Development of a DNA Biodosimeter for UV Radiation

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Abstract: Ultraviolet (UV) radiation has a strong influence in the damage of deoxyribonucleic acid (DNA). In this work, the possibility of a DNA UV radiation dosimeter is evaluated. For that, calf thymus DNA samples, thin films and aqueous solutions, were irradiated with 254 nm wavelength light during different periods of time, being the damage caused by the irradiation analysed by both UV-visible and infrared spectroscopies. As the DNA is a polyelectrolyte, the pH of the DNA samples was also considered as a variable. Results demonstrated that damage in DNA takes place in both thin films and solutions when irradiated at 254 nm, as revealed by a consistent decay in measured absorbance values. However, DNA solutions were seen to give more reliable as the induced damage is easily measured. For this case, the absorbance at 260 nm was seen to exponentially decrease with the irradiation time as a result of radiation damage with the kinetics damage strongly dependent of pH. Consequently, the lifetime of such dosimeter device can be chosen by changing the pH of aqueous solutions.

1 INTRODUCTION

The use of radiation for medical procedures, in particular for diagnostic and therapy purposes, has dramatically increased over the years (Yu, 2017). Mechanisms of justification of procedures and management of the patient dose are employed to avoid unnecessary or unproductive radiation exposure in diagnostic and interventional procedures. Dose constrains are appropriated to comforters and carers, and volunteers in biomedical research but regarding the therapeutic applications, it is not considered appropriate to apply dose limits or dose constraints, because such limits would often do more harm than good (ENEA2012).

The effects induced on biological systems by electromagnetic radiation are due to the energy transfer into the medium with absorption of the radiation (Bernhardt, 1992, Bronzino, 1995, Moulder, 2007), and are characterized by a series of events which differ (and are classified) according to their reaction time scale, leading ultimately to biological damage (Bernhardt, 1992). These events can thus be divided into three groups: 1) Physical –interactions between the charged particles and the tissues atomic structures, which leads to ionization and concomitant formation of ionic radicals, in an extremely short time frame (around 10^{-18} s); 2) Chemical – formation of ion pairs through an ionization process, which leads to formation of free radicals and chemical bonds rupture (around 10^{-6} s); and 3) Biological – follows from bond rupture and is characterized by altering the proper physiology of cells or even cells death (Moulder, 2007); the time that biological damage takes place after chemical bonds rupture is usually long, ranging from a few hours to several days, weeks, months, or even years.

When a cell is irradiated there are two types of changes which can occur, directly on the cellular component molecules or indirectly on water molecules, causing water-derived radicals. Radicals react with nearby molecules in a very short time, resulting in breakage of chemical bonds or oxidation of the affected molecules. The major effect in cells is DNA breaks (Gomes, 2014, Fretelde, 1993, Su, 1994, Xu, 1994, Storhatf, 1999, Podgorsak, 2006). Ionizing radiation can also lead to structural changes in several macromolecules present in cells. In nucleic acids, changes are essentially loss or damage of bases, thymine dimmers formation, single or double strand

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breaks and also DNA-protein dimmers formation (Kielbassa, 1997, Ravanat, 2001).

DNA is featured an interesting anionic polyelectrolyte having a unique double helix structure (Fretelde, 1993) that can be used for many purposes. For example, on the basis of hydrogen bonding properties of DNA base pairs, oligonucleotide probes have been recently designed to detect tumour gene and various biosensors were also proposed (Caruso, 1999, Lvov, 1993). Also, DNA aqueous solutions are of special interest, mainly in the development of biological sensors (Su, 1994, Xu, 1994).

Moreover, the DNA sequence defines the genetic information that commands the development of any living being and its main vital functions (Wilkins, 1953, Franklin, 1953, Gomes, 2014). Since DNA plays an important role in the maintenance of the genetic information, any modification in this macromolecule has significant effects at the cellular level (Lindahl, 1993; Beckman and Ames, 1997). Thus many efforts have been taken to delineate the mechanisms of formation and the chemical structures of the DNA modifications produced by genotoxic compounds, including also ionizing (X, gamma, heavy ions) and non-ionizing (ultraviolet (UV) and visible light) radiations (Ravanat, 2016).

