Applied Radiation and Isotopes 69 (2011) 1698–1701

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Reference systems for the determination of ^{10}B through autoradiography images: Application to a melanoma experimental model

A. Portu [a,b,*], M. Carpano [a], A. Dagrosa [a,b], S. Nievas [a], E. Pozzi [a], S. Thorp [a], R. Cabrini [a,c,d], S. Liberman [a], G. Saint Martin [a]

[a] Centro Atómico Constituyentes (CAC), Comisión Nacional de Energía Atómica (CNEA), Av. Gral. Paz 1499, AC: B1650KNA, San Martín, Buenos Aires, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Rivadavia 1917, AC: C1033AAJ, Ciudad Autónoma de Buenos Aires, Argentina
[c] Facultad de Odontología, Universidad de Buenos Aires (UBA), M.T. De Alvear 2142, Ciudad Autónoma de Buenos Aires, Argentina
[d] Laboratorio de Microespectrofotometría (LANAIS-MEF), CONICET-CNEA, Argentina

ARTICLE INFO

Available online 8 March 2011

Keywords:
Autoradiography image
Standard material
Melanoma tumour
^{10}B concentration

ABSTRACT

The amount of ^{10}B in tissue samples may be determined by measuring the track density in the autoradiography image produced on a nuclear track detector. Different systems were evaluated as reference standards to be used for a quantitative evaluation of boron concentration. The obtained calibration curves were applied to evaluate the concentration of ^{10}B in melanoma tumour of NIH nude mice after a biodistribution study. The histological features observed in the tissue sections were accurately reproduced by the autoradiography images.

1. Introduction

The knowledge of ^{10}B concentration in tumour and normal tissue becomes mandatory when a boron neutron capture therapy (BNCT) treatment is considered. Furthermore, if the local distribution of boron in tissue can be quantitatively determined, an important step is given in the understanding of the mechanisms involved in tumour uptake of boron compounds. Autoradiography techniques with nuclear track detectors have demonstrated their usefulness for the determination of boron distribution in biological materials for BNCT (Wittig et al., 2008).

When heavily ionizing particles penetrate into a solid state nuclear track detector (SSNTD), narrow paths are formed along the ions trajectories. These paths can be amplified by an appropriate chemical attack in order to visualize them with an optical microscope (Fleischer et al., 1975). Different plastic films can be employed as tracking media to determine the distribution of boron in compounds. For this purpose, radiography is produced by the ionizing particles generated in the sample (containing ^{10}B atoms), which is put in contact with the detector (Armijo and Rosenbaum, 1967). By irradiation with thermal neutrons, the ^{10}B(n, α)^{7}Li reaction takes place and the resulting α and Li particles which arrive at the detector surface may originate tracks. After etching the detector, an autoradiography image of the object is formed by the etch pits.

The image produced in the detector by a tissue section containing a certain ^{10}B amount can provide a map of the distribution of this element. An autoradiography image with high density of tracks can be obtained by irradiating the foil with high neutron fluence. Thus, a qualitative evaluation of boron spatial distribution can be inferred from the analysis of the differences in shades of grey (Altieri et al., 2008).

Moreover, the concentration of ^{10}B in tissue samples may be inferred by measuring the track density in the detector. For this purpose, some standard material with a known ^{10}B concentration must be used as a reference. The alpha and Li particles ranges (in g cm^{-2}) in the reference material used to calibrate the technique must be virtually the same as the one in the samples to be measured (Durrani and Bull, 1987). In this way, it is possible to assume that equal track density in the sample and the material implies equal boron concentration. Thus, calibration with standards of the same nature as the samples to be measured is recommended for a quantitative analysis.

