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DETECTION OF ^{10}B DISTRIBUTIONS IN HISTOLOGICAL SAMPLES BY NCAR USING THERMAL AND COLD NEUTRONS AND PHOTOLUMINESCENT IMAGING PLATES: NEW RESULTS

J. Rant[1*], D. Gabel[2], G. Bayon[3], H.Yanagie[4], H. Kobayashi[5], E. Lehmann[6], G.Kuehne[6], J.Skvarč[1] and R. Ilić[1]

[1] J.Stefan Institute, 1000 Ljubljana, Slovenia
[2] Chemistry Dept., University of Bremen, POB 330 440, D-28334 Bremen, Germany
[3] CEA Saclay, F91191 Gif sur Yvette Cedex, France
[4] Institute of Medical Science, Univ. of Tokyo, 4-6-1 Shiroganedai, Tokyo 108, Japan
[5] Institute of Atomic Energy, Rikkyo University, 2-5-1 Nagasaka, Kanagawa, 240-01, Japan
[6] Paul Scherrer Institute, Villigen, Switzerland

The Neutron Capture Autoradiography (NCAR) using various Solid State Nuclear Track Detectors (SSNTDs) is a well established and accurate method to detect and measure the distributions of ^{10}B in the ppm range on macroscopic and microscopic level in biological samples, such as histological sections of tumours loaded with ^{10}B compounds used for BNCT (e.g. 1,2). Recently a new technique of NCAR using sensitive photoluminescent Imaging Plates (IP) has been proposed to detect ^{10}B distributions in histological sections (3), exploiting excellent detection properties of IP systems such as very high detection sensitivity and quantum detection efficiency, broad linear response and dynamic range, very small image distortion, reusability of IP and possibilities of digital autoradiography. The advantage of IP-NCAR vs. NCAR with SSNTDs should be the much lower neutron fluence (10^7-10^9 vs. 10^{10}-10^{13} n/cm^2 with SSNTDs) , no intermediate chemical treatment (track etching) and direct and fast computational handling and evaluation of the digitized autoradiographic image. However, the spatial resolution of the present available IP detection systems is somewhat lower (~0.04 mm) than with SSNTDs (~0.01 mm). Another problem with IP NCAR is rather high sensitivity of IP to all types of ionizing radiations. Therefore the background of direct and induced gamma-rays as well as of epithermal and fast neutrons has to be filtered out of thermal neutron beam to be used for IP-NCAR. To improve the signal/background ratio and to increase the detectibility of ^{10}B we propose to use clean cold neutron or clean thermal neutron beams for the IP-NCAR of ^{10}B distributions in histological samples in BNCT experiments (4,5). In the present work the recent results of experiments in IP-NCAR with cold neutrons from the neutron radiographic channel of the ORPHEE reactor in Saclay and with the rather clean thermal neutron beam of the NEUTRA neutron radiography facility of the PSI (Villigen) will be presented. For the calibration of the NCAR histological samples of chicken liver with well known concentration of ^{10}B were used and the limit of ^{10}B detection in tissue samples down to at least 5 ppm has been demonstrated.

1. Introduction

Neutron Capture Autoradiography (NCAR) using various Solid State Nuclear Track Detectors (SSNTDs) is a well established and accurate method to detect and measure the distributions of ^{10}B in the ppm range on macroscopic and microscopic level in histological sections of tissues loaded with ^{10}B compounds used for BNCT [1,2]. The SSNTD techniques require relatively high neutron fluences, chemical etching and somewhat time consuming quantitative evaluation. Two exposures are normally needed- one for B distribution visualization (10^{12}-10^{13}n/cm^2) and second, 2-3 orders of magnitude lower, for quantitative boron determination by various track counting techniques. Recently a new

