FEASIBILITY ASSESSMENT OF A FLUORESCEIN DOSIMETER

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Summary

The objective of this work was to ascertain if fluorescein (a simple fluorescent molecule), could be used to create an economic radiation dosimeter. The degradation of fluorescein in water when exposed to ionizing radiation and the subsequent decrease in fluorescence intensity was investigated as an indicator of absorbed radiation. Cuvettes containing fluorescein concentrations ranging from 0.2-10μM were irradiated with doses ranging from 0.01-100 Sv using a 6MV linear accelerator (Varian Clinac® 600C). The resulting fluorescent signals were quantified generating dose-response type curves. The decrease in the fluorescence response based on absorbed dose was found to follow a 1st order decay model. In terms of detecting an absorbed dose, a concentration of 0.2 μM fluorescein was shown to have the greatest sensitivity with a detection limit of 0.05 Sv. It is recommended that future work investigate lower concentration fluorescein solutions, in addition to varying water chemistries for potential increases in sensitivity.

1.0 Introduction

Ionizing radiation is a significant concern for human health because it can cause cellular damage or mutations, which may lead to adverse health effects. Radiation dosimeters are useful tools which can detect and quantify the amount of ionizing radiation absorbed by an object, organism or tissue. When quantifying the dose imparted on tissues some conventional inorganic dosimeters exhibit a difference between dose measured by the dosimeter and the actual dose absorbed by the tissue. This occurs because inorganic dosimeters are chemically different from tissues [3]; therefore they interact with radiation in a different manner. As a result, the difference between dosimeter dose and tissue dose is known to vary with the particle type, rate, and energy of the imparted radiation, resulting in the use of dose conversion factors to determine the actual dose absorbed by tissues.

$$D_T = D_D C_{R,E} \tag{1}$$

Where D_T is the tissue dose, D_D is the dose measured by the dosimeter, and $C_{R,E}$ is the dose conversion factor. Figure 1 illustrates the varying dose conversion factors of some common conventional inorganic dosimeters.

It was hypothesized that a dosimeter based on fluorescein dissolved in water, unlike inorganic dosimeters, would be tissue equivalent. The advantage of this proposed dosimeter is that it would negate the requirement of a conversion factor to obtain D_T.

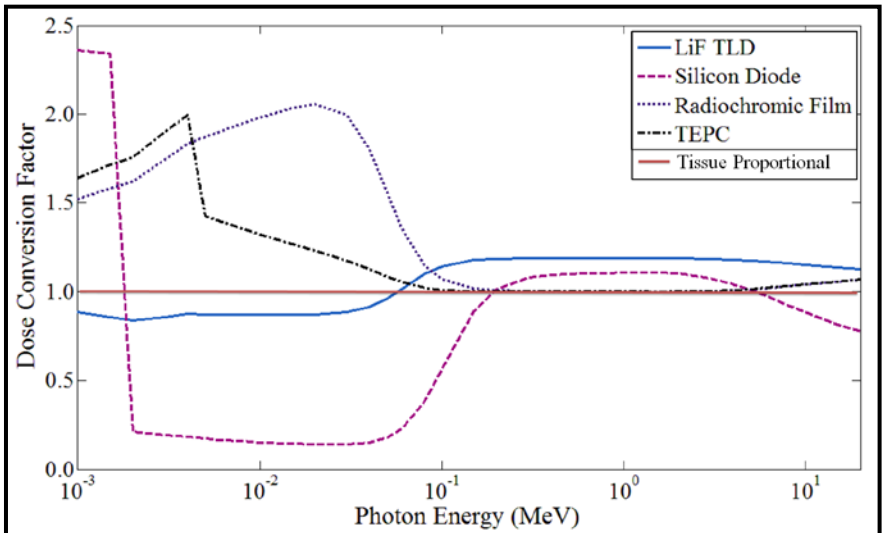

Figure 1: Dose conversion factors of some common conventional dosimeters. Adapted from [1].

1.1 Fluorescein Dosimeter

The proposed dosimeter utilizes the fluorescent property of fluorescein, a relatively inexpensive organic dye, as an indication for the quantity of absorbed dose. The fluorescein dosimeter consists of an aqueous fluorescein solution with known fluorescence intensity. When irradiated, the fluorescein will degrade resulting in a decrease in fluorescence intensity. As the fluorescein is suspended in water, it will degrade *via* mechanisms similar to those within the cytoplasm of cells. Therefore, the proposed dosimeter could be tissue equivalent, negating the requirement for a dose conversion factor ($C_{R,E}$).

