An investigation into ultra-sensitive substituted leucomalachite dye derivatives for use in the PRESAGE® dosimeter

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Abstract. A comparison between a commercial leucomalachite green (LMG) dye and three newly synthesised derivatives incorporated into the PRESAGE® dosimeter was carried out to determine their effect on the sensitivity and post-response photofading of the dosimeter. For all of the substituted LMG derivatives (with either methoxy, chlorine or bromine substituents), the sensitivity of the resulting dosimeters to radiation dose increased significantly and was dependent on the type of LMG derivative used, with the bromine substituted derivative showing the highest sensitivity increase (450%) followed by chlorine and methoxy substituted derivatives (340%, 200%, respectively) relative to commercial LMG. All LMG dyes investigated showed similar post-response photofading characteristics except the methoxy-LMG derivative, which showed a slight improvement in post-response photo-retention.

1. Introduction
The PRESAGE® dosimeter is a unique 3D radiochromic dosimeter with great potential for clinical applications [1, 2]. Unlike polymer gel dosimeters [3], the PRESAGE® dosimeter is a clear solid polyurethane matrix containing leucomalachite green (LMG) dye as a reporter component and halogenated carbons as a radical source [4, 5]. Free radicals generated from the radiolysis of the halocarbon bond upon irradiation oxidize the LMG dye leading to a change in absorbance [6]. The change in absorbance is linear with respect to the absorbed radiation dose [5]. Some of the attractive features and advantages of the PRESAGE® dosimeter over gel dosimeters include its lack of sensitivity to oxygen and diffusion [5]. Furthermore, the radiological tissue-equivalent [7, 8] PRESAGE® dosimeter is solid, easily handleable, can be fashioned into any shape and requires no supporting container. As a part of a series of PRESAGE® dosimeter component-specific optimization studies [4, 9], the aim of this study was to investigate several new LMG dye derivatives. The LMG dye is one of the critical components of previous PRESAGE® dosimeter formulations, however, the commercial LMG dye currently suffers from a number of potential limitations mainly related to its sensitivity to radiation dose and post-response photofading. The new LMG derivatives were specifically developed to overcome these limitations.
2. Materials and Methods

2.1. PRESAGE® fabrication
The chemical agents used in this study were: Polyurethane resin (Crystal Clear 200, Smooth-On, Easton, PA USA), which are supplied in two parts (Part A and Part B); The radical initiator, tetrabromoethane, and commercial LMG dye (Sigma-Aldrich, St Louis, MO); The newly synthesised LMG derivatives, referred to as MeO-LMG, Cl-LMG and Br-LMG (Heuris Pharma, LLC (Skillman, NJ)). In order to directly compare between the LMG dye and its derivatives, the concentration of the radical initiator and dye were kept constant throughout and only the type of leuco dye was varied. The dosimeters were prepared in poly(methyl methacrylate) spectrophotometer cuvettes with a wall thickness of 1 mm and internal dimensions of 1 x 1 x 4.5 cm[^10]. Detailed description of the fabrication process is described elsewhere [4].

2.2. Absorbance change measurements
Absorbance change measurements were acquired using a dual-beam Perkin Elmer Lambda 25 UV-VIS spectrophotometer (Perkin Elmer, Waltham, MA, USA). The absorption spectrum over the visible wavelength region (470–750 nm) with 1 nm intervals was initially used to determine the absorption maxima of the PRESAGE® dosimeters with LMG and its derivatives. The absorption of each PRESAGE® cuvette was measured pre- and post-irradiation. To investigate the influence of the different leuco dyes used on post-response photofading, absorption acquisitions were conducted at different time intervals (1, 3, 6, 24, 48, 92, 120, and 168 hours post-irradiation). All PRESAGE® cuvettes were kept in a cold (ca. -18 °C) and dark environment pre- and post-irradiation to avoid accidental exposure to ultraviolet or visible light.

2.3. Irradiation
The PRESAGE® dosimeter cuvettes were irradiated using a 6 MV medical linear accelerator (Elekta Synergy, Crawley, UK), using a dose rate of 5 Gy/min and various radiation doses (0, 0.5, 1, 5, 10, 20 and 30 Gy). The field size was set to 10 x 10 cm. The cuvettes were sandwiched between two solid water phantoms with the top one measuring 1.5 cm in thickness. The FSD was set to 100 cm from the solid water surface.

3. Results and Discussion
The molecular structure of the commercial LMG dye and its derivatives employed in this study are shown in figure 1. Upon irradiation the color formation occurs when the methine proton (C-H) is cleaved leading to the formation of a colored cation. In the three new derivatives, one of the aryl protons of LMG was substituted with either methoxy (MeO), chlorine (Cl) or bromine (Br) groups (figure 1).