* Retired from JSI

technique of NCAR using sensitive photoluminescent storage phosphors (Imaging Plates or IP) has been proposed to detect heavy charged particles from the $^{10}B(n, \alpha)^{7}Li$ reactions for the topography of ^{10}B distributions in histological sections [3]. The new method exploits excellent detection properties of IP systems such as very high detection sensitivity and quantum detection efficiency, broad linear response and dynamic range, very small image distortion, reusability of IP and possibilities of digital autoradiography and immediate quantitative evaluation [4,5]. A severe problem for using IP in NCAR is their high sensitivity to all types of ionizing radiation and hence the background of direct and induced gamma-rays as well as of epithermal and fast neutrons has to be suppressed or filtered out of the neutron beam. To improve the signal/background ratio and to increase the detectability of ^{10}B we proposed the use of rather gamma ray free cold neutron beams for the IP-NCAR of ^{10}B distributions in histological samples [6]. In the present work the first results of preliminary experiments in IP-NCAR with cold neutrons (IP-CNCAR) from the neutron radiographic channel of the ORPHEE reactor at Saclay are presented. In the first batch of experiments the cold neutron source was rather remote from the IP laser scanner (at IJS, Ljubljana). The time lag between the IP neutron irradiation and the read out amounted several days greatly increasing the background due to cosmic and environmental radiation and preventing the correct assessment of the detectability of the method. The number of NCAR images produced in total was only 12, produced in 3 separate sets of irradiations. However, the preliminary results were encouraging enough and enabled a comparison with the existing NCAR techniques. In the second batch of only 2 day experiments the IP scanning was performed in Saclay immediately after the irradiation, greatly reducing the gamma-ray background. New standards of freeze dried histological samples of chicken liver with known B concentrations were prepared instead of previous standards made of the filter paper. In the paper experiments are described and some characteristics of the IP-NCAR using cold neutrons for B detection are presented.

2. Methods and Materials

Neutron Capture Autoradiography

The procedure for NCAR with IP is practically the same as used in NCAR with SSNTDs. The freeze-dried whole body histological sections of mice were put directly on the unprotected surface of the imaging plate, wrapped by light tight thin plastic cover and sealed after evacuating air by vacuum pump. By this way an intense tight contact between the IP and the sample was achieved. The use of thin light tight plastic foil as irradiation cassette is essential for IP NCAR as the IP has to be protected from the light (in particular UV) and all types of ionizing radiation. The use of thin low Z "cassette" materials with a low neutron absorption cross section is important to minimize the production of neutron and γ-ray induced secondary ionizing radiation. The wrapped IP/sample assemblies were air mailed to Saclay and after neutron irradiation immediately returned back for reading. The time lag between the preparation of the sample/IP assembly and read out was typically 6-8 days and the time elapsed after the neutron irradiation was 4-6 days. Due to transport time the signal faded for a factor 2-2.5 and the background due to cosmic and environmental radiation increased for about factor 25! This drawback was removed by performing experiments and read-out of IP directly in Saclay during 2 days of experiments.

Cold Neutron Source

The neutron irradiations were performed on the neutron radiographic facility at the cold neutron guide G4 of the research reactor ORPHEE (Saclay, France). The cold neutron flux is

approximately 9.10^8 n/cm^2 s and the average energy of the neutrons is 0.003 eV. The neutron beam has a square profile 25 mm by 150 mm. The profile of the neutron beam is skewed for about 7 % in both X- and Y- axis. The samples are irradiated by moving the irradiation rig along the X-axis and the neutron fluence has to be corrected relevant to the irradiation position along the vertical axis. Neutron fluences for IP based cold neutron capture autoradiography (IP-CNCAR) varied between 1.0 - 5.0 10^9 n/ cm^2. The γ-ray background is considered to be negligible [7]

Imaging Plate Detection System

The imaging system used consists of FUJI Photo Film imaging plate type BAS IP-TR, FUJI BAS 1500 laser scanner at IJS (FUJI BAS 1800 scanner at Saclay) and Raytest UV IP eraser. The digital images are handled, processed and analyzed by a personal computer and printed by Epson Stylus Photo printer. The image handling, processing and analyzing software is TINA 2.0 supplied by Raytest (Germany) and Photoshop ADOBE 3.0.

The IP-TR type consists of a plastic support plate (12.7 cm X 12.7 cm) covered with 0.05 mm thick layer of crystals of photostimulable storage phosphor BaFBr:Eu^{2+} combined in a plastic binder. The phosphor layer is intended for the registration of ^{3}H soft beta radiation and is hence unprotected and susceptible for surface damage and wetting. The thickness of the active layer of the phosphor is much thicker than the range of α and ^{7}Li particles in the phosphor (few μm).

