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Tetrazolium salt monomers for gel dosimetry I: Principles

Kalin I Penev¹,², Meng Wang¹ and Kibret Mequanint¹,³

¹Department of Chemical and Biochemical Engineering, 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 salts (TS) have been previously used for radiochromic dosimetry in solutions, films and three-dimensional (3D) gelatine-based gels. However, widespread application for 3D dosimetry has not been achieved due to the required high concentrations and associated high costs of the TS dimer used in prior research. Through careful selection of TS monomer, sensitivity-enhancing additives and inert gel forming material, we report the preparation of a non-diffusing, chemically stable, 3D dosimeter with linear sensitivity between 0 and 80 Gy with submillimolar requirements for the active TS.

1. Introduction
The use of tetrazolium salts (TS) in liquid and film dosimetry of ionizing radiation is well-established [1, 2]; whereas, three-dimensional (3D) dosimetry has been reported in a single PhD thesis [3] and the resulting patent [4]. The underlying chemistry in all TS dosimeters is the radiochromic reduction of a colourless TS into an intensely coloured formazan dye. To increase the radiation chemical yield of the reaction, addition of a hydroxyl radical quencher is necessary. Previously, various radical quenchers such as alcohols, sodium formate and Triton X-100 have been used [1, 4]; where Triton X-100 may also affect the sensitivity by micelle formation [4, 5]. TS film and gel dosimeters showed: (i) no apparent signal diffusion as the formazan dye is insoluble in water and remains trapped within the film or gel matrix, (ii) good chemical stability, and (iii) limited or no inhibitory effect of the dissolved oxygen [1-4]. However, widespread use TS for 3D dosimetry has not been established, due to the perceived low sensitivity and high relative cost of TS [6]. Particularly, the cost of the compound of choice in recent reports [2-4] – a tetrazolium dimer, known as nitrotetrazolium blue chloride (NBT) – is prohibitive at the required concentrations of up to 4 g/L (5 mM). By contrast, the original research on TS dosimeters in solution [1] used less expensive tetrazolium monomers. Here we report the first use of TS monomers for gel dosimetry. Figure 1 presents the chosen compounds: nitrotetrazolium chloride (NTC) (1) and bisnitrotetrazolium chloride (BNC) (2). NTC has been used in solution and film dosimetry [1]; it is not commercially available, but can be prepared relatively easily [7]. BNC has not been tested previously, and is more sensitive to reducing agents than NTC [8]. As gel-forming material, both gelatin and gellan gum were tested. Gellan gum is a microbially produced polysaccharide which forms clear hydrogels with high mechanical strength and thermal stability [9]. As hydroxyl radical quenchers, we used sodium formate and propylene glycol. Monovalent alcohols were excluded due to their high volatility and Triton X-100 may be investigated in future research. The effects of the concentrations (C) of the chosen TS
and additives on the dose sensitivity ($S$) were fitted to a first-order saturation equation (1), under the assumption that the $S$ reaches a maximum:

$$S = S_0 + S_{\text{max}} \frac{C}{K_{1/2} + C}$$

(1)

Where $S_0$ is the sensitivity of the in the absence of additives ($S_0 = 0$ at $C_{TS} = 0$), $S_{\text{max}}$ is the maximum increase of sensitivity and $K_{1/2}$ is the half-saturation constant, at which $(S - S_0) = 0.5S_{\text{max}}$.

![Figure 1](image)

**Figure 1.** Evaluated tetrazolium salts: 1 = 2-(4-nitrophenyl)-3,5-diphenyl-2H-tetrazolium chloride = nitrotetrazolium chloride (NTC), 2 = 2,3-bis(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride = bisnitrotetrazolium chloride (BNC).

Here (part I), we present the initial characterization of NTC- and BNC-gel dosimeters in cuvettes, using gelatine and gellan gum, while part II of the paper discusses the characterization of a specific BNC-gellan gum composition, studied in clinically relevant volumes.