1.2 Degradation of Fluorescein

There are two possible mechanisms *via* which ionizing radiation imparts energy to a tissue: direct action of ionizing radiation and indirect action of ionizing radiation. Direct action of ionizing radiation involves the interaction between radiation (photons in this case) and a target molecule (DNA for example). Indirect action of ionizing radiation is when the radiation interacts with the water molecules in the cytosol of cells and causes the formation free radicals, which can degrade the DNA via oxidation reactions [2]. As the fluorescein is suspended in an aqueous solution, it is suggested that it will be subjected to simular degradation mechanisms as the DNA within a cell. The action mechanisms of ionizing radiation on fluorescein are illustrated in Figure 2.

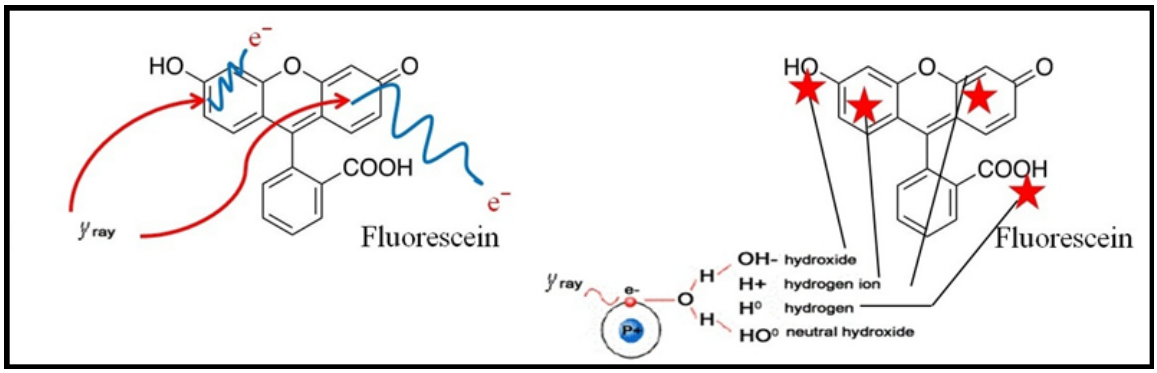

Figure 2: Proposed fluorescein decay by direct (left) and indirect (right) action of ionizing radiation.

2.0 Experimental Methods

To determine the feasibility of the proposed fluorescein dosimeter as a radiation dosimeter, the decay of fluorescence intensity as a function of the absorbed dose was investigated. Additionally, the stability of fluorescein solutions under possible alternate decay mechanisms such as degradation from sunlight, UV light, and instrument photobleaching was assessed.

2.1 Sample Preparation and Fluorescence Measurement

To prepare the samples, 10μM stock solutions were created using powder-form free acid fluorescein from MP Biomedicals, using deionized water in 2L volumetric flasks. The stock solutions were sonicated using a Fisher Scientific FS 110 Ultrasonic Bath for ten minutes, and then serial diluted to produce desired sample solutions ranging from 0.001-10μM. 1 mL samples were placed in clear BRAND® cuvettes and a Perkin-Elmer LS 50 B fluorometer* was used to measure the fluorescence of the sample solutions.

2.2 Stability Experiment

The stability experiment consisted of triplicate sample cuvettes filled with 0.2μM solutions, placed on a windowsill, in a dark cabinet, dark refrigerator, and a Chromato-Vue® UV viewing cabinet with a 365nm lamp. The samples were read bimonthly using the fluorometer to determine if the fluorescence signature had altered.

2.3 Irradiation Experiment

For the irradiation experiment, a Varian Clinac 600C EX Series linear accelerator was used to distribute doses of 0, 0.01, 0.05, 0.1, 0.5, 1, 3, 5, 10, 50, and 100 Sv at a dose rate of 13.2 $Sv \cdot min^{-1}$ for 100, 50 and 10 Sv, and 2.2 $Sv \cdot min^{-1}$ for lower doses. Quadruplicate solutions of 0, 0.2, 0.4, 1.0, and 10 μM fluoresceine were irradiated in 7mL high-density polyethylene vials for each dose giving a total of 55 combinations, and 220 samples total. To eliminate variations in the optical properties of cuvettes and the fluorometer, each irradiated sample was further separated into four separate 1.5mL cuvettes and then measured on the fluorometer.