The BAS 1500 He-Ne laser (632.8 nm) scanner has a pixel size 0.1 mm X 0.1 mm, (0.05 X 0.05 mm with BAS 1800) scanning speed 14 μs/pixel and 12 bit digitalization (4096 gray levels). Scanners with pixel size 25 μm X 25 μm and digitalization of 16 or 32 bits are commercially available. The inherent resolution of the system is lower than the pixel size, only about 0.2 mm (with advanced scanners 0.05 mm).

Standards and histological samples

The standard samples with known concentration of ^{10}B were prepared by pouring 10 μl drops of sodium borocaptate (BSH) solution in water on the filter paper (B impurity below detection limit 3 ppm) forming stains of ~1 cm^2. The B content of the BSH solutions was measured by plasma emission spectroscopy. The measured ^{10}B concentrations (in ppm) were: 841±28, 248±8.7, 84.6±1.5, 25.2 ±0.32, 8±0.3. Boron concentration on the filter paper were determined by digesting the paper samples in 65% HNO_3, diluting by pure water for factor 10 and measuring again by plasma emission spectroscopy. The measured ^{10}B concentrations were 483±15., 176±3.0, 73.5±6, 19.1±2.0 and 7.3±4. ppm. The B detection limit was at ~4 ppm. It would be useful to measure B content in this indicator by a PGAA method nondestructively.

New standards made of thin (0.025 mm) histological freeze dried chicken liver with known ^{10}B concentration were supplied by one of us (D. Gabel). The concentration values were (in ppm): 150, 100, 50, 25, 10, 5.0, 2.5, 1.75, 1.0, 0.5 and 0.1.

Three different samples of whole body histological sections of a mouse were supplied by two of the co-authors:

-Whole body section of the tumor bearing mouse sacrificed 3^h after the intravenous injection of ^{10}BSH solution (1.5246%, PGAA result) and another section of a mouse sacrificed 6^h after injection of ^{10}B conjugated liposome/PEG liposome. In the former sample high B concentration was found in the liver and to smaller extent also in the tumour and kidney. In the later case the B was localized only in the tumour at low concentration, as confirmed by subsequent NCAR with SSNTDs. The thickness of the samples was about

0.04 mm and were freeze-dried mounted on 3M scotch tape. Details of the preparation of the samples can be found in the literature of the relevant group [3,8].
- Whole body section of the mouse with B16 melanoma, sacrificed 1^h after the intraperitoneal injection of 2 mg ^{10}BSH. The thickness of the sample was 0.05 mm.

3. Results and Discussion

The IP-CNCAR images of new chicken liver ^{10}B standards are presented in Fig. 1a. The surface distribution of B in each liver standard is not uniform and an average signal from the whole surface area of the liver section has to be determined using tools of the TINA software and correlated with the B content. The average signal of photostimulated luminescence (PSL) above the average of the local background is proportional to the B concentration of the relevant standard. The calibration curve relating PSL signal above background to B content is presented in Fig. 1b.
From Fig. 1a it is evident that B content of at least about 5 ppm can be easily detected. The detection limit is in this case possibly still below 5 ppm, since the relevant standards with B concentrations below 5 ppm can be observed and the signal can be discerned from background noise. This can be concluded also from Fig. 1b, where the points with the B concentration of 0.1 to 5 ppm are showing significant scatter in the signal. However, the calibration curve (PSL signal vs. ^{10}B conc.) does not cross axes at 0 indicating the presence of some unresolved background. Increased nitrogen concentration in liver in comparison with the support layer would offer possible explanation. An experimental verification of the B concentration values by a reliable analytical and non-destructive method (e.g. PGAA or SSNTD NCAR) would be most helpful, while nitrogen content in the liver vs. support layer can be evaluated by the selective autoradiography with SSNTDs [10].
In IP-(C)NCAR the background due to protons from neutron captures in nitrogen and heavy particles and gamma rays from neutron absorption in hydrogen, oxygen and Li and B impurities in tissue is rather high and signal/background ratio is much worse than with NCAR using SSNTDs. The SSNTDs are sensitive only for heavy charged particles above certain threshold energies and offering the possibility of particle track discrimination by properly selecting the etching conditions[1], etchant [8,9] and in addition by automatic image analysis by measuring track dimensions, their shape and optical properties [2,10]. However, for complete visual and quantitative image evaluation in SSNTD NCAR two exposures are needed. Computerized track discrimination and counting requires fluences more than 2 orders of magnitude lower (~2. 10^9 n_{th}/cm^2) than required for visual image observation (~10^{12} n_{th}/cm^2) in order to prevent track overlapping. With IP-(C)NCAR as digital autoradiographic method quantitative evaluation and visual observation can be performed simultaneously.
In Fig. 2 the IP-CNCAR image of whole body section of a mouse prepared to put high B concentration in the body organs is presented. The distribution of B in the organs and tissues is clearly observed as well as differences in B content of different organs. In Fig. 2 the high B concentration is in liver and at much smaller concentration in tumor, bladder and intestines. Using an appropriate calibration curve for B concentration monitoring prepared in the same way as actual samples and using similar tissue it could possible immediately to determine the B content in selected regions. The spatial resolution is fair (0.2 mm with BAS 1500) but with modern scanners (e.g. FUJI BAS 5000) it can be improved down to ~ 40 μm, close to that of the whole body NCAR using SSNTD (10-20 μm) [1,2].

