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EXECUTIVE SUMMARY OF THE THESIS

Development and experimental methods for ultra-high dose-rate radiation therapy using alanine dosimetry

LAUREA MAGISTRALE IN NUCLEAR ENGINEERING - INGEGNERIA NUCLEARE

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1 Introduction

Radiotherapy is currently one of the main techniques used to treat cancer, however tolerance of healthy tissue remains the main limitation of this type of treatment. Recently, innovative work has demonstrated that ultra-high dose-rate irradiation, known as FLASH, has a major effect in sparing healthy tissue while preserving anti-tumor efficacy [1]. This new technique presents great metrological challenges, thus research on the dosimetry aspect of FLASH irradiation (i.e. monitoring, quality control, absolute dosimetry) is needed to secure its development and the enabling of the translation to clinical applications. Electron paramagnetic resonance (EPR) dosimetry uses solid materials such as alanine (an amino acid with a density close to that of water), which have great potential for application in radiotherapy thanks to their highly favorable dosimetric properties. Irradiation of this type of material leads to the generation of paramagnetic species, the number of which is linearly proportional to the absorbed dose. The dose can be determined by evaluating the absorption spectrum of alanine's unpaired electrons at a specific resonance frequency in a variable magnetic field. The alanine/EPR technique is not yet widely used due to a number of implementation difficulties, such as cost, complexity and low sensitivity for low doses ($<$5Gy). Indeed, to this day, no standard for FLASH dosimetry exists yet. This thesis work is therefore part of an effort to improve methods for ionizing radiation dosimetry under specific conditions (high linear energy transfer and high dose-rates). In this thesis, different detectors have been used (in particular ionization chambers in conventional mode and radiochromic films) as well as alanine/EPR dosimeters, and then compared for various types of beams (protons, electrons) and conditions (different energies, conventional, FLASH, etc.). Preliminary measurements have been analyzed in collaboration with the Institut Curie and the IRSN (Institut de Radioprotection et de Sûreté Nucléaire) in France, demonstrating the potential of alanine on this type of beams.

2 Literature overview

The first step of the research was to understand the state of the art of FLASH radiotherapy, high dose-rate dosimetry and alanine/EPR dosimetry.

2.1 FLASH radiotherapy

FLASH radiotherapy (FLASH-RT) is an emerging technique characterized by ultra-high dose-rate irradiation (i.e. mean dose-rate far over 40 Gy/s). The "FLASH effect" is a biological effect that offers enhanced tumor control and reduced damage to healthy tissues. Several studies have investigated this effect, yet still no definite answer has been found. Favaudon et al. [1] suggested that FLASH-RT induces transient hypoxia, reducing radiation-induced damage to normal tissues. Boscolo et al. [2] demonstrate that since FLASH-RT generates a high density of free radicals in a short time frame, recombinations will be increased and oxidative damage in normal tissues will be reduced. Similarly, the research of Limoli et al. [3] suggests that FLASH enhances endothelial integrity, reducing inflammatory response and lipid peroxidation.
Further research is needed to find the causes of FLASH effect, and standardized dosimetry can help in achieving it.

2.2 Dosimetric challenges in FLASH-RT

One of the major barriers to the preclinical implementation of FLASH-RT is the need for standardized dosimetry protocols. Gomez et al. study [4] showed that the ion chamber, the reference detector for conventional-RT, is not adequate for FLASH because it shows increased charge recombination leading to significant underestimation of the delivered dose. Diamond detectors and radiochromic films, on the other hand, are promising for FLASH applications and have performed well at such ultra-high dose-rates. The latter, as described by Dunn et al. [5], has shown independence from dose-rate variations. However, it requires careful calibration and storage, and its reading involves a precise off-line procedure.

