New directions for tetrazolium - gellan gum gel dosimeters

Kalin I. Penev¹,² and Kibret Mequanint¹,³
¹Department of Chemical and Biochemical Engineering, the University of Western Ontario, London, ON, N5A 5B9, Canada
²Modus Medical Devices Inc., 1570 N Routledge Park, London, ON, N6H 5L6, Canada
³Biomedical Engineering Program, the University of Western Ontario, London, Ontario, N6A 5B9, Canada
E-mail: kmequani@uwo.ca

Abstract. Tetrazolium salt-based dosimeters are non-diffusing, gel dosimeters with excellent physical and chemical stability but with relatively low dose sensitivity and a dose rate dependence of the dose response. Both issues are tackled in this study by: (a) the introduction of a new tetrazolium salt with simple synthesis and dose response around 585 nm which may provide the pathway to more sensitive formulations and (b) producing gels with low gellan gum concentration, thus limiting the dose rate effects, and addition of thickening agents to restrain the liquid loss (syneresis) from the resulting gels.

1. Introduction
Tetrazolium salts (TS) are attractive as radiochromic sensor compounds owing to their low background coloration and diffusion-free gel dosimetry, as in gellan gum gel with “bisnitrotetrazolium chloride” (BNC) [1,2]. A version of this composition with 0.25 mM BNC has been commercialized as ClearView 3D Dosimeter by Modus QA (London, Canada) and has found application for the dosimetry of high-dose stereotactic treatments, e.g. [3]. More recently, we tested a series of tetrazolium salts containing strong chromophores [4]; however, as research in chemical dosimeters shows, hundreds of related compounds may need to be tested before the most favorable radiochromic chemistry is found [5]. With that in mind, we performed quantum chemical modelling of a series of candidate compounds [6], which revealed that changes at the group bound to the carbon atom of the tetrazolium ring have minimal effect on the electrostatic potential (and therefore, sensitivity) of the tetrazolium salt, but may either facilitate or hinder its synthesis and can shift the spectrum of the resulting formazan. Electron-donating groups, such as 4-methoxyphenyl, may be particularly useful, as they would allow for easy synthesis and a bathochromic spectral shift in absorbance (to higher wavelengths). In the current study we have compared that previously used BNC with its 5-(4-methoxyphenyl) analogue (Figure 1). We have also recently demonstrated that the sensitivity of BNC-gellan gum gels can be increased by addition of methylparaben to act as a radical scavenger, while the total solids loading can by cross-linking with lithium salts without compromising with optical clarity [7]. The resulting gels, while mechanically strong, show a tendency to release liquid (syneresis) when subjected to external force. Here, we test whether the addition of thickening agents can alleviate the syneresis without affecting the dose rate dependence or any other properties of the dosimeter. In short, the goal of this study is to present the preliminary investigation a new and promising tetrazolium salt.
and approaches to limit the dose rate sensitivity by decreasing the gellan gum concentration and to minimize syneresis by introduction of a thickening agent.

**Figure 1.** Tested compounds: 2,3-bis(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (BNC) and 5-(4-methoxyphenyl)-2,3-bis(4-nitrophenyl)-2H-tetrazolium chloride (MBC)

### 2. Materials and Methods

MBC was synthesized according to a previously published procedure using 4-methoxybezldehyde, 4-nitrophenylhydrazine and 4-nitroaniline according to [8], whereas all other materials were purchased from commercial suppliers and used without further modification: BNC as “2,3-Bis(4-nitrophenyl)-5-phenyltetrazolium chloride hydrate” (cat. no. B1047) from TCI America, gellan gum (cat. no. J63423) and methylparaben (cat. no. A14289) from Alfa Aesar, propylene glycol (cat. no. P355) from Fisher Scientific, and polyacrylamide (150 kDa, cat. no. 767379) from Millipore Sigma. The tested compositions are given in Table 1. Gel preparation, cuvette specification, storage conditions, optical readout and irradiation setup were as described previously [1].

|              | Gellan gum, wt.% | Propylene glycol, wt.% | Methylparaben, wt.% | Polyacrylamide, wt.% | [LiCl], mM | TS, [TS], mM |
|--------------|-----------------|------------------------|---------------------|----------------------|-----------|-------------|
| A1           | 1.25            | 10                     | 0                   | 0                    | 0         | BNC 0.25    |
| A2           | 1.00            | 10                     | 0.25                | 0                    | 0         | BNC 0.25    |
| A3           | 0.75            | 10                     | 0.25                | 0                    | 50        | BNC 0.25    |
| A4           | 0.75            | 10                     | 0.25                | 0.25                 | 50        | MBC 0.25    |
| B1           | 1.25            | 10                     | 0                   | 0                    | 0         | BNC 0.25    |
| B2           | 1.00            | 10                     | 0.25                | 0                    | 25        | MBC 0.25    |
| B3           | 0.75            | 10                     | 0.25                | 0                    | 50        | MBC 0.25    |
| B4           | 0.75            | 10                     | 0.25                | 0.25                 | 50        | MBC 0.25    |

As a predictor for syneresis, the liquid expulsion during centrifugation was measured under using Amicon Ultra-4 centrifugal filter units (10 kDa pore size, 3.0 cm² filtration area, cat no UFC8010) from Millipore Sigma filled with ca. 1.6 g of tested gel and centrifuged at 2000×g. For statistical purposes, the effect of the TS type was ignored in the study of syneresis: two samples from each A and one from each B composition were tested, providing sets of triplicates.