4. Conclusions

The digital NCAR using clean cold neutron beams and IP as detectors has a potential to detect ^{10}B in histological sections of tissue at the level lower than 10 ppm and with the spatial resolution of ~ 40 μm. Using calibration curves prepared by tissue standards with known B content and in the same way as actual histological samples for BNCT studies the method enables immediate and simple quantitative analysis. The advantage of IP-NCAR vs. NCAR with SSNTDs should be the much lower neutron fluence (10^7-10^9 vs. 10^{10}-10^{13} n/cm^2 with SSNTDs) , no intermediate chemical treatment (track etching) and direct and fast computational handling and evaluation of the digitized autoradiographic image. Further experiments in optimal experimental conditions are required to estimate accurately the detection limit of this technique of B in tissues and to estimate the signal/background ratio.

5. References

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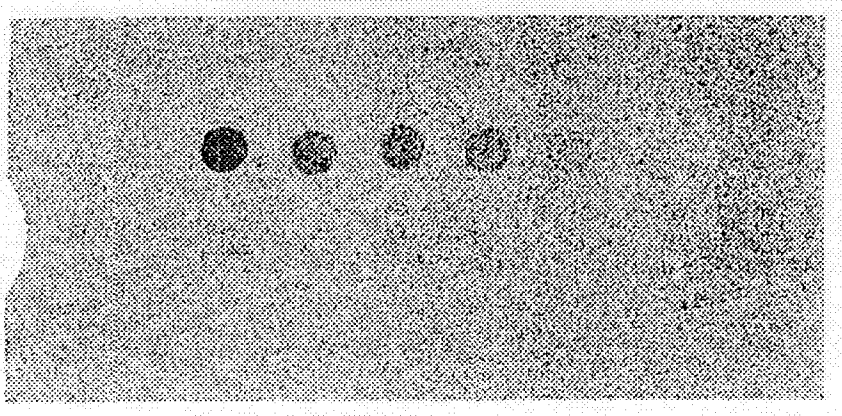

Fig. 1a IP-CNCAR of the histological samples of chicken liver with known ^{10}B concentration as calibration standards. In the upper row the concentration values in ppm of ^{10}B are (from left to right): 150, 100, 50, 25, 10, 5. In the lower row the concentration values are: 2.5, 1.75, 1.0, 0.5 and 0.1 !