2.3 Alanine Dosimetry and Electron Paramagnetic Resonance

Alanine dosimetry, combined with EPR spectroscopy, is already widely used for industrial applications, and has more recently been noted as promising for FLASH-RT. Indeed, alanine has already been used as a dosimeter for dose confirmation (together with radiochromic film) in the first FLASH irradiation of a patient [6]. Alanine is roughly tissue equivalent and has the ability to generate stable radicals upon irradiation, with a linear response over a broad dose range, from 1 Gy to more than 100 kGy [7]. Furthermore, alanine retains its dose-response linearity even at ultra-high dose-rates, going up to 3×10^{10} Gy/s [8]. As downside, alanine's accuracy decreases at low doses together with the signal-to-noise ratio (SNR), leading to uncertainties of several percents. In that context, reading procedures need to be optimized. In particular, in an investigation for FLASH dosimetry Gondré et al. [9] suggested protocols for alanine/EPR that reduce reading time and preserve a deviation to the reference of maximum 2%.

3 Materials and methods

3.1 Irradiation of the dosimeters

Several experiments were carried out at Institut Curie, using the proton therapy clinical gantry a conventional mode as well as an electron LINAC (linear accelerator) in both conventional and FLASH regimen. Conventional irradiations were monitored using dosimeters such as alanine and ion chambers (plane parallel PPC05 by IBA Dosimetry and Advanced Markus by PTW Dosimery), that served as benchmark for alanine measurements. The Technical Reports Series (TRS) N°398 [10] was used to estimate the absorbed dose from the chamber's current output and corrected from environmental and detector's characteristics. Alanine irradiation was performed both using a solid water phantom and in liquid water, as seen in Section 4.4, underlying the need of a waterproofing method for using alanine (extremely sensible to water and humidity): the body of a modified ion chamber, sealed with adhesive tape, was finally used and allowed reproducible measurements. In FLASH, since ion chambers are no longer usable, radiochromic films and diamond detectors (also calibrated using the TRS N°398 recommendations) were used as benchmark. The reading of the film is not trivial and to obtain accurate results the standard film reading protocol developed at the IRSN was used. The final aim was to produce a dedicated protocol for alanine/EPR dosimetry.

3.2 EPR dosimetry

EPR dosimetry refers to the estimation of the dose delivered to a medium through the evaluation of relative concentrations of the radical species generated by ionizing radiation. Radicals are molecules with unpaired electrons, giving rise to a magnetic moment, making the molecule paramagnetic. A simplified version of an EPR spectrometer consists of an electromagnetic wave source, an electromagnet, a resonant cavity and a detection system. It operates by applying a strong, static magnetic field to a sample while exposing it to microwaves. The sample's unpaired electrons, placed in a resonant cavity, absorb energy when the electron spin states reach resonance and an absorption spectrum will appear. In particular, EPR is sensitive to the Zeeman effect and hyperfine interactions. A waveguide directs microwaves from a Gunn diode source, and phase-sensitive detection, aided by modulated Helmholtz coils, amplifies and filters the signal.
The acquisition parameters of the detection system can greatly influence the shape of the spectrum as well as the SNR. They are the microwave power, which increases signal's intensity (improving the SNR) and causes signals saturation, the amplitude modulation, that also enhances the signal intensity but can distort the spectrum if too high. Furthermore, one could also vary the number of acquisitions, n, and their duration. SNR will vary as $\sqrt{n}$. Mass, size, shape, and orientation of the sample in the cavity will also influence the appearance of the spectrum, as well as environmental conditions like temperature.

3.3 Alanine dosimetry protocol

By weighing 10 randomly selected pellets a coefficient of variation of 0.22 % was obtained, thus the hypothesis was made that the mass variation does not significantly impact the results. To protect alanine's signal from environmental conditions, they are stored and read in a temperature and humidity controlled room.
An EPR recipe was created for this thesis and its setup for the EPR detection system's parameters was used for all readings. The overall protocol can be summarized as follows:

- Ensure the pellet height aligns with the cavity center.
- Verify that the cavity is clean.
- Select an appropriately sized glass tube-holder.
- Determine the saturation power manually (by measuring at different power levels) or using the Python script created for this purpose.
- Choose the recipe and start the measurement.
- Measure the cavity temperature during acquisition.
- For reproducibility evaluation, perform 10 single measurements, removing and reinserting the alanine each time instead of using accumulations.
- Record the internal marker signal (EPR reference signal) to confirm stability.
- Log the amplitude of each new EPR peak. If one or two measurements deviate significantly, redo them. Aim for a coefficient of variation below 1%.