Melanoma is an aggressive tumour which is poorly controlled when treated in advanced stages by the usual therapies. Tumours from different patients with the same histological diagnosis can show different responses to ionizing radiation. BNCT has also been used for the treatment of melanoma in many countries, including

* Corresponding author at: Departamento de Radiobiología, Laboratory TANDAR, Centro Atómico Constituyentes (CAC), Comisión Nacional de Energía Atómica (CNEA), Av. Gral. Paz 1499, AC: B1650KNA, San Martín, Buenos Aires, Argentina. Tel.: +54 11 6772 7150.
E-mail addresses: portu@cnea.gov.ar, agustina.portu@gmail.com (A. Portu), carpano@cnea.gov.ar (M. Carpano), dagrosa@cnea.gov.ar (A. Dagrosa), nievas@cnea.gov.ar (S. Nievas), pozzi@cnea.gov.ar (E. Pozzi), thorp@cae.cnea.gov.ar (S. Thorp), cabrini@cnea.gov.ar (R. Cabrini), liberman@cnea.gov.ar (S. Liberman), gisaint@cnea.gov.ar (G. Saint Martin).

0969-8043/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2011.02.049

Argentina (Menéndez et al., 2009). The analysis of the individual characteristics of this kind of tumours could help in understanding the variability of boron uptake observed in different cases of melanoma. An in vivo model was developed in our laboratory, by transplanting the human melanoma cell line MELJ into nude mice, measuring tumour growth and following them with infrared imaging and histological studies (Carpano et al., 2010).

In this work a calibration system for the determination of ^{10}B concentration using the autoradiography technique in polycarbonate nuclear track detector was set up and applied to analyse tissue samples from melanoma in nude mice.

2. Materials and methods

2.1. Reference systems

Lexan foils of 250 μm were chosen as SSNTDs. Different reference systems were evaluated in a preliminary study (Saint Martin et al., 2008) and those which were selected will be described in detail.

Small Lexan Cases (SLCs) of about 95 mm^3 were designed and assembled. They were filled with solutions of boric acid 99.99% enriched in ^{10}B in concentrations ranging from 0 to 100 $\mu g\,g^{-1}$ (ppm). The solution standards were analytically prepared and verified with Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) measurements. The SLCs were irradiated with thermal neutrons at the RA-3 reactor BNCT facility at fluences of 10^{12} n cm^{-2}. The foils constituting the SLCs were separated and etched for 2 min in PEW solution (30 g KOH+80 g ethyl alcohol+90 g distilled water) at 70 °C. Latent tracks were thus amplified up to microscopic level ($Ø_{tracks} \sim 1$ μm). The etching bulk velocity in Lexan had been previously measured and a value of 19.2 ± 0.6 $\mu m\,h^{-1}$ had been obtained.

Autoradiography images were acquired with a digital imaging system (Lanais MEF, CNEA-CONICET, Carl Zeiss MPM 800, 40 ×). A calibration curve of the track density (μm^{-2}) as a function of ^{10}B concentration was obtained.

On the other hand, low melting point (LMP) agarose 2% gels were prepared with enriched boric acid solutions of different concentrations. Homogeneous films of this material were placed on the surface of Lexan foils and then exposed to thermal neutrons at the previously mentioned conditions. The agarose films were carefully removed from the detector, then chemically processed and analysed in the same way as the SLCs. Agarose ($C_{24}H_{38}O_{19}$) films are easily produced and they have high water content, as well as tissue samples.

Both materials (aqueous solutions and agarose gels) are expected to show similar characteristics, due to the fact that they both have equivalent particle ranges, which are also similar to the ranges values in tissue (Ziegler et al., 2008). Agarose films could be eventually placed alongside the tissue samples, allowing the coexistence of a reference point and the unknown sample during the whole autoradiography process.

2.2. Biological samples

Female NIH nude mice, body weight 25 g, were implanted in the right back flank with 4×10^6 of a human cell line of melanoma (MELJ). At 35 days post-transplantation, the animals with tumours of 300–900 mm^3 were injected with BPA (0.14 M) at a dose of 350 $mg\,kg^{-1}$ b.w. (i.p.) and sacrificed at 2 h post-administration.

