Methylthymol blue in Fricke gels

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Abstract. The initial trial of methylthymol blue (MTB) as a chelator for ferric iron in Fricke gel dosimeters, used for three-dimensional (3D) dosimetry in cancer radiotherapy, is reported. MTB is a structural analogue of the conventionally used xylenol orange (XO); however, the absorbance spectrum of the ferric-MTB complex is shifted to higher wavelengths, which should allow for lower amount of light scattering during gel scanning. In this study, two gelatin substrates, two sources of XO and one source of MTB have been compared. The MTB-containing gels exhibited similar dose response and diffusion coefficient to the XO-containing gels at their wavelengths of maximum absorption (620 and 585 nm, respectively). In addition, the MTB gels gave an excellent dose response at 633 nm, which is an important wavelength that is already used with other 3D dosimeters.

1. Introduction
The principle of the ferrous sulfate – gelatin dosimeter (Fricke gel) is based on the ionizing radiation-induced oxidation of ferrous iron (Fe²⁺) to ferric iron (Fe³⁺) under acidic conditions in a gel material that retains the three-dimensional (3D) distribution of the delivered dose. Xylenol orange (XO) is a metal chelator that is typically added to Fricke gels to limit the diffusion and allow optical readout of the dose delivery [1, 2]. Other chelators have been tested to generally less favorable diffusion and dose response properties [2]. However, to our best knowledge, structural analogues of XO have never been tested in the Fricke gel dosimeter. We performed a structural search within the SciFinder® chemical database and found several potential compounds that could substitute XO in Fricke gels. Among them methylthymol blue (MTB) was found to be most promising: (1) the compound is very similar but larger than XO, which means that the diffusion of the Fe³⁺-MTB complex may be slower than the Fe³⁺-XO diffusion, (2) MTB is readily available as a commercial product, and (3) the maximum absorption Fe³⁺-MTB is shifted to the red at 620-625 nm, compared to 585-587 nm for Fe³⁺-XO [3]. Ultimately this means that the optical scattering in ferrous – MTB – gelatin (FMG) gels should be lower than that in ferrous – XO – gelatin (FXG) gels, and lower scattering means better image quality [4,5]. This report presents a comparison between XO and MTB as chelators in Fricke – gelatin gels.

2. Experimental
Two sources of gelatin were used in this study: 300 Bloom porcine skin gelatin (Gₚ) (Sigma-Aldrich, Canada, product no: G1890), and 300 Bloom bovine bone gelatin (Gₜ) (Rousselot International, Peabody, MA, USA), identical with the Eastman gelatin used in [5]. Xylenol orange as a disodium salt was supplied from Sigma-Aldrich (Fluka brand, product no: 52097), designated as Xₜ, and from TCI
America (Portland, OR, USA, product no: B0477), designated as Xₜ. Methylthymol blue, as a sodium salt was supplied for TCI America (product no: B0478), designated as Mₜ. Sulfuric acid (SA) and ferrous ammonium sulfate hexahydrate (FAS) were purchased from Caledon Labs (Georgetown, ON, Canada). All compounds were used without further purification in the preparation of Fricke gels in all possible combinations between gelatin substrate (Gₚ or Gₜ) and chelate (Xₛ, Xₜ or Mₜ) to the following common formulation (in mmol/L): SA:FAS:(XΟ or MTB) = 25:0.1:0.1 in 4% (w/v) gelatin. The gels were poured into polymethylmethacrylate cuvettes with 10 mm optical path length and left to solidify in at 10 °C overnight before irradiation on the next day.

Irradiations were performed on a Co-60 source (Eldorado 6, Atomic Energy of Canada Ltd.) at the London Regional Cancer Program (London, Canada). Cuvettes of each composition were exposed to 0, 5.0 and 20.0 Gy, and, semi-shielded, to 10.0 Gy for diffusion measurements. Optical scans were performed on a microplate reader (Infinite 200 Pro, Tecan Group Ltd., Switzerland). The semi-irradiated cuvettes were scanned at twelve points along the long axis of the cuvettes at three different times, starting one hour after irradiation, as previously described [6]. The diffusion profiles were fitted to the complementary error function to determine their ‘curvature parameters’ (n), as in [7]. The diffusion coefficients (D) were determined from the slope of n against time (t).

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D = \frac{1}{\sqrt{4 \pi t}} \frac{dn}{dt} \approx 0.212 \frac{dn}{dt} \tag{1}
\]

3. Results and discussion

Figure 1 depicts the typical behavior of the gels in porcine gelatin. As seen, the initial absorbance in the green part of the spectrum for FMG gels is much lower than that of FXG gels; however, after irradiation, the signal is stronger and red-shifted, forming the saturated green color seen on the right.

