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The preparation method of cells with boron nanoparticles to determine boron by direct current arc atomic emission spectrometry

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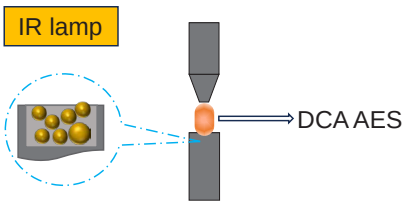
An approach to quantify boron in cells after administration of boron nanoparticles (BNPs) stabilized in hydroxyethyl cellulose (HEC) was developed. *In vitro* experiments with human glioblastoma cells and BNPs stabilized in HEC were carried out. Cell samples with BNPs were dried on graphite powder under an infrared lamp and analyzed by direct current arc atomic emission spectrometry (DCA AES).



Keywords: boron nanoparticles, BNCT, atomic emission spectrometry, glioblastoma cells, boron determination, sample treatment.

The synthesis of boron-containing drugs with required properties will be a key to successful cancer treatment using boron neutron capture therapy (BNCT). Boronophenylalanine (BPA) and sodium boroncaptate (BSH), widely used as a boron delivery agent, have low selectivity and accumulate relatively poorly in tumors.[1] There is an active search for new suitable boron compounds. It has been proposed to use an inorganic boron compound[2] or boric acid,[3–5] as well as binding of BPA,[6–9] BSH,[9–12] or other boron compounds[13,14] to various clusters such as liposomes or polymers. Nanoparticles of elemental boron (BNPs) are another potential drug for BNCT.[15] The digestion method for boron quantification depends on the type of nanoparticles.[16–19] We offer universal sample preparation method for samples containing any nanoparticles, including samples after phagocytosis by cells. Previously, a similar approach was used to determine silicon in silicon nanoparticles stabilized in an organic solvent,[20] and to trace elements in micro- and nanoparticles from coal dust.[21] Direct current arc atomic emission spectrometry (DCA AES) was used to quantify boron in any BNPs produced by any method.

The synthesis of BNPs by ultrasonic treatment in an aqueous medium was described earlier.[22] The size of BNPs was determined by transmission electron microscopy (TEM). Individual spherical boron particles with a size of 70 ± 10 nm were observed (Figure 1). The presence of particle associates was also noted, which content did not exceed 5%. The size of BNPs determined by small-angle X-ray scattering (SAXS) was 61 ± 9 nm (Figure S1, Online Supplementary Materials). BNPs were stabilized in a solution of hydroxyethyl cellulose (HEC) to give an injectable form. The BNP concentration was 100, 210, and 500 mg dm^{-3} (BNPs/HEC composition **1**, **2**, and **3**, respectively).

The human glioblastoma U87 MG (ATCC® HTB-14™) cell line was stored in the SPF-vivarium cryobank at the Institute of Cytology and Genetics (Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia). Composition **2** (V = 0.5 ml) was added to U87 cells, and the ratio of BNPs to nutrient medium was 1 to 10. Cells were incubated at 37 °C in an atmosphere with 5% CO_2 for 24 h. Cells and nutrient medium were separated and placed in the tubes of 10^6 cells (for details of *in vitro* experiment design, see Online Supplementary Materials). The *in vitro* experiment design suggests that BNPs are located within or associated with cells.

The BNPs/HEC composition, cells, and nutrient medium were placed on graphite powder with 4 wt% sodium chloride[23,24] (spectral buffer) and dried by an IR lamp at ~80 °C. The resulting mixtures were stirred and placed into the craters of graphite electrodes; then, the DCA AES analysis was performed (for details, see Online Supplementary Materials).

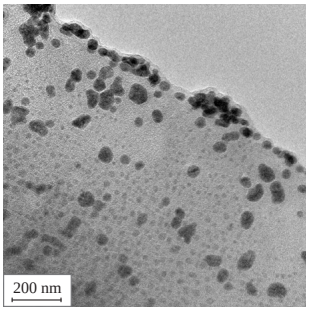


Figure 1 TEM micrograph of boron nanoparticles.

Dilution of the original BNPs/HEC compositions with water leads to an uneven distribution of BNPs in the resulting solution apparently due to agglomeration. Boron recoveries were determined to be 36% for composition **1** and 26% for composition **3**. Dilution was carried out by adding pure spectral buffer after initial drying and mixing of BNPs/HEC compositions. It was found that the HEC residue does not affect the stability of the arc discharge and the nature of the analyte entry into the arc plasma. No acid digestion of the HEC residue is required. Without any acid treatment, recoveries are close to 100% with relative standard deviation (RSD) values not exceeding 10%.