Following excision, nonfixed tumours were divided in to two parts: the first one was measured by ICP-OES using the sample preparation protocol and measurement method as described in a previous work (Liberman et al., 2004). The second one was sectioned at 50 μm in a Lipshaw USA cryostat. The sections were mounted on Lexan foils to perform autoradiography. The samples to be used for qualitative analysis were irradiated at 10^{13} n cm^{-2}, and those undergoing quantification with 10^{12} n cm^{-2}. A lower fluence is chosen for the quantitative analysis in order to avoid track overlapping which could lead to miscounting errors.

Between the irradiation and the etching attack, the tumour sections were haematoxylin-stained and photographed using a CCD camera (Olympus DP70). Then, the tissue sections were removed using trypsine and the detector foils were etched under the same conditions as those used for standard samples. The number of tracks per surface unit was measured, and boron concentration was determined from calibration curves.

3. Results and discussion

Both the SLCs assembly and the agarose films showed homogeneous distribution of tracks, gave reproducible results and showed minimal background. Good linear relationship was found between track density and ^{10}B concentration for both systems, as observed in Fig. 1. The fitting parameters of the calibration curves are given in Table 1.

The autoradiography images of most melanoma tumour sections showed a uniform track density, revealing a homogeneous distribution of boron in the sample. This fact is in accordance with the histological observations, which presented no significant structural variation along the tissue section. Fig. 2 shows the tissue section stained with haematoxylin and its corresponding autoradiography image. The latter reflects the scratches and irregularities present in the tissue section, but it does not reveal significant differences in shades of grey.

^{10}B concentration measurements in tumour samples performed by ICP-OES and by autoradiography are presented in Fig. 3. Concentration values for these melanoma samples are

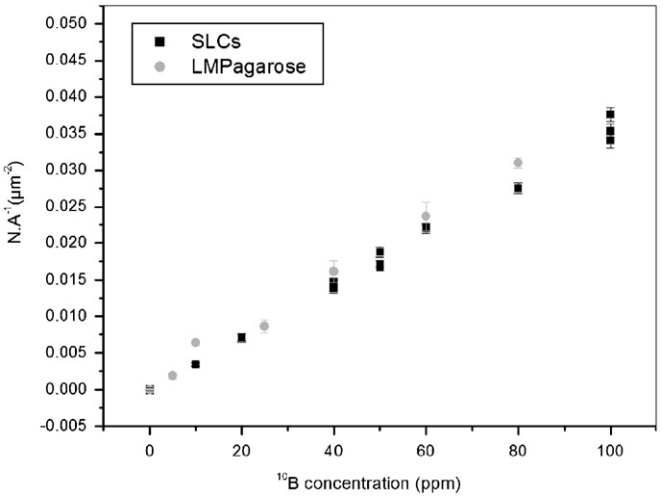

Fig. 1. Number of tracks per unit area ($N\,A^{-1}$) versus ^{10}B concentration for the developed references systems: Small Lexan Cases (SLCs) and low melting point agarose films (LMP agarose).

Table 1
Parameters of calibration curves obtained for reference systems. $NA^{-1} = A + B[^{10}B]$.

System	A (10^{-5})	B (10^{-5})	R
SLCs	−4.22	35.6	0.998
Agarose	−3.58	37.8	0.997

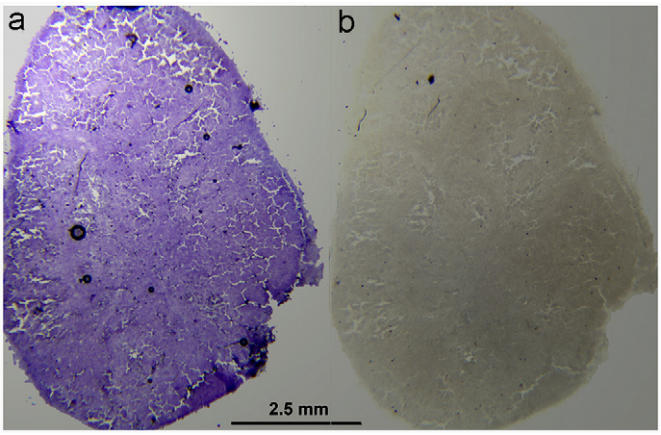

Fig. 2. Haematoxylin-stained tumour section (a) and its corresponding autoradiography image (b) (1.25 ×).

