transmission electron microscope (40000 magnification).

The structure of boron nanoparticles was studied by X-ray diffraction using a Bruker D8 Advance diffractometer (CuK_α radiation (λ = 1.5418 nm), Ni filter, focusing Ge monochromator, LYNXEYE detector). The measurements were carried out in the transmission mode in the range of scattering angles 2θ = 10°–50° with sample spinning at 90 rpm. Prior to X-ray diffraction studies, hydrosol samples were frozen in liquid nitrogen and freeze-dried with a Martin Christ Alpha 2-4 LSC dryer to give a highly dispersed powder. A weighed sample of the powder was placed between amorphous poly(ethylene terephthalate) films. The processing of X-ray diffraction patterns (normalization, corrections for non-coherent and background scattering) was done using the DIFFRAC EVA and Origin 15 Pro programs.

Figure 2 shows the X-ray diffraction patterns of boron powders before ultrasonication (Fig. 2a) and after the third stage (Fig. 2b). The presented data indicate that the elemental boron occurs in these particles in the amorphous state (halo at 2θ = 17°–24° and 30°–42°) both before the ultrasonic treatment and after three stages of treatment. In addition to amorphous elemental boron, the particles contain polycrystalline boric acid. In the initial powder, the boric acid impurity has a triclinic crystal lattice with the reflections *010* (2θ = 14.60°), *100* (2θ = 14.98°), and *002* (2θ = 28.02°). Presumably, the ultrasonication results in partial dissolution of boric acid during the cavitation-induced fragmentation of particles; drying of the hydrosol after three stages of sonication results in recrystallization of the acid in a hexagonal system (reflections: *100* (2θ = 14.50°), *003* (2θ = 27.97°), and *201* (2θ = 30.72°)).

The results indicate that the multistage ultrasonication is an efficient and easily scalable method for the preparation of elemental boron nanoparticles.

Since the boron nanoparticles are meant, first of all, for the therapy of cancer by neutron irradiation, the following biological tests were carried out: determination of the cytotoxicity of boron nanoparticles,

accumulation of boron in cancer cells, and evaluation of the BNCT efficiency using clonogenic assay.

Model experiments were performed for T98G human glioma cells, which are often used to study the effects of ionizing radiation, including neutron radiation. [8]. The T98G human glioma cells were provided by the Institute of Cytology, Russian Academy of Sciences (St. Petersburg) and cultured in the Iscove's DMEM medium (IMDM, SIGMA 17633 with L-glutamine and 25 mH HEPES buffer) with the addition of 10% fetal bovine serum (Thermo Scientific HyClone SV30160.03 HyClone UK Ltd.) at 37°C in 5% CO_2 atmosphere.

The cytotoxicity of the boron nanoparticles was determined using a Cell Titer 96 AQueous One Solution colorimetric assay (Promega corp., Madison). The T98G cells (4×10^4) were placed into each well of a 96-well plate with 100 μL of the medium and incubated for 24 h. The medium was replaced by a medium with boron nanoparticles (5–10 nm particle size) in concentrations of 0–250 μg of boron/mL and incubated for 24 h. Then the medium was removed and the cells were washed with a buffer solution. A solution (2 mL) of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) with PMS (Cell Titer 96 AQueous One Solution) was mixed with the medium (10 mL), and the mixture (100 μL) was added into each well. The plates were incubated for 2 h and placed in a Thermofisher Scientific Multiskan FC plate reader to measure the absorbance at 490 nm. The data are presented as the percentages of surviving cells after incubation with the drug relative to incubation without the drug (Table 1).

The cytotoxicity data indicate low toxicity of nanoparticles for boron concentrations up to 250 ppm (μg/mL of the medium solution) (50 ppm of boron-10), which is far more than the required therapeutic range (~20 ppm); this confirms the applicability of nanoparticles for neutron irradiation experiments.

The boron content in the T98G cells was quantified by inductively coupled plasma atomic emission spectroscopy (ICP AES) on a Thermo Fisher Scientific iCAP 6500 instrument [2]. The cells were incubated with boron nanoparticles (5–10 nm particle size) in the growth medium for 24 hours. After incubation, the cells were washed with phosphate buffered saline (PBS) and removed from the plastic substrate with 0.05% trypsin solution (Trypsin-EDTA, Nacalai Tesque, Inc.). Then the samples with boron nanoparticles were boiled in 70% nitric acid for 3 h up to the formation of boric acid, which was detected using the instrument. The instrument was calibrated against standard solutions of boric acid with known concentrations (Boron Standard for ICP, TraceCERT, 1000 mg of H_3BO_3/L of H_2O, Sigma) (Table 1).

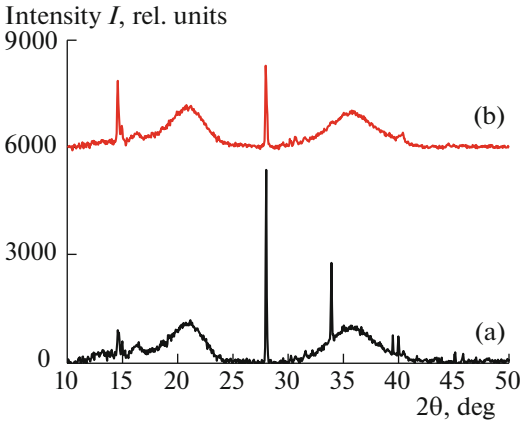

Fig. 2. X-ray diffraction patterns of boron powders (a) before ultrasonication and (b) after the third stage of ultrasonication.

On exposure to a medium with 40 μg/mL concentration of boron-10, boron nanoparticles are accumulated in T98G glioma cells in considerable amounts of up to 3.9 μg/10^6 cells, which significantly exceeds the accumulation of boron phenylalanine BPA (boron-10), which is 0.37 μg/10^6 cells.

The experimental preclinical study of the synthesized boron nanoparticles, approximating the real

Table 1. Evaluation of the cytotoxicity of boron nanoparticles (NPs) against T98G cells; colony forming ability of T98G cells in the presence of boron NPs after neutron irradiation; accumulation of boron nanoparticles in T98G cells

No.	Concentration of boron NPs, μg/mL of the medium	Colony forming ability of T98G cells, pre-incubated with boron NPs, after neutron irradiation, %	Cytotoxicity of boron NPs against T98G cells, %	Accumulation of boron NPs in T98G cells, μg/mL of the medium (ICP AES data)
1	0	100.0	100.0	0.0
2	50 (boron-10:10 ppm)	84.5	92.0	48.5
3	100 (boron-10:20 ppm)	52.0	88.0	98.6
4	150 (boron-10:30 ppm)	40.0	86.0	145.0
5	200 (boron-10:40 ppm)	29.0	84.0	197.5
6	250 (boron-10:50 ppm)	23.0	85.0	246.0

clinical conditions, was carried out using epithermal neutrons from an accelerator [9] at the Institute of Nuclear Physics, Siberian Branch, Russian Academy of Sciences (Novosibirsk). Irradiation parameters: proton current for the neutron-generating target of 1.8 mA; energy of 2.0 MeV; neutron fluence 7.2×10^{11} cm^{-2}. According to clonogenic assay data, neutron irradiation for 1 h of the tumor cells pre-incubated in a medium containing boron nanoparticles (10, 20, and 40 ppm expressed in terms of boron-10) leads to a significant suppression of cell viability (Table 1).

The results obtained in this study may be used to produce therapeutic drugs for BNCT based on the boron-10 stable isotope.

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