4 Results and Discussion

4.1 EPR parameter optimization

An experiment was performed to understand the effects of the spectrometer parameters on the pellet reading. The pellet used for the investigation was irradiated with 100 MeV protons at 40 Gy. The first parameter under analysis was the modulation amplitude, which was varied between 0.2 mT and 1 mT. The signal was read using the Python script that was developed for this thesis, and a formerly computed calibration curve was used to obtain the dose. The fit of the results lays on a linear curve, y = ax + b, where the R^2 value is equal to 0.9917. Ideally, the best choice of modulation amplitude would be the one with minimum relative standard deviation (RSD) which was found at 0.8 mT. Moving onto to the microwave power, which was varied between 1 mW and 20 mW. In this case too the fit of the amplitude and dose variations was also a linear curve, with R^2 equal to 0.97. In this case too the best choice of microwave power would be the one with minimum RSD, which was found at 5 mW. Next, field sweep time was analyzed by varying it between 5 to 240 seconds. The best choice of time sweep is the one with minimum RSD, which decreases with increased time sweep. From a practical standpoint, a 60 seconds sweep time would maintain good SNR while keeping the measurement quick. Finally, temperature was investigated. The room's temperature was changed between 17 °C to 30 °C with a subsequent cavity temperature variation between 28 °C to 38 °C. Fitting amplitude to temperature between 28.77°C and 37.11°C, a linear correlation was again found with an R^2 value of 0.99. Using one of the calibration curves created, and interpolating dose from amplitude, one can correlate the dose read and the temperature of the cavity. In particular, the dose value decreased by -0.28 Gy for every degree Celsius of temperature increase.

4.2 Calibration curves

Four different calibration curves were created during this study, using proton beam irradiation under different conditions. Doses to alanine ranged from 0 to 40 Gy. The first investigation was done creating calibration curves for alanine irradiated in a solid water phantom and in liquid water. The fit of the data (linear $y = ax + b$) yielded for both phantom and water irradiation a R^2 of 0.99. To estimate if there is any significant difference between the curves, a Monte Carlo approach was created ad-hoc and performed. By generating 1000 mock datasets from a normal distribution (with means and uncertainties taken from experimental data), each dataset is fitted with a linear model using Python. The differences in slopes and intercepts between the two calibration curves are then analyzed. Confidence intervals (e.g: 95% confidence level) are constructed, and if they include zero, it indicates no significant difference between the two curves at the considered level of significance. The slope difference between the phantom and water calibration curves is statistically significant at 95% confidence, indicating a real difference in their relationships. However, the intercept difference is not statistically significant, suggesting that any observed variation is likely due to random fluctuations. In particular, the slope of the calibration curve is the key parameter, as it reflects factors like LET and secondary radiation effects, whereas the intercept can vary, for example, with the number of data points. Differences in slope between water and phantom curves indicate changes in the surrounding environment, affecting radiation interactions. The same procedure was followed for different energy protons irradiation: 100 MeV and 226 MeV, performed in phantom. The data was fit linearly for both energies and in both cases had $R^2 = 0.99$. Applying again the Monte Carlo method, the analysis shows that neither the slope nor the intercept differences between the calibration curves at the two energies are statistically significant at 95% confidence. The result might suggest that energy differences between the 100 MeV and 226 MeV beam do not have significant effect on the calibration curve.

4.3 Conventional and FLASH irradiation

Pellets irradiated in FLASH (ultra-high dose-rate, up to 10^6 Gy/s) and conventional mode with 7 MeV electrons were also analyzed, using the FLASH LINAC at Institut Curie. The detectors compared in conventional irradiation were ion chamber, films, alanine and diamond. In FLASH, only the last three were compared, and irradiations were performed at 7 Gy and 18 Gy. In Tables 1 and 2 the results of the conventional irradiation are outlined.