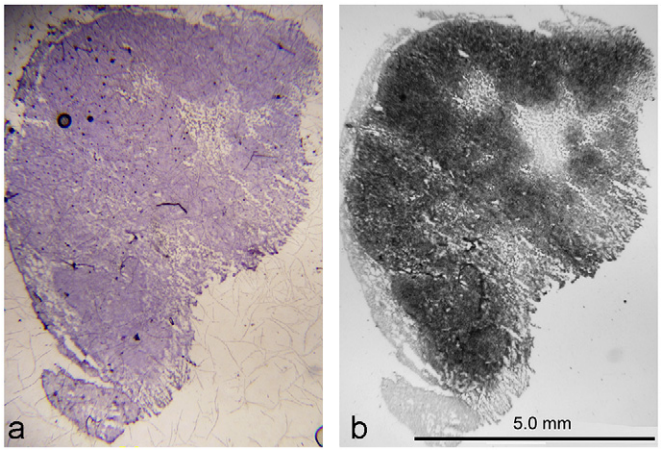

Fig. 4. Haematoxylin-stained tumour section (a) and its corresponding autoradiography image (b) (1.25 ×).

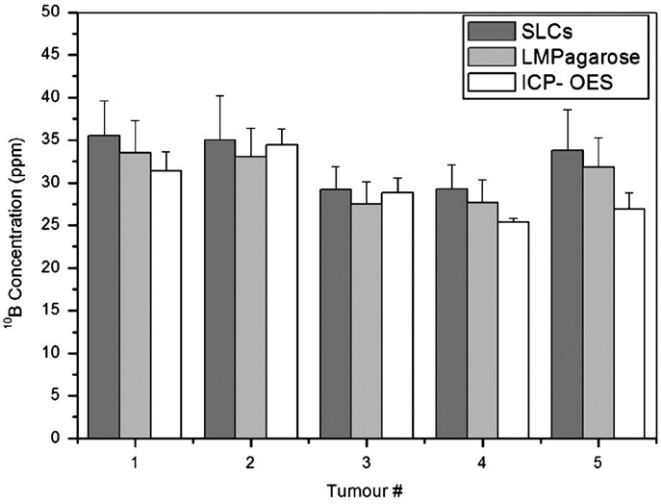

Fig. 3. ^{10}B average concentration values for different tumour samples measured by autoradiography with SLCs and agarose calibration curves and by ICP-OES.

