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Inter-comparison of boron concentration measurements at INFN-University of Pavia (Italy) and CNEA (Argentina)

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HIGHLIGHTS

- Intensive BNCT research collaboration is ongoing between Argentina and Pavia BNCT groups.
- An inter-comparison of three boron determination nuclear techniques: alpha-spect, NCR and QTA.
- Real tissue samples were used together with a variety of boron compounds.
- Results showed a good agreement and no statistical discrepancy for liquid and tissue samples.
- This work contributed to a better understanding of the issues related to each technique.

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ABSTRACT

An inter-comparison of three boron determination techniques was carried out between laboratories from INFN-University of Pavia (Italy) and CNEA (Argentina): alpha spectrometry (alpha-spect), neutron capture radiography (NCR) and quantitative autoradiography (QTA). Samples of different nature were analysed: liquid standards, liver homogenates and tissue samples from different treatment protocols. The techniques showed a good agreement in a concentration range of interest in BNCT (1–100 ppm), thus demonstrating their applicability as precise methods to quantify boron and determine its distribution in tissues.

1. Introduction

In order to improve the knowledge of the physiological behavior of ^{10}B in Boron Neutron Capture Therapy (BNCT), it is necessary not only to determine gross ^{10}B concentration, but also to study its microdistribution in tumor and surrounding tissue. Most of the techniques presently employed allow only global determination of boron in tissue samples, as for example inductively coupled plasma optical emission spectroscopy/mass spectrometry (ICPOES/ICP-MS) and prompt gamma neutron activation analysis (PGNAA) (e.g., Wittig et al., 2008). Another technique is alpha spectrometry (alpha-spect), developed at the University of Pavia (Italy), which allows ^{10}B concentration measurements in thin tissue samples based on charged particle spectroscopy. Coupling the gross results with the histology of a contiguous section and with boron imaging of another subsequent section, it is possible to reconstruct ^{10}B concentration and distribution in heterogeneous samples (Bortolussi and Altieri, 2013). Only a small number of techniques allow precise microdistribution studies of boron, one of them is secondary ion mass spectrometry (SIMS) (e.g. Cruickshank et al., 2009; Chandra et al., 2014) and another one is neutron autoradiography. A quantitative neutron autoradiography (NCR) protocol based on CR-39 solid state nuclear track detector (SSNTD) has been set-up in the Pavia BNCT group (Postuma et al., 2015). In the BNCT group of Argentina, another quantitative neutron

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autoradiography technique (QTA) using Lexan™ as nuclear track detector had been developed and validated with ICP-OES and ICP-MS methods (Portu et al., 2011), and was applied to tissue samples evaluation in association with a qualitative analysis of tissue samples (QLA, Portu et al., 2013). These methods allow a more precise spatial determination of boron at tissue level, which could provide useful information for a localized dosimetry.

Several experiments have been performed and are ongoing in collaboration between the BNCT groups in Italy and Argentina (e.g. Bortolussi et al., 2010; Farías et al., 2014; Trivillin et al., 2014; Molinari et al., 2015). In view of the increasing amount of shared research to be performed in the two laboratories, an inter-comparison protocol of boron determination methodologies is required. In this work we present an extensive comparison between the alpha-spect, the NCR and the QTA. The aim of this study was to analyse samples of increasing degree of complexity, in terms of preparation procedure, matrix effects and correction factors that may be required. Most of the samples employed were calibration standards. Boron-containing liver and kidney samples were also measured: healthy organs were selected due to their almost uniform boron distribution. This feature enables the obtention of several tissue sections with the same concentration in order to be measured with the different techniques. Liver was chosen because ex-situ BNCT for hepatic metastases is a current project of the Argentinean BNCT group (Miller et al., 2009), based on the first experience at the University of Pavia (Zonta et al., 2009). Kidney was chosen because it accumulates large amounts of boronophenylalanine (BPA), thus it was possible to analyse two tissues with different boron concentrations from each animal.