The effects of ionizing radiation on DNA have been investigated in detail during the last three decades but one of the most common environmental health hazards that cause highly toxic effects is the UV radiation (Kielbassa *et al.*, 1997, Ravanat *et al.*, 2001, Yu and Lee, 2017). It should be referred here that UV radiation is classified as UVA (315-400 nm), UVB (290-315 nm), and UVC (280-100 nm). Most UVC is absorbed by the ozone layer, and only UVA and UVB compose ground level UV radiation (Kalinnowski, 1999, Caruso, 1999). This is because, firstly, certain biomolecules such as proteins and nucleic acids have chromophores that absorb in the UV region of the spectrum. Under high UV fluxes, these molecules are photo-chemically degraded or transformed, resulting in impairment or even complete loss of biological function. The magnitude of damage caused by these so-called direct or primary mechanisms is determined by the amount of radiation absorbed (absorbance cross-section) and the quantum yield of photo-damage (molecules damaged per photon absorbed).

One class of UV toxicity effects is caused by a series of indirect mechanisms. UV is absorbed by some intermediate compound (photosensitising agent) either inside or outside the cell to produce reactive oxygen species (ROS) (Vincent, 2000). The resulting high energy oxidants such as hydrogen peroxide, superoxide or hydroxyl radicals can then diffuse and react with other cellular components with sites of damage that can be well away from the site of photo-production. Regarding genetic damage, nucleic acid bases absorb maximally in the UVC range, with peak absorbance around 260 nm, and exhibit a tail that extends well into the UVB (Vincent, 2000). This absorbed energy results in the first excited singlet state, with a lifetime of only a few picoseconds. Most of this energy is dissipated by radiation less processes inside the molecule, but a small fraction is available for a variety of chemical reactions. This can result in the photo-damage of nucleotides (Vincent, 2000), with a two- to four-fold greater effect on pyrimidines (thymine and cytosine) relative to purines (adenine and guanine). In addition, three principal photoproducts are formed by the UV-induced reactions: (a) 5,6-dipyrimidines, which are cyclobutane-type dimers, generally referred to simply as pyrimidine dimers; (b) photohydrates; and (c) pyrimidine (6—4) pyrimidones, often referred to as (6—4) photoproduct (Vincent, 2000). For example, skin aging, eye damage, and skin cancer are some of the most harmful effects known. This is because of increased production of cellular reactive oxygen species and by direct DNA damage, and if the DNA damage is not properly repaired, will lead to mutations and interferes with many cellular mechanisms (e.g. replication, transcription, and the cell cycle) (Yu, 2017).

If one intends to develop a device which allows the measurement of light dose based on biological materials, it should be chear that there are three kinds of biologic markers: exposure (dose), effect and susceptibility markers. Biologic markers of effect record biologic responses in individuals who have been exposed to a genotoxic agent, but markers of dose do not necessarily indicate effects. Superimposed on this are susceptibility markers; those that could be used to identify persons who are at increased risk of developing a disease that could be triggered by a radiation exposure. Included here might be organisms whose ability to repair DNA damage is limited (National Research Council, 1995).

Biological dosimetry does not measure the exposure in real time but the biological changes induced by radiation. There are both indicators of exposure or effects. Often the two aspects overlap as in the case of deterministic effects induced by high-doses, as for the acute radiation syndrome clinic that is characterized by damages in skin, haematopoietic, gastrointestinal, and cerebrovascular systems. In the case of stochastic effects, induced by low doses, the biomarkers used to measure the absorbed dose, not

always imply a clear detriment of health. It has been, however, often demonstrated that an increase in the frequency of these indicators is associated with an increased risk of radiation-induced cancer and may be indicative of radio-sensitivity (Giovanetti, 2012). According to Giovanetti et al, 2012, for a biodosimeter to be effective the following features are determinant: 1) measurement on tissues or fluids easily obtainable; 2) the effect must be specific of radiation; 3) response should vary directly depending on the dose; 4) it has to measure also chronic or repeated exposure; 5) it must be possible to measure retrospectively exposure also after years and 6) the measurement must be simple, fast or automated.

A simple method of analyse the effect of UV radiation on DNA is the measurement of AC electrical conductivity of DNA thin films (Gomes, 2012). Such study revealed that electrical conduction arises from DNA chain electron hopping between base-pairs and phosphate groups being the hopping distance a value of 3.3899±0.0002Å which coincides with the distance between DNA base-pairs. Moreover, the loss of conductivity of DNA samples follow the decrease in phosphates groups with irradiation time, suggesting the use of DNA based films for UV radiation sensors (Gomes, 2012). Based in these achievements, in this paper, a new biological dosimeter based radiation-induced lesions in DNA is proposed, where the damage caused by radiation is obtained by UV-visible (UV-Vis) and infrared spectroscopies and related to radiation exposure.