3.0 Results and Discussion

The results of the stability experiment indicated that fluorescein is strongly sensitive to exposure to sunlight and that it will degrade over time. On the other hand, storage in a dark cabinet indicated stability for an extended period of time (+1 month). The instrument bleaching was found to be statistically insignificant over 200 scans.

The results of the irradiation experiment yielded non-linear decreases of fluorescence response as a function of absorbed dose. An analysis of variance (ANOVA) verified a statistically significant trend ($p\text{-value}<0.01$); the fluorescence intensity decreased as a result of ionizing radiation. Figures 3 and 4 below show the graphical representation of results for 0.2 and 1.0 μM solutions, respectively.

* Excitation wavelength of 488.0nm, emission wavelength of 512.5nm, and slit width of 2.5 Å.

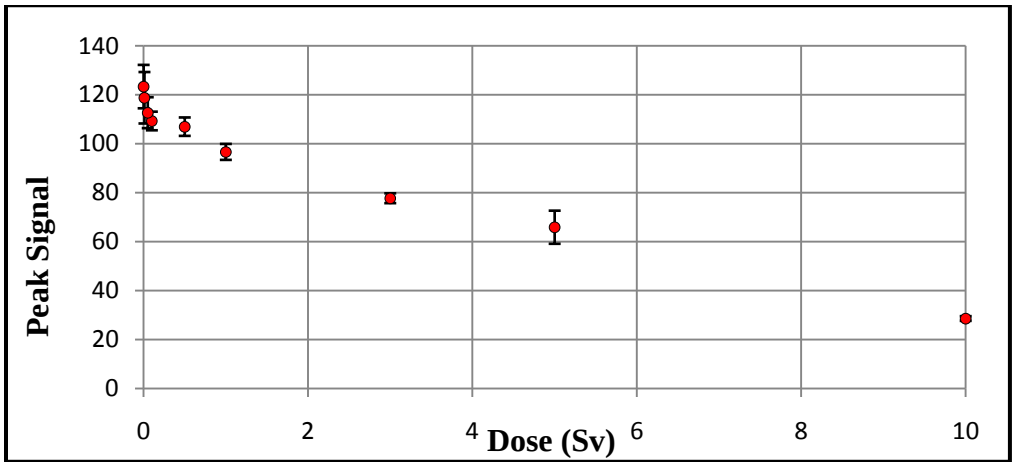

Figure 3: Decay curve of the 0.2 µM fluorescein solution

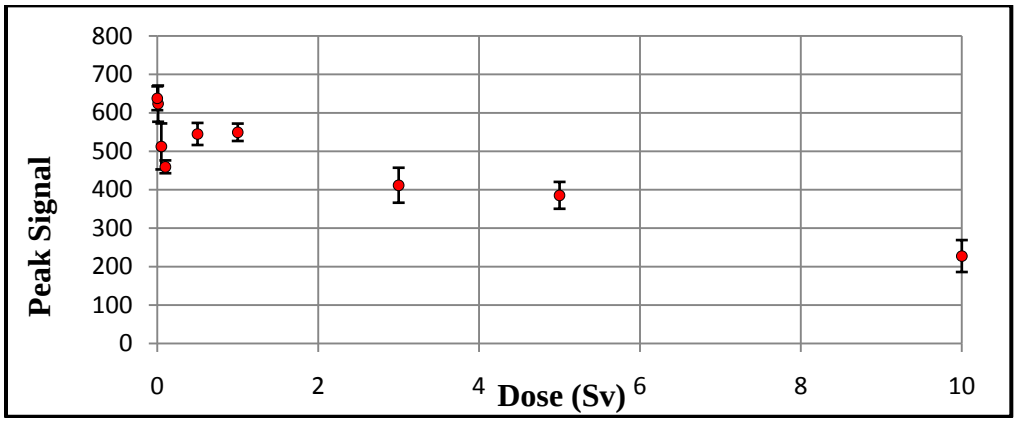

Figure 4: Decay curve of the 1.0 µM fluorescein solution

The data was fit to decay models of 0, 1st, and 2nd order to attempt to characterize the reduction in the fluorescence intensity as a function of dose. Table 1 lists R^2 values for the fit of each decay model. The 1st order model, in most cases, best characterized the reduction of fluorescence intensity.

Table 1: R^2 values for different decay model fits.

