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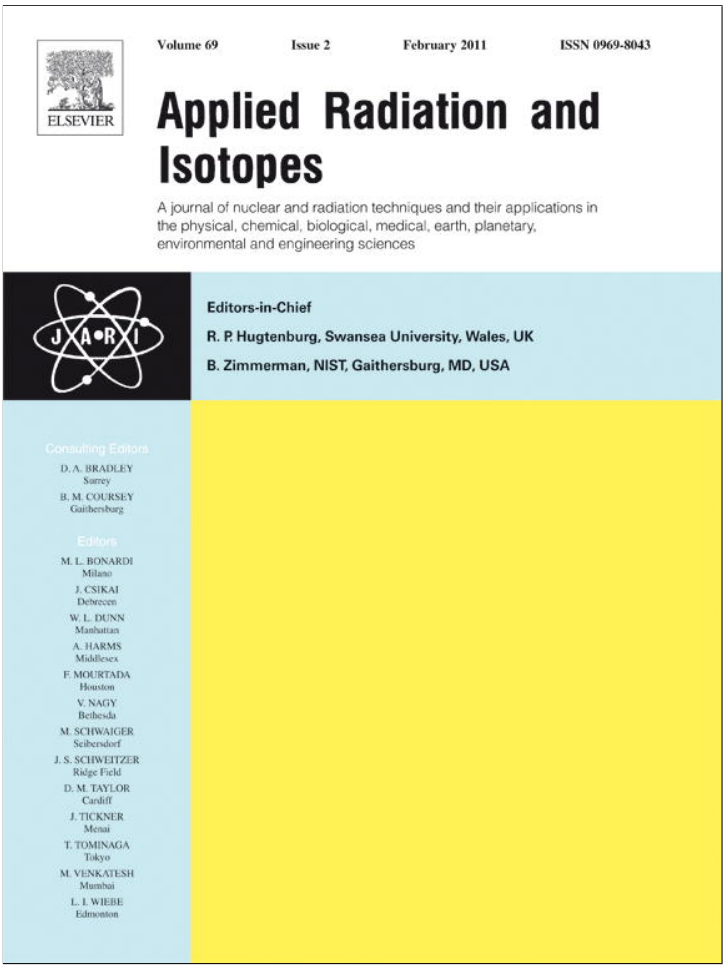

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# Study of DNA damage with a new system for irradiation of samples in a nuclear reactor

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ABSTRACT

In this paper, we report results of a quantitative analysis of the effects of neutrons on DNA, and, specifically, the production of simple and double breaks of plasmid DNA in aqueous solutions with different concentrations of free-radical scavengers. The radiation damage to DNA was evaluated by electrophoresis through agarose gels. The neutron and gamma doses were measured separately with thermoluminescent detectors. In this work, we have also demonstrated usefulness of a new system for positioning and removing samples in channel BH#3 of the IEA-R1 reactor at the Instituto de Pesquisas Energéticas e Nucleares (Brazil) without necessity of interrupting the reactor operation.



## 1. Introduction

In an attempt to better understand the nature of DNA damage by radiation, several groups of researchers (Divyasree et al., 2010; Xudong and Ying, 2008; Yokoya et al., 2008; Śmialek et al., 2008; Sharma et al., 2008) have carried out numerous studies.

With the same motivation, we undertook a study of in vitro DNA damage by neutrons in the radial beam channel BH#3 of the IEA-R1 Brazilian Reactor at the Instituto de Pesquisas Energéticas e Nucleares (IPEN). We also compared the effects of the mixed "thermal n+γ" radiation in the reactor and the $^{60}$Co radiation.

Neutrons produce various effects in a DNA molecule: strand breaks, damaged base groups and some others. Studies of the process of breaking DNA by particles with different LET are very important for understanding radiation-induced death of cells. It is well known that unrepaired double-strand breaks (DSBs) are the main cause of cellular death under irradiation (Friedberg, 2003). Therefore, knowledge of DSB radiation yields under controlled conditions will allow other researchers to better quantify other effects that occur in cellular radiation death.