Table 1: Results of conventional irradiation on the active dosimeters.

Detector type	Measurement	Uncertainty
PPC05	7.73 Gy	0.15 Gy
Markus	7.67 Gy	0.70 Gy
Diamond	7.55 Gy	0.14 Gy

Where [amp] signifies amplitude units and it is the dimensionless unit of measure of the EPR dosimetry system. For FLASH the results are the ones shown in Tables 3 and 4. The uncertainty for the diamond detector was considered to be the same as in conventional irradiation.

4.4 LET effects

The last aim of this thesis was to evaluate LET possible dependence for proton beams, both experimentally and using Monte Carlo simulations. Some insights were already briefly offered with the different energy irradiations in the section 4.2. Furthermore, Bragg peak experiments were performed: alanine and ion chamber were

Table 2: Results of conventional irradiation on passive dosimeters, with each film dosimeter paired with the alanine dosimeter it was irradiated with.

Film Dosimeter	
Measurement [Gy]	Uncertainty [%]
7.39	5.2
7.50	5.2
7.49	5.2
Alanine Dosimeter	
Measurement [amp]	Uncertainty [%]
0.0026	0.9
0.0027	1.2
0.0027	1.7

Table 3: Results of FLASH 7 Gy irradiation.

Detector type	Measurement	Uncertainty [%]
Diamond	7.44 Gy	1.9
Film	7.17 Gy	5.2
	7.15 Gy	5.2
Alanine	0.0025 [amp]	2.6
	0.0026 [amp]	2.2

Table 4: Results of FLASH for 18 Gy irradiation.

Detector type	Measurement	Uncertainty [%]
Diamond	18.56 Gy	1.9
Film	19.94 Gy	5.2
	19.92 Gy	5.2
Alanine	0.0020 [amp]	0.5
	0.0020 [amp]	1.4

irradiated in a water tank and bench-marked, lowering them deep in the water with the aim of building the Bragg Peak shape by reading the detector's signals. The beam was composed of 100 MeV protons, with an entrance dose of 10 Gy and peak dose of around 40 Gy. The experiment was also simulated through Monte Carlo codes using Matlab and TOPAS (Geant4), to interpret the Bragg Peak behavior and LET distribution (also referred to as TEL) in alanine. On the same plot, Figure 1 the experimental points are also shown. The simulation used 100 MeV protons. The alanine dosimeter was created for this thesis, by investigating its geometry, composition and density.
Figure 1 showcases experimental and simulated data for both ion chamber and alanine.

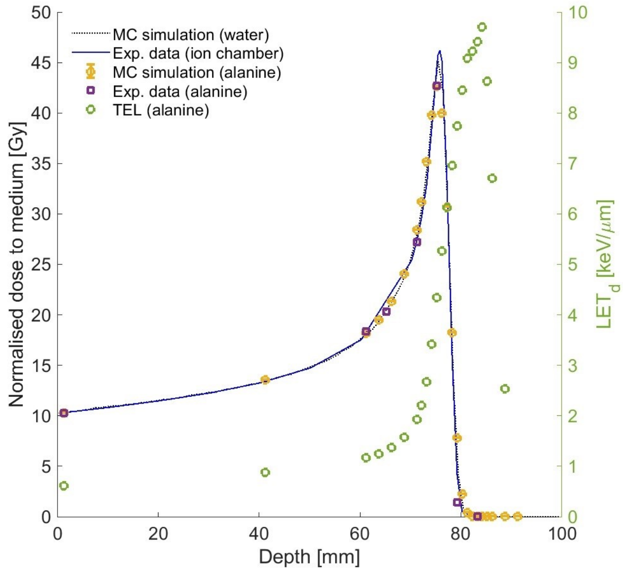

Figure 1: Bragg Peak shape for ion chamber, alanine and water, with LET (TEL) distribution in alanine.