R^2 Values of Modified Graphical Fits			
Concentrations (µM)	0 Order Fit	1st Order Fit	2nd Order Fit
10	0.9823	0.9999	0.9804
1	0.817	0.8986	0.9229
0.4	0.9596	0.9791	0.9491
0.2	0.9593	0.9868	0.9355
0	N/A	N/A	N/A

Also, a Dunnet's test was used to estimate the critical dose (lowest dose/detection limit) at which a statistically significant change in the fluorescent response could be observed. The detection limit (p-value<0.01) was found to be 50 Sv for 10 µM; 0.05 Sv for 1.0 µM (reported as a preliminary result due to the inconsistent trend as seen in Figure 4); 3 Sv for 0.4 µM; and 0.05 Sv for 0.2 µM.

3.1 Preliminary Dosimeter Design

The 0.2 µM solution was selected for the conceptual dosimeter design, because it exhibited the lowest detection limit. The preliminary dosimeter design, Figure 3, consists of fluorescein solution (0.2 µM) in de-ionized water, macro BRAND UV-transparent disposable cuvettes, BRAND cuvette caps, and an opaque polypropylene (PP) container and lid (with carbon black, UV stabilizers and anti-oxidant additives). The sealed cuvette contains the fluorescein solution and is attached to the PP lid. The cuvette is then inserted into the PP container. The cuvette may be withdrawn and can be inserted directly into a fluorometer. Material cost for a single dosimeter unit in this design is approximated at $1.65 per unit.

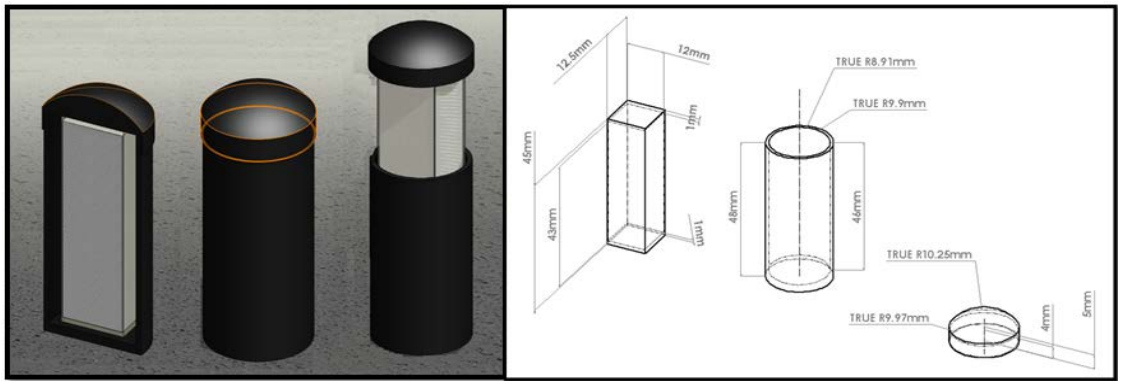

Figure 5: Proposed preliminary design of the fluorescein dosimeter.

4.0 Conclusion and Recommendations

Fluorescence intensities decayed with increased radiation dose, roughly characterized by a 1st order decay model. The detection limit of the proposed 0.2 µM solution was 0.05 Sv, which would not suitable for a personal protective dosimeter, but may be applicable in higher dose applications (*e.g.*, food irradiation). It was noted that fluorescein degraded with prolonged exposure to sunlight.

It is recommended that future research be conducted in order to improve the detection limit of the fluorescein dosimeter, so that it may become feasible as a tissue proportional personnel protective dosimeter. It is recommended that a fluorometer with higher resolution and sensitivity be utilized in future experimentations. Additionally, effects of pH, temperature, ionic strength, and other possible fluorescence-enhancing additives should be investigated for fluorescence signal amplification. Moreover, further investigation of the effect of radiation particle, energy, and dose rate dependences should be determined. Also, degraded fluorescein should be investigated for possible fluorescence recovery.

5.0 References

[1] Wood, T. (2012, March). *Development of a Novel DNA Dosimeter*. Royal Military College of Canada, Kingston, Ontario.
[2] Hall, E. J., & Giaccia, A. J. (2006). Radiobiology for the radiologist (6th ed.). Philadelphia: Lippincott Williams & Wilkins.
[3] National Institute of Standards and Technology 2010 Composition of TISSUE, SOFT (ICRU) http://physics.nist.gov/cgi-bin/Star/compos.pl?matno=262 (accessed 10 February 2013)