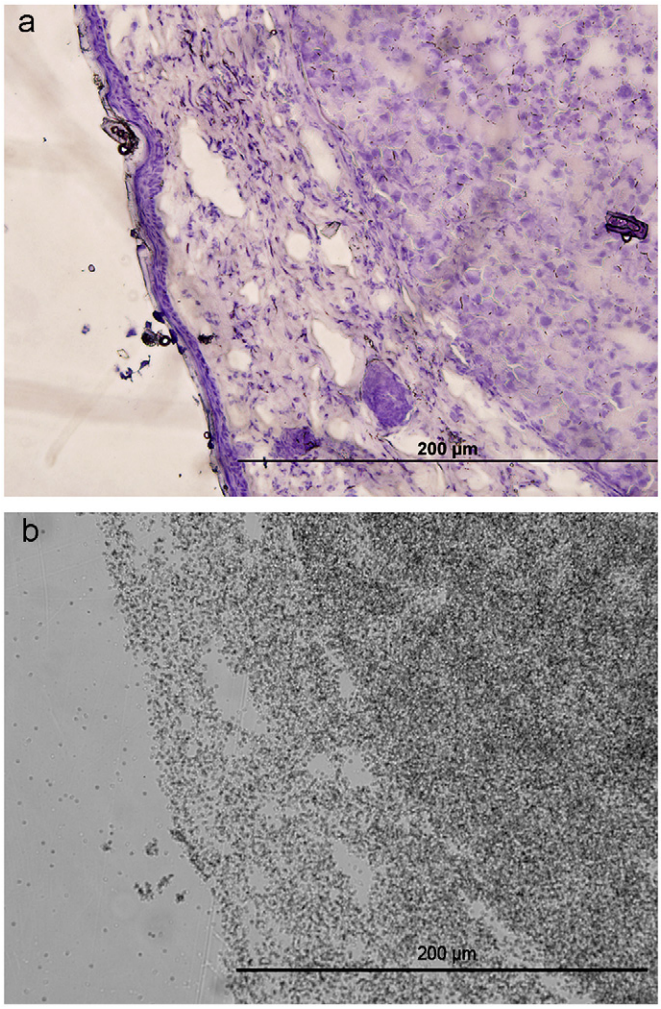

Fig. 5. (a) Tumour section border (detail from Fig. 4a) and its corresponding autoradiography image (b) (20 ×).

within 25 and 35 ppm and this fact reflects the biological variability between animals subjected to equal experimental conditions. Differences between values measured by both methods are around 10%. The uncertainty in the boron concentration determination using our calibration curves is mainly of 3 ppm, though there is one point of about 5 ppm.

It can be seen that SLCs results systematically exceed agarose estimations. However, these differences are always under the experimental deviation and are of about 2 ppm. The results of all measurements were corrected considering the background evaluated in control samples. In agarose and tissue samples the evaporation mechanism was considered as reported for α-spectrometry measurements (Bortolussi, 2007).

There were also some samples that, although coming from the same protocol, showed nonuniform distribution of tracks. This fact indicates differences at the histological level, as can be observed in Fig. 4. There are zones of the section which present holes. These regions could be ascribed to necrotic areas that were torn when cutting the sample under the used cryostat conditions. These sectors can also be localized in the autoradiography image, where it is possible to observe areas of the same shape with no tracks. In this sense, the qualitative autoradiographies allow a clear overview of the relative ^{10}B distribution, by comparing differences in shades of grey. Another fixation and cutting procedure should be assayed to preserve the necrotic areas for quantification.

When observing pictures of the section presented in Fig. 4(a) at higher magnification, we can see tumoral tissue surrounded by skin (Fig. 5a). The corresponding autoradiography image (Fig. 5b)

shows a lower track density in this region, in comparison with the one observed on viable tumour. A quantitative analysis was performed with the mentioned technique for this case, in order to evaluate these two types of tissue. The obtained tumour-to-surrounding skin ratio was 3.0. Though this result is somewhat lower than the ratio calculated with average ICP measurements performed in tumour and skin samples by Carpano et al. (2010), which is around 3.6, the comparison is acceptable taking into account differences between animals and even between sections of the same tumour. More samples should be measured in order to perform a statistical analysis.

The main advantage of autoradiography is the capability of analysing different structures in the same section. Thus, features that may stay undetected at macroscopical level, thus impairing their isolation for ICP determinations, could be quantified using this imaging technique.

4. Conclusions

As shown by the preceding data, both the SLCs and agarose films showed suitable conditions to be used as reference systems and could be successfully applied for the determination of ^{10}B concentration in tissue samples. Although the autoradiography cannot be considered as an analytical technique, very satisfactory results were obtained with the developed method.

The first studied tumour sections exhibited a homogeneous structure and the autoradiography images confirmed histological observations. Besides, there was good agreement between ICP-OES and autoradiography measurements.

Samples with histologically different regions were also analysed and this heterogeneity was evidenced in the autoradiography image. This fact demonstrates the capability of the technique in revealing the histological variations, reflected as differences in shades of grey. The quantification of different regions of tissue was also performed, but further work will be done in order to analyse more samples coming from this model.