The radiation damage was quantified in terms of single (SSB) and double (DSB) breaks in the DNA strand. Radiation damage to DNA can be evaluated by horizontal electrophoresis in agarose gels, which separates three forms of DNA molecules, namely, supercoiled (S), linear (L) and circular(C). Supercoiled DNA (form I) is undamaged plasmid; circular DNA (form II) results from single-strand breaks (SSBs) and linear DNA (form III) is a product of double-strand breaks (DSBs). Each fraction of the irradiated plasmid DNA was quantified by agarose gel electrophoresis. The yields of SSB and DSB induced by ionizing radiation were derived from measurements of the fraction of S and L plasmids (Van Touw et al., 1985; Hempel and Mildenberger, 1987; Cowan et al., 1987).

The aim of this paper is to report some results of our studies of the damage to DNA molecules caused by mixed "thermal n+γ" radiation, which were obtained with a new convenient system of positioning of biological samples into the radiation field in the radial beam hole.

We studied effects of mixed thermal neutron and γ-ray irradiation of plasmid pBsKS+ (2961 bp) DNA in the absence and in the presence of an OH· radical scavenger, glycerol, at pH 7. Plasmids are a convenient model system to study DNA damage because their sizes are well defined, quantifications of their SSBs by gel electrophoresis are relatively easy and accurate, chemical environment of the DNA can be precisely controlled, and there is no biological repair process.

## 2. Materials and methods

### 2.1. Irradiation facility

Neutron irradiations were performed in the radial beam channel #3 (BH#3) of the Brazilian Reactor IEA-R1. The beam channel #3 is

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261.5 cm long, and its internal diameter is 20.32 cm. A cylindrical cavity for the sample holder is 30 cm long and 12.8 cm in diameter. The channel area of the reactor is surrounded by shielding, which is made of concrete and paraffin to isolate it from the experimental room. Fig. 1 shows a diagram of the facility.

A weakness of this and many other similar facilities is that samples can be positioned to and removed from the irradiation channel only when the reactor is shut down. As the reactor is shut down only approximately once a week, one can perform only one irradiation a week because there is no system to position and remove samples in the radial channel without interrupting reactor operation. In order to be able to perform a series of consecutive radiobiological experiments while the reactor is in continuous operation, we have designed and constructed a new mechanical system for changing samples in the irradiation position while the reactor is working.

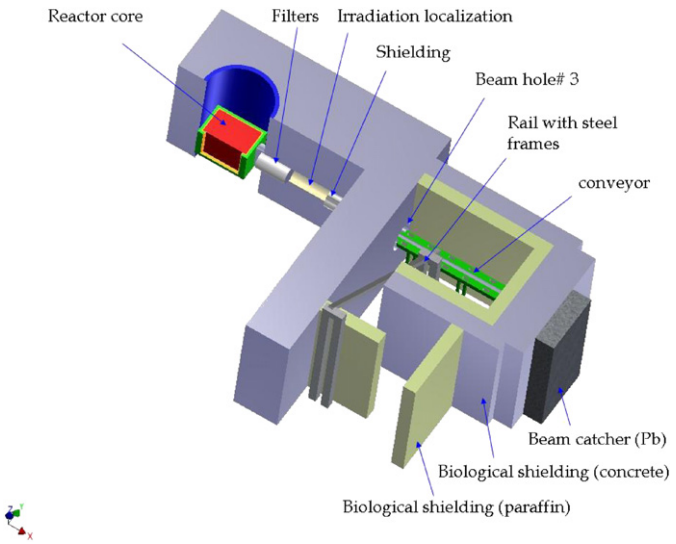


**Fig. 1.** A diagram of the channel #3 area of the IEA-R1 reactor of Instituto de Pesquisas. Energéticas e Nucleares.

The new system (Fig. 2) consists of: (i) a subsystem for sample positioning, (ii) a transporting subsystem and (iii) a subsystem for monitoring. The sample positioning subsystem consists of a sample holder, a mechanical arm with a claw, roller bearings, a pulley system, a sample carrier (semi-tube), an aluminum U-shaped track rail and a mechanical switch. The transport subsystem comprises a reactor channel conveyor, two microswitches, an electric motor and an electric control pad with two buttons that activate the reactor channel conveyor for sample entry/exit into/out of the reactor irradiation zone. The monitoring system has a small video camera installed above the reactor channel.

Each part of the system is described in detail in another paper (Gual et al., in press). The system was constructed in the shop of Instituto de Física de la Universidad de São Paulo, Brazil.

Advantages of this innovative design are: easy maneuverability and simplicity of operation; minimal manufacturing cost (under US $1000); low maintenance cost; a possibility to irradiate samples regardless of the reactor shutdown schedule; high throughput; a possibility of safe remote manipulation; a capability of irradiating samples up to 500 g in weight. The launch of this system had immediate effect on the research in radiobiology, dosimetry and other areas performed in the facility. Many types of experiments have been using the constructed system (Coelho et al., 2008; Mangueira et al., 2010).