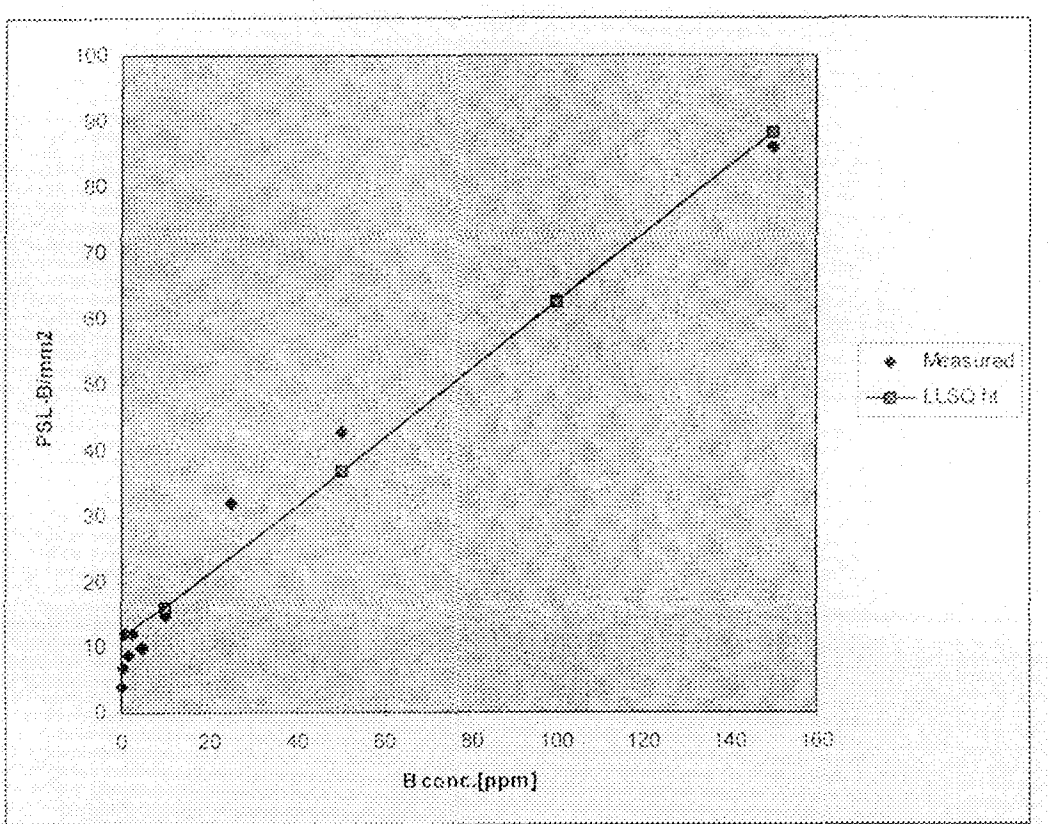

Fig. 1b. Calibration curve realting background corrected PSL signal events (PSL/mm^2) with the ^{10}B concentration in the samples (see Fig. 1a). Note significant scatter of the results around and below 5 ppm.

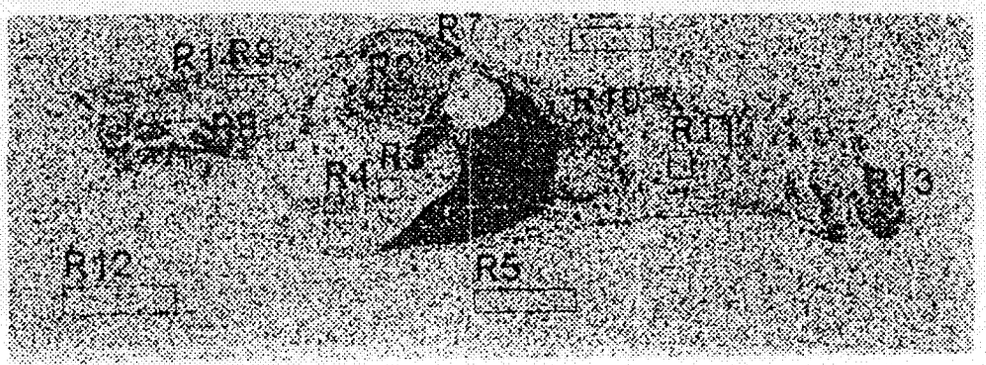

Fig. 2 IP-CNCAR of a mouse sample, sacrificed 3 hours after intravenous injection of ^{10}B BSH solution (2000 ppm). Experimental conditions are different from those during the calibration. FUJI type TR IP was scanned after 7 days waiting time! Cold neutron exposure at reactor ORPHEE: 5. 10^9 n/cm^2. In areas R1-R12 the signal PSL events/mm^2 was evaluated to estimate the B content in the tissue.