2. Materials and methods

In this section, the three techniques to be inter-compared are briefly presented.

Alpha-spect is based on the spectrometry of the reaction products of the neutron capture reactions. Sections of 60 μm thick are obtained from frozen tissue samples and then irradiated in a thermal neutron field in front of a silicon detector, which collects the charged particles emitted by neutron capture in nitrogen and boron. The obtained spectra have a characteristic absorbed shape because tissue section thickness is higher than the emitted particles (protons, alpha and lithium ions) ranges in tissues. The boron concentration can be calculated from the alpha particle contribution to the final spectrum in a certain energy range, taking into account the cross section of neutron capture, the stopping power of particles in tissue and the geometrical efficiency of the set-up. The system is calibrated by means of a certified boron-doped silicon standard (NIST). The standard has a ^{10}B depth profile, with a maximum at 0.188 μm. The uncertainty of the alpha spectrometry is around 18%.

Both the NCR and the QTA are based on the possibility of the SSNTD to register the damage produced by an incident heavy ion, which can be amplified by a chemical attack (etching). Thus, if the ions are originated in a tissue section put in contact with the detector, the image produced by the nuclear tracks reveals the spatial distribution of the emitter element in the sample (Fleischer et al., 1975).

NCR and QTA were optimized both for liquid and for solid tissue samples. Track density was evaluated and converted into boron concentration by calibration curves previously obtained in the two laboratories (Postuma et al., 2015; Portu et al., 2011).

NCR employs polyallyldiglicol (CR-39) as track detectors, and the etching is performed with PEW solution at 70 °C for 10 min. QTA employs polycarbonate (Lexan), and the etching is performed also with PEW solution at 70 °C for 2 min.

It should be noted that important differences exist between the three techniques. Alpha-spect values are obtained from the absorbed spectrum, while boron concentration is calculated in neutron autoradiography by comparison of a track density value with a calibration curve. Tracks are counted from images obtained with an acquisition system connected to a light microscope. In the case of the QTA, all tracks are counted with no morphological discrimination. In fact, under the established etching conditions, Lexan does not register the proton tracks originated by neutron capture in nitrogen atoms present in tissue (Saint Martin et al., 2011), thus measured tracks are only produced by alpha particles and lithium ions. As CR39 has a lower detection threshold in terms of linear energy transfer (LET), a set-up was developed in Italy in order to reduce the background signal, tuning the etching conditions to prevent the detection of protons (Postuma et al., 2015). Moreover, the image analysis consists in selecting the tracks according to certain morphological parameters: the ratio of the radii (ratio between maximum and minimum distance from the border to the center of the track) and the track area. The first parameter is an estimator of track roundness, approaching to one for tracks nearly circular. Imposing a threshold on the radii ratio, non-circular objects like artefacts are not considered. The second parameter depends on the ionizing particle energy (Nikezic and Yu, 2004): by imposing a threshold on this value, artefacts smaller than the physical tracks can be excluded.

The irradiations took place at the research reactor TRIGA Mark II at LENA laboratory at the University of Pavia (Italy) and at RA-3 reactor of the National Atomic Energy Commission, Buenos Aires (Argentina).

The technical details about the NCR and QTA parameters are summarized in Table 1.

Different samples have been prepared and measured:

(a) Aqueous standards: two sets of borated solutions with nominal concentrations of 10, 50 and 100 ppm of ^{10}B, one in each laboratory (named ITA and ARG). ITA solutions were prepared with enriched boric acid (99%), while ARG solutions were obtained by diluting a certified 1000 ppm boric acid solution (MERCK).

Table 1
Characteristics of NCR and QTA neutron autoradiography techniques.