Although there are some difficulties that need to be solved, e.g. the correct visualization of necrotic areas to enable the quantitative analysis, the technique has been successfully set up. In future studies, new applications to other biological models will also be performed.

Acknowledgments

This work was partially supported by Grant PAE 22393, ANPCyT. The authors want to thank Dr. Gabriela Leyva for the sample evaporation analysis and Dr. Omar Bernaola for useful advice.

References

Altieri, S., Braghieri, A., Bortolussi, S., Bruschi, P., Fossati, F., Pedroni, P., Pinelli, T., Zonta, A., Ferrari, C., Prati, U., Roveda, L., Barni, S., Chiari, P., Nano, R., 2008. Neutron autoradiography imaging of selective boron uptake in human metastatic tumours. Appl. Radiat. Isot. 66, 1850–1855.

Armijo, J.S., Rosenbaum, H.S., 1967. Boron detection in metals by alpha-particle tracking. J. Appl. Phys. 38 (5), 2064–2070.

Bortolussi, S., 2007. Boron Neutron Capture Therapy of Disseminated Tumours. Ph.D. Thesis, Dipartimento di Fisica Nucleare e Teorica, Università degli Studi di Pavia.

Carpano, M., Dagrosa, A., Nievas, S., Santa Cruz, G.A., Cabrini, R., Juvenal, G., Pisarev, M., 2010. In-vivo studies towards optimizing the application of BNCT to the individual treatment of malignant melanoma. In: Proceedings of the 14th International Congress on Neutron Capture Therapy, October 25–29, 2010, Buenos Aires, Argentina, pp. 111–114.

Durrani, S.A., Bull, R.K., 1987. Solid State Nuclear Track Detection. In: ter Haar, D. (Ed.), Principles, Methods and Applications. International Series in Natural Philosophy. Pergamon Press.

Fleischer, R.L., Price, P., Walker, R.M., 1975. Nuclear Tracks in Solids. University of California Press, Berkeley.

Liberman, S.J., Dagrosa, A., Jimenez Rebagliati, R.A., Bonomi, M.R., Roth, B.M., Turjanski, L., Castiglia, S.I., Gonzalez, S.J., Menendez, P.R., Cabrini, R., Roberti, M.J., Batistoni, D.A., 2004. Biodistribution studies of boronophenylalanine-fructose in melanoma and brain tumour patients in Argentina. Appl. Radiat. Isot. 61, 1095–1100.

Menéndez, P.R., Roth, B.M.C., Pereira, M.D., Casal, M.R., González, S.J., Feld, D.B., Santa Cruz, G.A., Kessler, J., Longhino, J., Blaumann, H., Jiménez Rebagliati, R., Calzetta Larrieu, O.A., Fernández, C., Nievas, S.I., Liberman, S.J., 2009. BNCT for skin melanoma in extremities: updated Argentine clinical results. Appl. Radiat. Isot. 67, S50–S53.

Saint Martin, G., Portu, A., Bernaola O.A., Liberman S., Cabrini R.L., 2008. Evaluación de sistemas de referencia, para la cuantificación de ^{10}B en cortes de tejido utilizando SSNTD. XXXV Reunión Anual de la AATN, Bs, As, Argentina.

Wittig, A., Michel, J., Moss, R.L., Stecher-Rasmussen, F., Arlinghaus, H.F., Bendel, P., Mauri, P.L., Altieri, S., Hilger, R., Salvadori, P.A., Menichetti, L., Zamenhof, R., Sauerwein, W.A., 2008. Boron analysis and boron imaging in biological materials for boron neutron capture therapy (BNCT). Crit. Rev. Oncol. Hematol. 68 (1), 66–90.

Ziegler, J.F., Ziegler, M.D., Biersack, J.P., 2008. SRIM-2008.4. The Stopping and Range of Ions in Matter. 1984, 1989, 1998, 2003, 2008.