2. Materials and Methods

Gels were prepared using the following materials: NTC (synthesized in-house [7]), BNC (TCI America, USA, cat. no: B1047), propylene glycol (PG) (Caledon Laboratories, Canada, cat. no: 6840), sodium formate (SF) (Caledon, cat. no: 7840), gelatin (Gl) (Sigma-Aldrich, cat. no: G1890), and gellan gum (Gn) (Alfa-Aesar, cat. no: J63423, lot no: C01Z014, gel strength: 434 g/cm²) to final concentrations of 0.05 – 2.50 mM NTC or BNC, 0 – 20 % (v/v) PG, 0 – 200 mM SF, and 5% (w/v) Gl or 1.25% (w/v) Gn. Samples were poured into 4 mL poly(methyl methacrylate) cuvettes, closed with polyethylene caps, and placed overnight in a refrigerator. Cuvettes were irradiated the following day on a Co-60 source (at 100 cGy/min) at 21 – 23 °C, in triplicates for the initial testing, and between 0 and 80 Gy in subsequent tests. Absorbance measurements were performed at 22 °C on a Cary 60 spectrophotometer (Agilent). To create dose-gradients, cuvettes were semi-shielded by a lead block, irradiated to 20 Gy, and scanned on a microplate reader (Infinite 200 Pro, Tecan). The dose gradient data was fitted to an analytical approximation of the complementary error function, erfc(x) [10]. All optical measurements are reported as the linear attenuation coefficient, $\mu$ (cm⁻¹). Individual compositions are designated as #1TS/#2XX/#3Gx meaning: #1 mM of the respective TS, #2 mM or % (v/v) of the additive, and #3 % (w/v) of the gel-forming agent.

3. Results and Discussion

Figures 2 and 3 show the effects on concentration of the TS and two additives on the dose sensitivity of NTC and BNC dosimeters in gelatin and gellan gum, respectively, at the wavelengths of maximum attenuation. BNC-containing gels showed higher sensitivity to dose, even at lower concentrations of the tetrazolium salt, as $K_{1/2}$ for BNC is significantly lower than $K_{1/2}$ for NTC (Figure 2A). Addition of radical quencher led to a two- to three-fold increase of sensitivity (Figure 2B). Sodium formate showed a stronger positive effect on the sensitivity than propylene glycol. However, sodium formate could not be tested in gellan gum at high concentrations due to strong cross-linking by the sodium ion (Na⁺) resulting in formation of translucent gels.
Figure 2. Dose sensitivity of NTC (494 nm) and BNC (535 nm) in 5% (w/v) of gelatin as a function of: (A) tetrazolium salt concentration ([TS]), and (B) radical scavenging additive concentrations at a constant [TS]. Data is fitted to Equation 1.

As shown in Figure 3, in the absence of additives the dose sensitivity in gellan gum was linear in relation to the TS concentration, indicating very high values for $K_{1/2}$. However, multi-fold increase of sensitivity was seen in the presence of 10% (v/v) propylene glycol. Additionally, the background attenuation of all NTC-gelatin gels was stable for at least 24 h (data not shown). BNC-gelatin gels, however, became darker at rates as high as $6.0 \times 10^{-3} \text{cm}^2\text{h}^{-1}$ for 1.0BNC/20PG/5Gl. By contrast, the BNC-gellan gum gels showed excellent stability (Figure 4).

Figure 3. Dose sensitivity of NTC (at 494 nm) and BNC (at 535 nm) in 1.25% (w/v) gellan gum gel in: water (solid symbols) or 10% (v/v) PG (open symbols). Data is fitted to Equation 1.

BNC-gellan gum compositions were chosen for the remainder of the study. As seen in Figure 4, the 0.1BNC/10PG/1.25Gn composition showed stable background attenuation and dose response with no
diffusion observed for at least 36 hours at room temperature. However, at 1.0 mM BNC some increase of attenuation was observed over the course of the 36 hours, but the diffusion remained undetectable. Excluding the spontaneous darkening at 1.0 mM BNC, the signal development for both compositions took less than 30 minutes for doses up to 80 Gy (data not shown), and the observed colour changes were analogous – from colourless to purple. Figure 5 gives the attenuation spectra at 0.1 mM BNC, and the dose response for both compositions between 0 and 80 Gy. Maximum dose response was observed at 535 nm. The achieved dose sensitivity (~4×10⁻³ cm⁻¹Gy⁻¹) is on par with previously reported minimally-diffusing gel dosimeters [5]; however, our gels exhibit no apparent diffusion and have very high chemical stability.

![Figure 5. A, B: attenuation spectra and change of attenuation at doses from 0 to 80 Gy in 0.1BNC/10PG/1.25Gn; C: linearity of the dose response for two compositions at λ_max = 535 nm.](image)

4. Conclusions
We have successfully demonstrated the use of tetrazolium salts for preparation of 3D, non-diffusing, radiochromic gel dosimeters, with linear dose response in the range of 0 to 80 Gy, sensitivity on par with other minimally diffusing gel dosimeters, and greatly enhanced chemical stability. In part II, we discuss the use of one such composition in bulk-volume samples and clinically-relevant settings. Our future research includes the synthesis and testing of novel tetrazolium salts for enhanced sensitivity of the non-diffusing gel dosimeters in either gelatin or gellan gum.

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6. References