2 MATERIALS AND METHODS

Ultra-pure water and DNA hydrophilized in sodium salt form (DNA sodium salt from calf thymus, CAS 73049-39-5, acquired from Fluka®) was used for the preparation of DNA aqueous solutions. Its dissolution is favoured by the presence of sodium ion (counter-ion), allowing the preparation of aqueous solutions with anionic character. The concentration of the DNA solutions was 0.025 mg/mL DNA. The pH value of the DNA aqueous solution was 6, these solutions are also designated as natural solutions or pHN. In order to obtain DNA solutions with pH=9 and pH=3, the pH was adjusted to basic or acid with NaOH (1M) and HCl (1M), respectively.

Cast films were obtained by the drop casting method, i.e, depositing some drops of the DNA aqueous solutions with different pHs onto calcium fluoride (CaF_2) solid supports. These samples were placed in a desiccator during several hours to dry.

Solutions and cast films were irradiated for different periods of time by means of a 254 nm UVC germicide lamp, model TUV PL-L 55W/4P HF 1CT from Philips®, at an irradiance of 1.9W/m^2, in a ventilated chamber at room conditions.

The DNA damage was monitored in aqueous solutions by measurements of UV-Vis spectra after each irradiation period in a spectrometer (UV 2101PC, Shimadzu®) while the thin films were characterized with a Fourier transform infrared (FTIR) spectrometer Thermo Scientific Nicolet-model 530 (Waltham, MA, USA).

3 RESULTS AND DISCUSSION

According to Schuch et al (Schuch 2013), to develop a reliable system for measure the UV light dose, one have to search for material that would present the most adequate features: (i) high transmittance to UVB and UVA wavelengths; (ii) resistance to environmental adversities; (iii) possibility of framing the shape of the template according to the aim of the experiment; and (iv) low cost. Having into account such advices and the conclusions achieved by Gomes et al (Gomes, 2012), it seemed that the use of DNA thin films should be interesting for the development of a UV dosimeter. Consequently, DNA cast films deposited onto CaF_2 and quartz were prepared from DNA aqueous solutions with pH 3, 6(N) and 9. These films were irradiated with 254 nm UV radiation for different periods of time and the UV-vis and infrared spectra were measured for the different irradiation times. As expected, in the absence of water, the changes caused by radiation are minimal as can be inferred from the infrared spectra of the DNA cast films prepared from DNA aqueous solutions (pHN) before and after UV irradiation for 15 h, displayed in Figure 1. The observed peaks in the spectra are in accordance with Gomes et al (Gomes, 2009) where the infrared absorbance peaks were systematically assigned to the respective DNA groups. Accordingly the range of wavenumbers contained between 1250 and 900 cm^{-1} are associated with the phosphate backbone region while 1500–1250 cm^{-1} and 1800–1500 cm^{-1} wavenumber regions are associated to DNA bases vibrations influenced by the sugar component and to DNA bases, respectively (Gomes, 2009).

Since UV radiation has effect on DNA phosphates groups as demonstrated by Gomes et al (Gomes, 2015), the values of absorbance at 1097 cm^{-1}, assigned to the presence of symmetric PO_2^- stretching of backbone in the DNA molecules (Gomes, 2009), were plotted in Figure 2 as a function of the

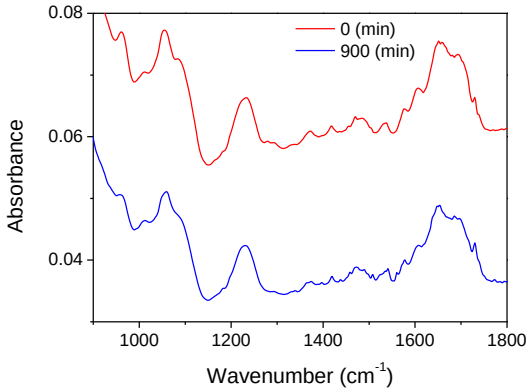

Figure 1: FTIR spectra of DNA casted films prepared from solutions at natural pH (pH 6) conditions before and after irradiation with UV-light at 254nm wavelength during 900 min (15 h).