Figure 2 presents the background absorbance, dose sensitivity, and the non-linearity of the dose response over a wide range of wavelengths (550 to 650 nm). In both porcine and bovine gelatin, the gels containing XO sourced from TCI America show the lowest sensitivity and linearity; nevertheless, even with these gels the error of linearity is less than 1% around the Fe³⁺-XO absorbance maximum (585 nm). The Sigma-Aldrich – sourced XO could be used at a wide range of wavelengths but the sensitivity of the dosimeter drops fast at wavelengths over 585 nm. By contrast, the FMG gels show both high sensitivity and good linearity on both sides of the absorbance maximum at 620 nm, and could potentially be used at 633 nm – a wavelength that can be generated by He-Ne lasers and is already used for scanning of 3D dosimeters (viz. PRESAGE°).
Figure 2. Background absorbance, dose sensitivity, and non-linearity of dose response, expressed as the complement of the correlation coefficient to unity (1-\(R^2\)).

Figure 3 (left) presents the diffusion profiles for the FMG gels, at three times with \(t = 0\) at one hour after irradiation. As expected, the diffusion slopes become shallower with time; hence, the curvature parameters (\(n\)) increase. The linear dependences between \(n\) and time (figure 3, left) give the diffusion coefficients, according to equation (1).

Table 1 gives a snapshot of the data from figure 2 together with the estimated diffusion coefficients from figure 3. The diffusion properties of dosimeters do not show a consistent behavior between the porcine and the bovine gels, and since the experiments were not conducted in multiples definitive conclusions are hard to make. However, the average diffusion coefficients of the porcine and the bovine gels (0.573±0.069 and 0.341±0.011 mm\(^2\)h\(^{-1}\), respectively) are significantly different (\(p = 0.005\)). In a previous study we found the diffusion coefficient of an FXSGP gel with 25 mM SA to be 0.476±0.011 mm\(^2\)h\(^{-1}\) [6]. This value is not statistically different from the average porcine gel diffusion from this report (\(p = 0.094\)). Based on these results, we can conclude that the diffusion in FMG gels is not lower than that in FXG gels (as hoped), but it is of equal magnitude or slightly higher.
Figure 3. Diffusion profiles in the FMG gels (left) and linear fitting of the curvature parameter ($n$) against time for all tested compositions (right).

Table 1. Properties of the dosimeters are characteristic wavelengths.

| Gel code | Gelatin type | Chelator | Wavelength (nm) | Bckgr. abs. $\times 10^3$ | Abs. change $\times 10^3$ (Gy$^{-1}$) | $1 - R^2$ | Diffusion coeff. (mm$^2$h$^{-1}$) |
|----------|--------------|----------|-----------------|--------------------------|-------------------------------------|-----------|-------------------------------|
| FX$_2$G$_P$ | porcine | XO$_{Sigma}$ | 585 | 218 | 16.1 | 5$\times$10$^{-6}$ | 0.497 |
| FX$_2$G$_P$ | porcine | XO$_{TCI}$ | 585 | 215 | 13.3 | 0.003 | 0.589 |
| FM$_2$G$_P$ | porcine | MTB$_{TCI}$ | 620 | 221 | 17.5 | 7$\times$10$^{-6}$ | 0.632 |
| FM$_2$G$_P$ | porcine | MTB$_{TCI}$ | 633 | 196 | 15.7 | 1$\times$10$^{-4}$ | n.d. |
| FX$_2$G$_B$ | bovine | XO$_{Sigma}$ | 585 | 373 | 10.6 | 0.001 | 0.351 |
| FX$_2$G$_B$ | bovine | XO$_{TCI}$ | 585 | 322 | 9.1 | 0.002 | 0.329 |
| FM$_2$G$_B$ | bovine | MTB$_{TCI}$ | 620 | 354 | 12.5 | 5$\times$10$^{-5}$ | 0.341 |
| FM$_2$G$_B$ | bovine | MTB$_{TCI}$ | 633 | 323 | 11.7 | 2$\times$10$^{-4}$ | n.d. |

4. Conclusions
The substitution of xylene orange with methylthymol blue in Fricke-gelatin gels produced dosimeters with comparable properties, and an absorbance maximum shifted to the red. Due to that, the new dosimeters are expected to cause less optical scatter in optical computed tomography systems and may be used with existing light sources, set at 633 nm for the scanning of other 3D dosimeters. In addition, there was a noticeable difference in the behaviors of dosimeters prepared with porcine skin gelatin and the bovine bone gelatin. It is possible that the gelatin substrates are hydrolyzed to different extents by the presence of sulfuric acid, which may account for their different properties. We plan on examining those effects in the future. Further research on the ferrous–methylthymol blue–gelatin gel will focus on the stability and reusability of the dosimeter, the effects of the sulfuric acid concentration, and presence of ferrous iron stabilizing dopants.

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6. References
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