Gamma irradiations were performed in a Gamma Cell 220 Excell irradiator (MDS Nordion, Inc., Canada) at IPEN.

### 2.2. Dosimetry

Dose rates were measured with paired Harshaw thermoluminescent dosimeters. One of them, TLD-600 ($^{6}$LiF:Mg,Ti with 96.5% of $^{6}$Li) was sensitive to both neutrons and γ-rays, while the other one, TLD-700 ($^{7}$LiF:Mg,Ti with 9.99% of $^{7}$Li), was sensitive only to from γ-rays. The neutron and γ-ray dose rates were 58 and 35 Gy/h, respectively, for a 2 MW reactor operation power.


**Fig. 2.** The new system for sample delivery in the channel #3 of the IEA-R1 reactor.

The neutron flux and γ-ray dose rate were calculated using the Monte Carlo MCNPX transport code (Waters, 2002) and compared with experimental measurements (Gual et al., 2010). The optimal filter sets that provided a high thermal neutron flux and a low level of fast-neutron and γ-ray contamination were found by Monte Carlo simulations described in a previous work (Gual et al., 2004).

### 2.3. Radiobiological procedures

Plasmid pBsKs+ was isolated from *Escherichia coli* cells. DNA concentration (88 ng/μL) was determined by measuring absorbance at 260 nm with a spectrophotometer. DNA plasmid was purified by cesium chloride centrifugation, a detailed procedure of which has been described in a previous paper (Gouveia, 2004). Neutron irradiations in the IEA-R1 reactor of IPEN were conducted at the reactor power 2 MW. Approximately 98% supercoiled DNA plasmid was used (25 μL samples in 0.5 mL polypropylene Eppendorf tubes).

After irradiation, the three forms of DNA were separated by horizontal electrophoresis in 0.8 g of agarose gel at pH 8. The total electrophoresis time was 2 h at 5 V/cm. Before each run, 3 μL of 0.5% bromophenol blue was added to 10 μL of each sample. The DNA forms were localized in the gel under UV light after being stained with 0.5 μg/mL ethidium bromide. Gel images were recorded with EAGLE EYE II system (Stratagene, San Diego, CA, USA). The procedure consists of illuminating the gel with a UV transilluminator and capturing the resulted ethidium-DNA emission through a filter coupled to an image intensifier and a video camera connected to a personal computer. To account for the weaker binding of ethidium bromide to supercoiled plasmid than to its open circular and linear forms, intensities of the supercoiled bands were magnified by a factor of 1.7 (Spotheim-Maurizot et al., 1990; Stankus et al., 1995). The relative fractions of DNA in each plasmid form were quantified with the gel image analysis program GELANALYS (Milian, 2006).

A theoretical model developed by Van Touw et al. (1985), Hempel and Mildenberger (1987) and Cowan et al. (1987) makes it possible to calculate the radiation chemical yields of SSB and DSB from the slopes of dose-response curves. The slopes represent the probability of inducing one SSB or one DSB per plasmid and per dose unit. These procedures have been described by Spotheim-Maurizot et al. (1990).

## 3. Results and discussion

Figs. 3 and 4 show *G* values for SSB and DSB as functions of the OH· radical scavenger concentration in comparison with the corresponding values for γ-irradiation with $^{60}$Co. The results shown in the figures are given in the units of breaks/Gy Da. In this case, *G* is normalized to the total number of the base pairs of the DNA molecule and to the dose, representing the probability of a break per base pair per Gy. This form of expressing the results is useful for comparisons with results of experiments with different plasmids reported in the literature.

It is estimated that 60–70% of cellular damage may be done indirectly by OH· radicals, which cause radiolysis of water around the DNA molecule (Powell and McMillan, 1990).

The indirect effect is predominant in dilute solutions, and, therefore, it has been studied extensively in a variety of aqueous model systems (Purkayastha et al., 2005). As our experiments showed that $G_{SSB} > G_{DSB}$, the indirect effect appears to be predominant in all cases, which could be expected for a dilute solution.