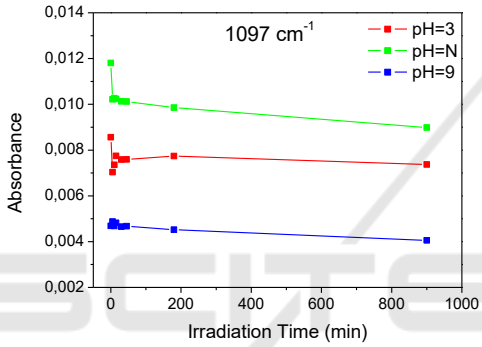

Figure 2: Absorbance at 1097 cm^{-1} after baseline subtraction *versus* irradiation time for the different DNA cast films prepared from aqueous solutions with different pHs.

irradiation time for samples prepared from DNA aqueous having different pH. Generally, an absorbance decay is observed. However, these measurements are always tricky due to baseline fluctuations and also if the molecules concentration seen by the beam is not identical–leading to absorbance deviations. To circumvent this drawback, the analysis of the effect of UV radiation at 254 nm was carried out on DNA aqueous solutions prepared at different pHs. Figures 3 a), b) and c) present the UV-vis spectra obtained for the DNA aqueous solutions with pH=3, pH=6 and pH=9, respectively, irradiated during different periods of time. The obtained results point out that the DNA solutions with pH=3 (Figure 3 a) tend to be more sensitive to higher times of UV light exposure since the absorbance at 260 nm for 900 minutes of irradiation was the lowest value found for the different DNA solutions studied. The baselines changes can be due to the light scattering of smaller molecules, originated by the cleavage of DNA molecule during the irradiation, as demonstrated by Gomes *et al*, 2012.

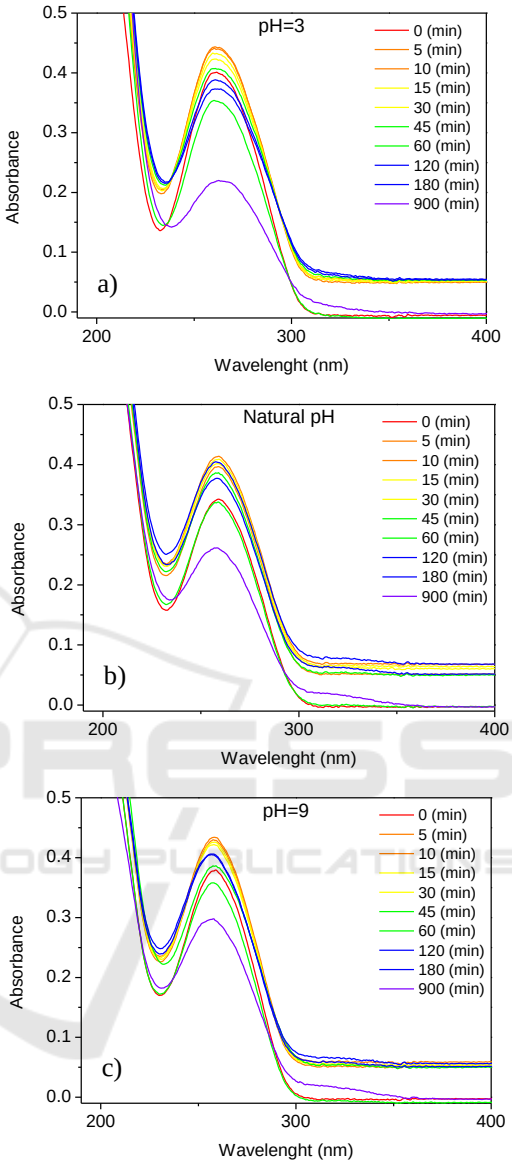

Figure 3: Absorption spectra of DNA solutions with: a) pH=3; b) pH=6 (natural) and c) pH=9; irradiated with 254 nm wavelength light for different periods of time.

The obtained results are in accordance with literature as similar behaviours and patterns are observed by Chen *et al*., 2009, where the disinfection of water was studied and they present the effect of UV radiation on the spores.