### 3. Results and Discussion

Figure 2 shows the background optical density, aging at room temperature, and dose response of compositions A1 and B1. The wavelength of maximum absorbance shifts from ca. 530 to ca. 585 nm, allowing the gels to be scanned with amber light and decreasing the optical background and scatter within the gel matrix. The rate of auto-reduction was independent of the composition and varied among samples at (5.1±1.2)×10⁻⁴ cm⁻¹ day⁻¹ in units of optical density at the respective wavelengths of
interest. Whereas the sensitivity of MBC gels appears lower, it should be noted that the material can be prepared readily and in high yields unlike BNC, which means that MBC may be used at an increased concentration in order to obtain more sensitive gels at little added cost. Figure 3 shows that decreasing the gellan gum concentration leads to lower dose rate dependence, while the presence of polyacrylamide has no apparent effect on the sensitivity.

Figure 2. Comparison of BNC and MBC dosimeters (A1 and B1): optical density and auto-reduction in panel (a) and dose response in panel (b). Irradiations on a Cobalt-60 source at ca. 100 cGy/min.

Figure 3. Dose rate effect of the dose response. Irradiation was performed using 6 MV x-ray to cuvettes positioned 5.0 cm under water, source-to-surface distance was 95.0 cm to the water surface.

Figure 4 supports the potential utility of compositions A4 and B4, as the addition of polyacrylamide improved the resistance of the gel to syneresis, as modelled according to the equation:

\[ w_{\text{loss}} = \frac{w_{\text{eq}}}{\tau + t_{c}} \]  

Where, \( w_{\text{loss}} \) is the mass loss, \( w_{\text{eq}} \) is the equilibrium mass loss, \( \tau \) is a characteristic time period, \( t_{c} \) is the cumulative centrifugation time, and \( w_{\text{eq}}/\tau \) is the initial rate of mass loss. The results are presented in
Figure 4 and Table 2. Comparing gels A3/B3 and A4/B4 shows that in the absence of polyacrylamide the gels cannot reach equilibrium water content at 0.75% gellan gum concentration. More importantly, the initial rate of mass loss is drastically decreased between the two compositions, indicating resistance to syneresis in composition A4/B4. It should be noted that in addition to polyacrylamide other thickening agents were tested with the goal of decreasing the rate of syneresis. For example, carboxymethylcellulose and Pluronic F-127 showed promising results but both were ultimately rejected due to decreased optical quality of the gel in the first case, and reaction with the tetrazolium salts in the second.

Table 2. Modelling results.

|    | $w_{eq}$, % | $w_{eq}/\tau$, %/min | $R^2$ |
|----|-------------|------------------------|-------|
| A1/B1 | 63±8 | 2.5±0.4 | 0.996 |
| A2/B2 | 79±5 | 4.2±0.1 | 0.998 |
| A3/B3 | 117±5 | 4.3±0.1 | 1.00 |
| A4/B4 | 92±5 | 2.9±0.0 | 1.00 |

Figure 4. Results from the syneresis study.

4. Conclusions
The use of polyacrylamide as a thickening agent in gellan gum gels presents a promising perspective for decreasing the total solids loading in the dosimeters, and indirectly lowering the dose rate effects. Further, the while MBC showed lower sensitivity than BNC, the bathochromic shift in absorbance and ease of preparation make this compound attractive for further studies.

Based on the promising results from the cuvette experiments, larger scale, complex dose-delivery plans will be tested using gellan gum – polyacrylamide gels at low total solids loading to prove the promising dose-rate effects, with both tetrazolium salts presented in this study.

5. Acknowledgements
We would like to acknowledge the help of Mr. Khalid Noori in performing experimental tests and data analysis for this work. Funding for this research was provided by the Collaborative Health Research Projects (CHRP).

6. References
[1] Penev K I, Wang M, Mequanint K 2017 J. Phys. Conf. Ser. 847 012048
[2] Ascención Y, Dietrich J, Mequanint K et al 2017 J. Phys. Conf. Ser. 847 012049
[3] Du D, Quinn B, Penev K et al 2019 Med. Phys. 46 E310
[4] Hazarika R, Penev K I, Mequanint K et al 2019 J. Phys. Conf. Ser. 1305 012036
[5] Adamovics J A, Coakley R J 2019 J. Phys. Conf. Ser. 1305 012028
[6] Penev K I, Bedada T and Mequanint K 2020 Chem. Phys. 535 110790
[7] Brzozowski P, Penev K I, Mequanint K 2021 Int. J. Biol. Macromol. (under review)
[8] Penev K I, Mequanint K 2015 J. Het. Chem. 53 1655