The radical scavenger present in the DNA solution decreases the yields of the strand break formations, and the effect intensifies with increasing radical scavenger concentration. This is due to the

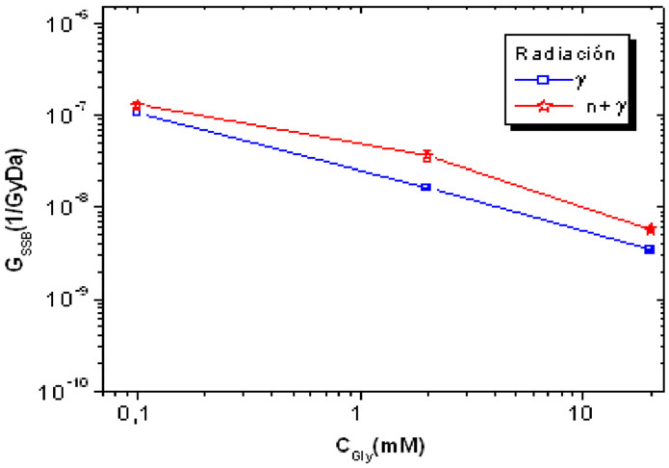


**Fig. 3.** DNA SSB yield as a function of the radical scavenger concentration. The initial DNA concentration was 88 ng/μL.

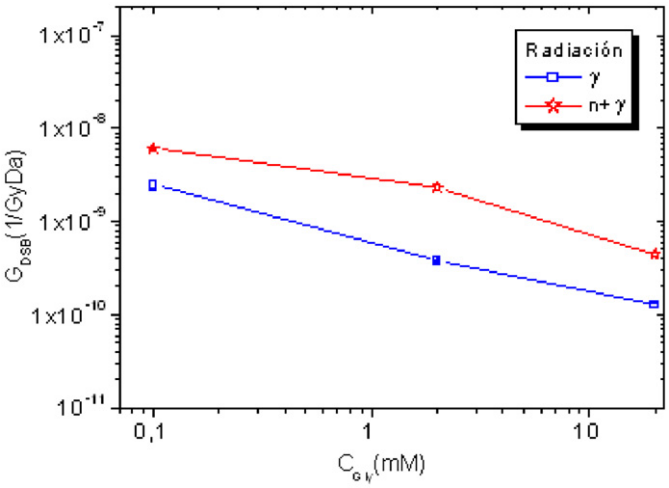


**Fig. 4.** DNA DSB yield as a function of the radical scavenger concentration. The initial DNA concentration was 88 ng/μL.

recombination process between the free radicals produced by water radiolysis and the added scavenger, which decreases concentration of OH· radicals attacking DNA. This, in turn, decreases SSB and DSB yields. A similar trend with increasing scavenger concentration was reported recently by Leloup et al. (2005), who exposed a simple model DNA molecule to various kinds of radiation.

Irradiation of cells with neutrons results in higher values of $G_{SSB}$ and $G_{DSB}$ than irradiation with $^{60}$Co γ-rays. The reactor beam is mixed: it contains thermal, epithermal and fast neutrons, in addition to γ-rays. Primary γ-rays are produced by the fission process, while secondary γ-rays result from inelastic collisions and capture reactions in the structural materials of the experimental facility. Also, it is well known that the secondary product of the capture reactions of thermal neutrons with the matter is γ-rays. Hydrogen nuclei have large thermal neutron cross section, and the interaction produces 2.2 MeV γ-rays by the reaction $^{1}$H(n,γ)$^{2}$H. On the other hand, the linear energy transfer (LET) is lower than 2 KeV/μm for the majority of γ-ray interactions, whereas the LET value for the mixed radiation (n+γ) fields is much higher.

In general, there are significant differences between the results of studies of DNA radiation damage reported by different laboratories. This is most likely because of the variations in the experimental conditions, such as the DNA concentration, the grade of

DNA purity, the type of the OH· radical scavenger and the used method of quantification. This work is a part of a broad accurate study of the effects of various types of radiation (protons, γ-rays and neutrons) on DNA molecules under identical conditions (same DNA molecule, same scavenger, same method of DNA damage detection and the same quantification technique). We have also obtained previously unavailable data for irradiation in the absence of a free-radical scavenger. A software GELANALIS was developed for a quantitative analysis of the electrophoresis image, which turned out to be the most important tool for accurate quantification of the products of DNA damage. An important reduction of uncertainties has been achieved, which has made it possible to extend the experimental studies to lower concentrations of the scavenger. The new design of the sample delivery system has enhanced research capabilities of the IEA-R1 Brazilian Research Reactor.

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