Research Article

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High Borate Networks as a Platform to Modulate Temporal Release of Therapeutic Metal Ions Gallium and Strontium

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Abstract: The effect of increasing substitutions of Ga$_2$O$_3$:Na$_2$O on the structure and contingent properties, of six quaternary high borate glasses was evaluated. Component ion release and particularly gallium ion release was studied post extraction, under simulated physiological conditions. Increasing substitutions of Ga$_2$O$_3$:Na$_2$O (i.e. 0:1 – 6:4) resulted in destabilization of the glass network, observed by increases in component ion release and half-life of release. However, at > 6:4 Ga$_2$O$_3$:Na$_2$O ratio, network stabilization appeared to occur, resulting in a decrease in ion release half-life and total ion release for B, Sr, and Ga at 720 h of extraction. A linear release profile for strontium was provided by each glass composition, and for gallium by composition GB202 (70B$_2$O$_3$:20SrO:6Na$_2$O:4Ga$_2$O$_3$) and GB203 (70B$_2$O$_3$:20SrO:4Na$_2$O:6Ga$_2$O$_3$) for up to 720 h. $^{11}$B MAS NMR reveals that the replacement of Na$_2$O with Ga$_2$O$_3$ (in the studied composition range) causes a linear increase of three-fold coordinated B[3] groups at the expense of B[4] groups. The data indicates the potential formation of GaO$_4$-tetrahedra, associated with network stabilization.

1 Introduction

Applications for bioactive glasses have, to a great extent, focused conventionally on the replacement, repair, and regeneration of hard tissues (i.e. bone and teeth) in orthopedic and oral/maxillofacial surgeries [1–3]. However, in addition to these applications, recent research has shown the considerable potential of glass-based biomaterials in soft tissue indications that include, but are not limited to, wound healing, peripheral nerve repair, laryngeal repair, and the treatment of hypervascular tumors (i.e. tumor embolization) [2–5]. Interestingly, and despite the exceptionally large number of non-crystalline solids (NCS) that can be synthesized from the useful elements on the periodic table of elements (estimates are upwards of 1.3 x 10$^{52}$ potential NCS), the compositional palette of bioactive glasses has largely focused on the silicate-based 45S5® glass, as a platform, for the continued development of new degradable bioactive glass systems [6–8].

However, more recently boron based-glasses, particularly those networks where B is the sole former, are increasingly investigated for medical applications [6–9]. Contrary to historical concerns regarding borate glasses’ low chemical durability, several compositions have recently displayed excellent potential in hard tissue and soft tissue augmentation/repair, specifically, enabling accelerated bone remodeling and healing of chronic wounds (e.g. diabetic ulcers) [1, 7, 9–12]. An interesting characteristic of vitreous boron oxides is its complete degradation. This, amongst other glass properties (e.g. glass transition temperature ($T_g$)) may be modulated with exacting control over composition [13, 14]. Composition-property changes in borate glasses are largely attributable to a concentration dependent conversion of B[3] to B[4] structural units as a result of the addition of modifying cations to the network. This response is ultimately related to the modifiers charge and its concomitant field strength [15–17]. Interestingly, a valuable feature of borate networks is that depending on the type and R-values (R being defined as the ratio of M$_2$O/B$_2$O$_3$ or MO/B$_2$O$_3$), cations, M, may shift between a charge compensation role and a modifying role within the glass network [17, 18]. This concentration dependent modulation of glass properties is particularly useful as (i) many modifying cations have therapeutic potential, e.g. strontium (Sr) [12] and gallium (Ga) [19], and (ii) their inclusion in borate networks may orchestrate their subsequent controlled release under physiological conditions.
whilst concurrently augmenting the degradation characteristics of the network as a whole. These features of borate networks may enable “…an emerging philosophy in tissue engineering is that rather than attempting to recreate the complexity of living tissues ex vivo, we should aim to develop synthetic materials that establish key interactions with cells in ways that unlock the body’s innate powers of organization and self-repair…”[20]: the controlled release of therapeutic metal ions (TMIs) in this regard is a promising strategy to enable this philosophy [3, 21].

With this data and philosophy as a back drop, the investigation of borate networks as functional delivery matrices for TMIs may offer new perspectives on the design and utility of borate glasses for medical applications [22–25]. In this context, the incorporation of Ga₂O₃ into different glass forming systems, including B₂O₃ has been investigated [26–28]. In a composition-dependent manner (i.e. in the presence of sufficient charge compensation) Ga₂O₃ has been shown to behave as a network former (NF), increasing the rigidity of the glass network and in some instances, decreasing glass degradation and component ion release [26, 29]. Ga is an interesting candidate for inclusion in a borate-based bioactive glass. It has been investigated as a therapeutic agent for the treatment of a number of disorders including: pathologic bone resorption, autoimmune disease, allograft rejection, infectious disease, Non-Hodgkin’s lymphoma, bladder cancer, and hypercalcemia [30]. For example, with respect to the latter, gallium nitrate is approved by the U.S. food and drug administration (FDA) for the treatment of malignancy associated hypercalcemia [31–33]. In addition, due to its antimicrobial potential, which has been associated with inhibition of a number of bacteria in vitro, including methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa, Ga has been incorporated into silica and phosphate based bioactive glasses as an infection control additive [19, 27, 28, 30, 31].