![Figure 1: Molecular structure of the leuco dyes investigated. R = the substituted part of the parent LMG.](image)

The absorption spectra of the LMG dye and its three derivatives after oxidisation are shown in figure 2. For all leuco dyes, the absorption maximum ($\lambda_{max}$) was found to peak at ca. 633 nm (red
region of the spectrum), which is a typical visible absorption $\lambda_{\text{max}}$ of the oxidized form of LMG (malachite green). This suggests that the new LMG derivatives could be used with current optical tomography imaging systems such as Vista™ (Modus Medical Devices Inc.) with optimum sensitivity.

Figure 2: Normalized absorption spectra of the PRESAGE® dosimeters with the four dyes used in this study showing absorption maxima at ca. 633 nm. Reference cuvettes (same composition but with zero radiation dose) for each formulation were used as a baseline to establish the zero value.

The measured absorbance changes at 633 nm wavelength versus the radiation absorbed doses for all of the dosimeters are displayed in figure 3. Absorption values were obtained by subtracting the relevant value of a reference cuvette for the same batch with zero radiation dose from that of the irradiated cuvettes. Consequently, the intercepts of the dose–response plots are nearly zero. In all cases a very good correlation coefficient linearity ($R^2 > 0.99$) for the dose response was observed for PRESAGE® compositions with all of the leuco dyes investigated over the applied radiation dose range.

Figure 3: Recorded absorbance changes as a function of absorbed radiation dose for the PRESAGE® dosimeter with LMG dye and its derivatives. Correlation coefficient parameters of each fitted line are shown in the bottom right inset. Error bars represent the standard deviation in the measurement and are smaller than most points. Photograph of the dosimeter cuvettes with LMG dye and its derivatives pre- and post- exposure to 30 Gy are shown in the top left inset.
It was found that the change in absorbance varied significantly between LMG and its derivatives (figure 3), with the dose–response curves for PRESAGE® with Br-LMG showing the highest sensitivity followed by those with Cl-LMG and MeO-LMG (450 %, 340 %, and 200 % increase, respectively) relative to the commercial LMG. The significant increase observed for halogen substituted LMG derivatives could be attributed to the fact that halogens (Cl, Br) are weak deactivators that would withdraw electron density and weaken the methine C-H bond making it easier for radicals to abstract the proton. These electronic effects would influence the dose sensitivity and post-response photofading. In addition, the 110% increase in sensitivity with Br-LMG than Cl-LMG could be attributed the lower C–Br bond dissociation energy (72.1 kcal.mol⁻¹) compared to C–Cl (83.7 kcal.mol⁻¹) [4, 11]. Thus, less energy is required to cause oxidation of Br-LMG than Cl-LMG. The 200 % increase in sensitivity observed with the MeO-LMG derivative is attributed to its methoxy substituent (OCH₃), which is known as a moderate activator whereby the lone pair of the oxygen would be expected to stabilize the colored cation intermediate formed after radical abstraction of the proton of the methine group.

The post-response photofading of the PRESAGE® dosimeters with different leuco dyes are shown in figure 4. The results demonstrate that, the LMG dye and its synthesised derivatives were fairly stable over a period of one week with MeO-LMG showing a slight improvement in the retention of the post-response absorption value over the period studied.

![Figure 4](image-url)

**Figure 4:** Variation in absorbance over time for the PRESAGE® dosimeters with the four dyes used in this study after exposure to 1 Gy. Error bars represent the standard deviation values in the measurement.

### 4. Conclusion

In this work, newly synthesised LMG derivatives were investigated for potential use in the composition of the commercial PRESAGE® dosimeter. In general, all new LMG derivatives showed significant improvement over the commercial version. In addition, they also had no influence on the typical characteristics of the PRESAGE® dosimeter. Therefore, where higher sensitivity is required, it is recommended that the commercial LMG used in the composition of the PRESAGE® dosimeter be replace by any of the newly synthesised derivatives, especially Br-LMG.
5. References

[1] Clift C et al 2010 Phys. Med. Biol. 55 1279-93
[2] Abdul Rahman AT et al 2011 Phys. Med. Biol. 56 4177-99
[3] Baldock C et al 2010 Polymer gel dosimetry Phys. Med. Biol. 55 R1-63
[4] Alqathami M et al 2012 Radiat. Phys. Chem. 81 867-73
[5] Adamovics J and Maryanski M J 2006 Radiat. Prot. Dosim. 120 107-12
[6] Guo P et al 2006 Med. Phys. 33 1338-45
[7] Brown S et al 2008 Appl. Radiat. Isot. 66 1970-4
[8] Gorjiara T et al 2011 Med. Phys. 38 2265-74
[9] Alqathami M et al 2012 Radiat. Phys. Chem. (in press)
[10] Rintoul L et al 2003 Appl. Spectrosc. 57 51-7
[11] Blanksby S J and Ellison G B 2003 Acc. Chem. Res. 36 255-63