LET (dose-averaged) behaved as expected, with an increase towards the end of the Bragg Peak, where energy deposition of the particle is at maximum, and a subsequent decrease in the distal part. A clear under-response of the alanine signal can be seen, which may be related to LET increase. Palmans et al. [11] also stated that the response of alanine is dependent on the type of radiation. To this end the relative effectiveness (RE) for protons was introduced in his paper, defined as the ratio of the detector's signals for the same amounts of absorbed dose by protons and by 60-Co gamma radiation.

$$RE(E_{\text{eff}}) = RE_0 + \frac{\Delta RE}{1 + e^{-C\cdot(\log(E_{\text{eff}})-\log(E_m))}} \tag{1}$$

where RE_0, ΔRE, C and E_m are tabulated parameters.
E_{eff} is the effective energy, estimated as the average of the entrance and exit energies. It can be considered as a measure for the average LET of the protons in alanine.
As seen in the paper, RE decreases with decreasing proton energy, or increasing LET. Indeed, Palmans et al. [11] demonstrated that at proton energies below 20 MeV, RE drops significantly and the Bragg peak occurs at low proton energies (typically few MeV), where the RE of ala-

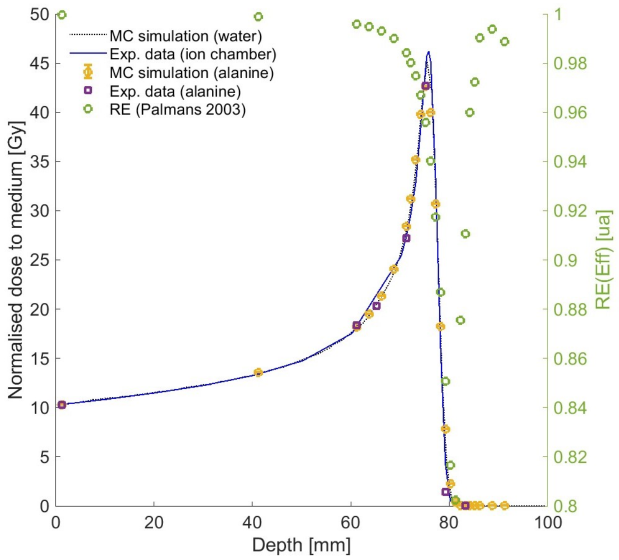

Figure 2: Bragg peak from experimental data and simulation with RE estimation.

nine decreases and this is in accordance to Monte Carlo simulations (Figure 2).
Furthermore, Waligorski et al. [12] predicted that at high LET, radical formation in alanine decreases due to increased radical recombination. Since protons at the Bragg peak have higher LET than at entrance, alanine's dose decreases, reflected by a reduction in RE, which cold be used in order to correct for this energy dependence..
The paper notes that ionization chambers do not exhibit the same RE drop because their response is based on direct charge collection, which remains relatively independent of LET effects.

5 Conclusions

This thesis characterized alanine as a dosimeter for both conventional and FLASH radiotherapy, comparing its performance with ion chambers (where possible), films, and diamond detectors. To do that, a standardized protocol for alanine irradiation, storage, and waterproofing was developed and tested for reproducibility and accuracy. Furthermore, EPR detection system's parameters were analyzed and a recipe was created to correlate signal amplitude with dose in the most optimal way. Python scripts were created to streamline data analysis. Calibration curves from different beam energies and media were created experimentally, data analysis was performed and the results were statistically compared. An inter-comparison of alanine, films and diamond was started for FLASH (ultra-high dose-rate) irradiation, highlighting the consistency of the detectors' response at various dose-rates. This will work as a foundation for further studies in order to demonstrate alanine's usefulness for FLASH applications. Finally, LET effects in alanine were studied using Bragg Peak experiments and Monte Carlo simulations within TOPAS, thanks to an alanine dosimeter model with custom material composition and geometry, confirming previously reported findings in the literature.

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