For a better comparison, the absorbance values at 260 nm, after removing the baseline (i.e. subtracting the value of the absorbance at 350 nm), were normalized, for each pH, and plotted as a function of the irradiation time in figure 4. Several attempts have

been done to find the best equation to model the experimental data. The normalized experimental data ($NAbs$) was found to be best fitted by an exponential like expression as follows:

$$NAbs = \frac{Abs_{260nm}}{Abs0_{260nm}} = exp\left(-\left(\frac{t}{\tau}\right)^{n}\right) \quad (1)$$

in which Abs_{260nm} is the absorbance at 260 nm, $Abs0_{260nm}$ corresponds to the initial (at the beginning of the experiments) absorbance at 260 nm, t the time in minutes, τ is the characteristic time or time constant and n is a constant which can be related with the order of the kinetics process (Raposo, 1997) with respect to radiation damage.

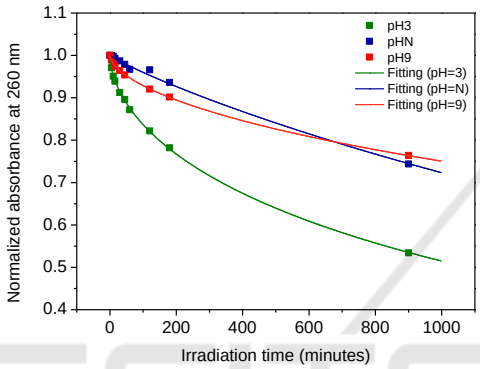

Figure 4: Normalized absorbance at 260 nm after removing the baseline *versus* irradiation time for the different solutions. The lines correspond to the fitting with equation (1).

Figure 5 shows the plot of the time constants in minutes for each pH. The results show that DNA solutions at higher pH (more basic) can be exposed to UV light during more time. Moreover, from equation (1) one can propose an expression for the dose level to which the sample has been subjected, as follows:

$$D = -\frac{C_D}{n} ln \frac{Abs_{260nm}}{Abs0_{260nm}} \quad (2)$$

in which D is the dose calculated by multiplying the irradiance by the irradiation time, C_D is the characteristic dose constant and n is the order parameter of the damage kinetics. These parameters as well as the characteristic time constants are presented in Table 1 for each pH investigated. From the obtained results one can conclude that DNA solutions can be suitable for the measurement of 254 nm wavelength light dose, being the lifetime of such dosimeter device dependent of solution pH. To develop a DNA based dosimeter device to cover also UV A and UV B region, DNA damage has also to be investigated in these UV regions. According with previous results (Gomes, 2015), damage is expected also take place with 300 nm wavelength light in such a way that the same procedure described here should be used to analyse the DNA damage when the solutions are irradiated with higher wavelength light.

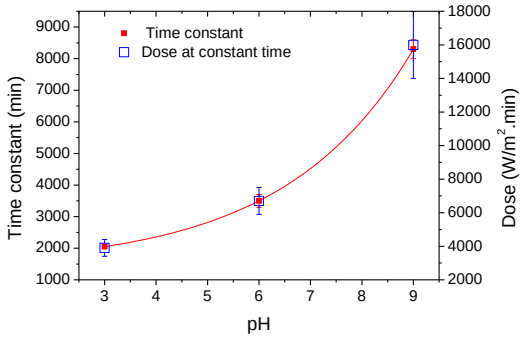

Figure 5: Time constant obtained by equation 1 *versus* pH of the solutions irradiated and estimated dose for the constant time for the solutions irradiated.

Table 1: Coefficients determined to each pH of DNA solution irradiated.

pH	τ (min)	C_D ($Wm^{-2}.min$)	n
3	2050±40	3890±70	0.571±0.005
6	3500±200	6700±300	0.90±0.03
9	8300±300	15800±600	0.590±0.007

4 CONCLUSIONS

In this work it was demonstrated that aqueous DNA solutions can be used to probe UV radiation at 254 nm and to evaluate the radiation dose at 254 nm, through absorbance measurements. The absorbance was seen to exponentially decrease with irradiation time being the damage kinetics parameter dependent of pH DNA aqueous solutions. This work also evidenced that the lifetime of such DNA dosimeter device can be chosen changing the pH of those solutions. In the future we intent to 1) irradiate the samples with a fixed wavelength of 300 nm in order to check the new kinetics damage; 2) check if there is a linear correspondence to the irradiation power; and 3) study the sensibility of the potential sensor.

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