	NCR (Italy)	QTA (Argentina)
SSNTD	CR39	Lexan
Calibration standards	Boron aqueous solutions liver homogenates	Boron aqueous solutions (SLCs)
Reactor	TRIGA Mark II (Pavia, Italy)	RA-3 (Bs As, Argentina)
Power	10 kW (aqueous solutions) 2 kW (homogenates and tissue samples)	8 MW
Neutron flux	$(5.80 \pm 0.05)10^7$ n cm^{-2} s^{-1} (aqueous solutions) $(1.10 \pm 0.05)10^7$ n cm^{-2} s^{-1} (homogenates and tissue samples)	$(9 \pm 1)10^9$ n cm^{-2} s^{-1} $(2.8 \pm 0.2)10^9$ n cm^{-2} s^{-1}
Irradiation time	30 min	1.5 min
Fluences	$1.04 \cdot 10^{11}$ n cm^{-2} (aq solutions) $1.6 \cdot 10^{10}$ n cm^{-2} (homogenates)	10^{11} n cm^{-2} 10^{12} n cm^{-2}
Etching solution	PEW 40 (45 g KOH, 125 g C_2H_6O, 130 g H_2O)	PEW (90 g H_2O, 80 g absolute alcohol, 30 g KOH)
Etching temperature	70 °C	70 °C
Etching time	10 min	2 min
Observed tracks	Alpha tracks	Alpha and lithium tracks
Quantification of tracks	Individual counting and morphological selection	Individual counting without differentiation
Magnification	115 ×	400 ×
Tracks diameter	< 6 μm	< 1 μm
Uncertainty	10%	10%

(b) Liver homogenates: prepared at University of Pavia with extruded healthy rat liver mixed with known concentration of BPA, ranging from 0 to 75 ppm. They were conserved at −80 °C and sectioned in a Leica cryostat. More details regarding the preparation process can be found in Gadan et al. (2012).

(c) Tissue sections: normal liver samples from BDIX rats injected with boronophenylalanine (BPA, 300 mg kg^{-1}) and Sprague Dawley rats treated with liposomes loaded with o-closocarboranyl beta-lactoside (LCOB) (Altieri et al., 2009) were sectioned at the University of Pavia. Rats were sacrificed after 4 h from BPA administration and 24 h after LCOB administration.

Samples (a) were quantified with both neutron autoradiography techniques (alpha-spect cannot be applied for liquid samples because of vacuum conditions in the irradiation facility) and the irradiations were performed using a specific setup for each methodology. In Italy, they were exposed to a neutron fluence of about 10^{11} n cm^{-2} in the liquid irradiation assembly with CR-39 (Gadan et al., 2012). In Argentina, they were irradiated with 10^{12} n cm^{-2} inside the Small Lexan Cases (SLCs) system (Portu et al., 2011).

Samples (b) and (c) were sectioned with a LEICA cryostat (thickness: 60 μm) and mounted on mylar disks for alpha-spect, on CR-39 for NCR and on Lexan for QTA. At least three sections per sample have been measured with alpha-spect. Fifty images were quantified for each sample in the case of NCR and QTA, and again three sections per sample were analysed to ensure statistical validity.

3. Results and discussion

Homogeneous autoradiography images were obtained from all the studied samples, and spectrometry measurements resulted compatible with the expected values.

Aqueous solutions (samples (a)) prepared in both laboratories generated similar autoradiography images. In Fig. 1, a comparison between ARG solutions measured by both autoradiography techniques (NCR and QTA) is shown. In order to compare the solutions, the QTA measurements of ITA solutions were compared with the NCR of ARG solutions, yielding similar values that are close to the nominal ones (Fig. 2).