Boron was determined by the spectral line of boron at 249.667 nm with a linear concentration range from 1 mg dm^{-3} to 100 mg dm^{-3} and at 249.772 nm with a linear concentration range from 0.1 mg dm^{-3} to 10 mg dm^{-3}. The boron limits of detection (LODs) were calculated using the 3s criteria: LODs = 3 × × $S_{background}$, where $S_{background}$ is a standard deviation of boron concentration in pure spectral buffer. The LODs values are 22 and 7 ng for the lines at 249.667 and 249.772 nm, respectively. The limits of quantification (LOQs) in cells are 22 × 10^{-6} ng for the 249.667 nm line and 7 × 10^{-6} ng for the 249.772 nm line. The LOQs in solution are 0.5 μg ml^{-1} and 0.18 μg ml^{-1} for lines at 249.667 and 249.772 nm, respectively. BNPs are not currently used in BNCT. Let us compare the obtained LODs with therapeutic doses of BPA (700 mg kg^{-1} body weight) and BSH (100 mg kg^{-1} body weight).[25] A patient weighing 60 kg is administered with 2.2 and 3.5 g of boron using BPA and BSH, respectively. So, the LODs we obtained are much lower than the boron amounts injected for BNCT.

The accuracy of the proposed DCA AES method was evaluated by spike experiment (Table S1, Online Supplementary Materials). The results obtained showed excellent recoveries (98–107%) and precision (standard deviation <15%).

The results of analyses of BNPs/HEC compositions **1**–**3** are presented in Table 1. The proposed DCA AES method makes it possible to provide the RSD at the level of quantitative analysis that is 10%. This is an excellent achievement for the DCA AES method as it typically has a higher RSD.

In vitro experiments are performed to demonstrate the accumulation of boron by cells in exploration of potential BNCT drugs. The *in vitro* experiments were carried out with the BNPs/HEC composition **2**. Cells in these experiments are washed to remove any BNPs that are not incorporated into them. The proposed method makes it possible to quantify the boron content in cells after BNPs administration. Cells and separated nutrient medium after boron accumulation were analyzed using the proposed DCA AES method. A different sample preparation in principle was also used, namely, dissolution in acid mixture and analysis of the resulting solutions by inductively coupled plasma atomic emission spectrometry (ICP AES)[26] (for details, see Online Supplementary Materials).

The results in Table 2 show that the boron content obtained by ICP AES is significantly lower than that obtained by DCA AES, both in cells and in the nutrient medium. This can be explained by the incomplete dissolution of boron nanoparticles during sample preparation for ICP AES analysis (for details, see Online Supplementary Materials). Using more severe dissolution conditions, such as microwave digestion system, will result in dilution of cell samples and accidental loss. We have proposed a simple sample preparation method to avoid this. Thus, Table 2 highlights the feasibility of the proposed method.

Table 1 Results of boron quantification by DCA AES in BNP/HEC compositions (n = 4, p = 0.95).

BNPs/HEC composition	Theoretical concentration/ mg dm^{-3}	Analytical line/nm	Measured concentration/ mg dm^{-3}	RSD (%)
1	1.0 × 10^2	249.667	(1.1 ± 0.3) × 10^2	10
2	2.1 × 10^2	249.667	(1.8 ± 0.4) × 10^2	8
3	5.0 × 10^2	249.772	(5.8 ± 1.0) × 10^2	7

Table 2 Comparison of the boron content (μg) in cells and nutrient medium determined by ICP AES and DCA AES (n = 3, p = 0.95).

Samples	ICP AES	DCA AES
Cells	(6.6 ± 1.2) × 10^{-3}	(2.5 ± 0.5) × 10^{-2}
Nutrient medium	(7.0 ± 0.5) × 10^{-4}	(3.0 ± 0.6) × 10^{-3}

We especially note that the ability to use a small volume for DCA AES analysis is an advantage over ICP AES. This may be a key aspect for experiments with cells. It can be concluded that the proposed method shows good analytical characteristics for the quantitative measurements of boron in biological samples after experiments with BNPs.

We offer a method for the boron quantification both in the form of nanoparticles and in the form of chemical compounds, such as BPA, BSH, and others. The proposed approach can be used to determine boron in blood samples, for example, for *in vivo* determination of BNP accumulation and biodistribution.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.10.040.

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