The differences between the results presented in Fig. 2 are related with the uncertainty in the solutions preparation process. On

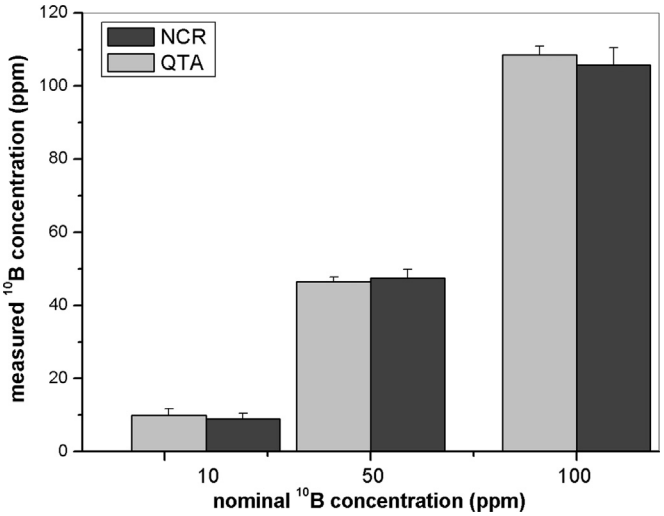

Fig. 1. Results of the NCR and QTA measurements performed in Italy (dark bars) and in Argentina (light bars) respectively on the same aqueous solutions (ARG), for the nominal values of 10, 50 and 100 ppm.

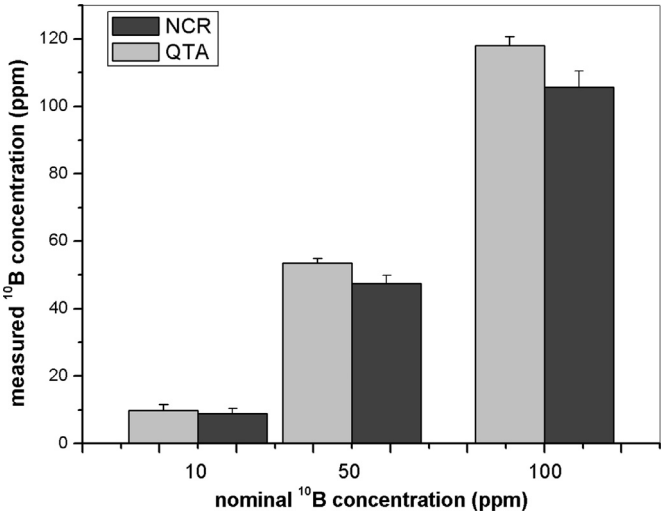

Fig. 2. Comparison of the measurements of aqueous solutions. The light bars are ITA solutions measured with the Argentinean calibration curve (QTA). The dark bars represent the ARG solutions measured with the Italian calibration curve (NCR), for the nominal concentrations of 10, 50 and 100 ppm.

the other hand, when the same set of samples was analysed with both methodologies, the agreement of the measurement results is very good (Fig. 1). The error values reported in Figs. 1 and 2 are smaller than the uncertainty of the technique because there is no need to apply evaporation corrections in aqueous samples so the error is only related to the statistical analysis and the interpolation in the calibration curves.

Liver homogenates (samples (b)) were firstly measured by NCR and QTA using the calibration curve obtained with liquid standards, applying a correction factor to take into account the evaporation of the section when it is deposited on the detector (Gadan et al., 2012). Thus, lower evaporation coefficients correspond to samples with higher water content. This factor was also necessary for alpha-spect. A value of 0.17 ± 0.01 was experimentally established for the liver homogenates (Postuma et al., 2015). In Fig. 3, the results of these measurements are shown, together with the nominal values. The differences in the measured values by the three techniques are not statistically significant across the whole range of concentrations.

The homogenates standards were then used to build another calibration curve for NCR, as described in Postuma et al. (2015), which served for the measurement of samples (c), of liver and

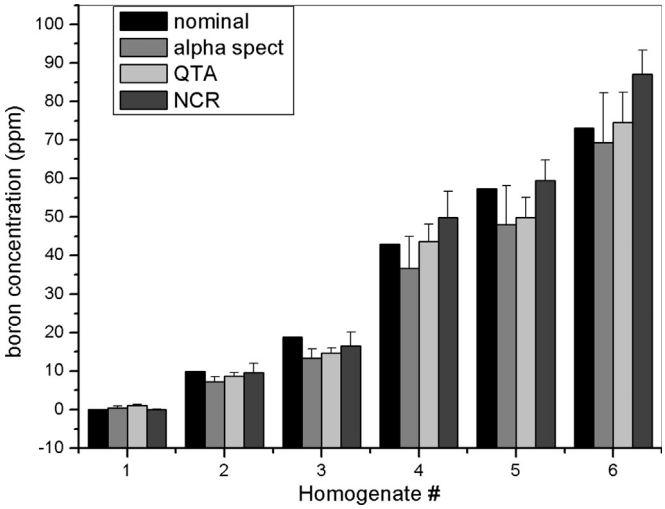

Fig. 3. Liver homogenates: comparison of the measurements results.

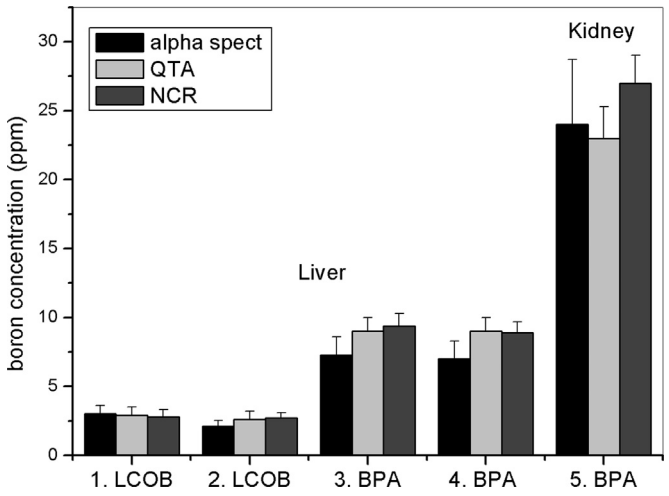

Fig. 4. Tissue samples (c): comparison of the results of the three measurement methods. LCOB: o-closocarboranyl beta-lactoside. BPA: boronophenylalanine.

kidney tissue. The resulting ^{10}B concentration values were compared to those obtained with QTA and alpha-spect. An evaporation coefficient of 0.31 ± 0.03 was experimentally determined for liver and kidney samples. This result was significantly higher than the mean value obtained for liver homogenates. The difference is due to the fact that liver homogenates were prepared by adding aqueous boron solutions to the extruded liver, resulting in higher water content in the sample.

The results of this comparison are presented in Fig. 4. As it can be observed, the liver uptake of LCOB-liposomes was about three times less than BPA. In case of BPA administration, kidney accumulated more than twice the amount of boron of the liver, as also found in the work of Chou et al. (2009). These results are consistent with previously reported values (e.g. Bortolussi et al., 2014). No statistical differences were observed between the three techniques. Besides, with this set of samples, the agreement between the results obtained with the studied techniques is achieved in the whole range of interest. The lower the concentration, the higher is the experimental error associated with the techniques. However, the agreement in the low concentrations zone (< 5 ppm) is still very good.

These results obtained in samples of different nature (liquids, homogenates and tissues), in presence of ^{10}B bound to different molecules (boric acid, BPA and carboranes) confirm the validity of the methods applied in two different laboratories, and the robustness of the analysis performed in order to assess BNCT dosimetry as precisely as possible. The inter-validation of the three techniques offer the possibility to exploit their different advantages and capabilities in the study of the behavior of different boron carriers in terms of ^{10}B gross concentration, level of uniformity in tumor, tumor to normal tissue concentration ratio, etc.

4. Conclusions

From the described findings, we can conclude that the compared techniques give compatible results of boron concentration both in tissue and liquid samples, with different boron chemical compounds: the measured samples with the three techniques do not show statistical discrepancy in boron concentration values.

This inter-comparison measurement campaign contributed to a better understanding of the multiple issues related to the application of each technique and paves the way for their application in BNCT protocols that are being carried out in collaboration between Argentina and Italy.

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