The broad-spectrum therapeutic capabilities of Ga are primarily attributed to interactions with Fe and in particular Fe proteins [32, 34]. These interactions are possible as Ga³⁺ shares certain physiochemical properties with Fe³⁺, which allows Ga to interfere with (i) Fe cellular uptake, (ii) Fe requiring enzymes (e.g. ribonuclease reductase and superoxide dismutase), and (iii) Fe signaling [30, 32]. It is these similarities that also allow Ga to bind, with high avidity to transferrin (TF); a glycoprotein that binds and keeps Fe/Ga nonreactive in circulation, delivering to cells bearing specific transferrin receptors (i.e. TFR1) [31, 35]. It is this TFR mediated pathway that appears to be the primary mechanism of Ga uptake by mammalian cells. The ability of Ga to perturb cellular Fe homeostasis, is particularly relevant as a considerable amount of data show that certain malignant cells (e.g. breast cancer) have a greater requirement for Fe compared to normal cells and that proteins involved in Fe import, export, and storage can be altered within these cancerous cells [36, 37]. In addition, disturbing Fe uptake and metabolism in pathogenic and opportunistic bacteria cells is a promising target for antibacterial strategies, particularly in the context of antibiotic resistance [38, 39].

Existing knowledge regarding the composition-structure-property relationships of binary alkali/alkaline-borate networks provides a framework for the research community to examine questions relating to the potential use of borate glasses for the controlled delivery of TMIs. With respect to the inclusion and subsequent release of Ga as a therapeutic ion from borate glasses, this paper will establish the effect of Ga inclusions on (i) the relative population of B[3] and B[4] structural units within a borate network, and (ii) the associated effects of structural changes on glass properties, including degradation and TMI release kinetics. Establishing these fundamental data will further allow researchers to investigate, determine, tailor, and subsequently utilize optimum therapeutic release profiles for specific indications.

2 Materials and Methods

2.1 Glass Synthesis

Six quaternary glass compositions (Table 1) were synthesized and prepared by weighing desired amounts of the analytical grade reagents, boron oxide, strontium carbonate, sodium carbonate and gallium (III) oxide (Sigma Aldrich, Canada). Individual glass formulations were mixed separately for 60mins to ensure homogeneity. Each homogenized glass precursor blend was placed and packed in 50ml Pt crucibles (Johnson Matthey, Noble Metals, Pennsylvania). The pack crucible was then placed in a furnace at room temperature. The furnace was heated (25°C /minute) to an initial dwelling temperature of 600°C and held for 60 minutes. The temperature was then ramped (25°C /minute) to a final dwelling temperature of 1,100°C and held for 75 minutes. On removal, each glass melt was rapidly quenched between two stainless steel plates. The resulting quenched glasses were ground separately within a planetary micro mill (Pulverisette 7, Fritsch, Germany) and sieved with ASTM E-11 compliant sieves (Cole Parmer, U.S.A) to obtain particles of <45 and 45 – 150 µm. Glasses were stored under vacuum in glass scintillation vials.
Table 1: Glass composition.

<table>
<thead>
<tr>
<th>Glass designation</th>
<th>B₂O₃</th>
<th>SrO</th>
<th>Ga₂O₃</th>
<th>Na₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td>GB200</td>
<td>0.70</td>
<td>0.20</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>GB201</td>
<td>0.70</td>
<td>0.20</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>GB202</td>
<td>0.70</td>
<td>0.20</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>GB203</td>
<td>0.70</td>
<td>0.20</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>GB204</td>
<td>0.70</td>
<td>0.20</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>GB205</td>
<td>0.70</td>
<td>0.20</td>
<td>0.10</td>
<td>0.00</td>
</tr>
</tbody>
</table>

2.2 Characterization of Glass Powders

2.2.1 Particle Size Analysis (PSA)

The particle size distribution of each glass was determined by using (as per manufacturers’ instructions) a Malvern mastersizer (MS) 3000 laser diffraction particle size analyzer. Deionized water was used to make glass powders suspensions. An obscuration value of between 5-8 % for each suspension was obtained. A blue (\(\lambda = 470\) nm) and red (\(\lambda = 632.8\) nm) laser were used to measure each glass suspension (n=5). Particle size distribution data is reported as the mean diameter \(D_{x90}\), \(D_{x50}\) and \(D_{x10}\), which represents particle diameters at 90%, 50% and 10% cumulative size, respectively.

2.2.2 X-Ray Diffraction (XRD)

XRD measurements were obtained with a Bruker D-8 Discover diffractometer, which was equipped with a Vantec-500 area detector and a Cu target X-ray tube. Powder specimens of each glass composition (<45 \(\mu\)m), were pressed into a square hollow steel wafer and scanned between \(10^\circ \leq 2\theta \leq 95^\circ\) with a step size \(2\theta = 0.02\).

2.2.3 Differential scanning calorimetry (DSC)

A simultaneous thermal analysis - STA 409 PC Luxx® (Netzsch-Geratetbau-GMBH, Germany). Glass specimens (n=3 per glass, <45 \(\mu\)m) was used to perform DSC. Each glass powder specimen was weighed to have a mass no >35 mg into a platinum crucible. Prepared specimens were heated at 10 \(^\circ\)C/min from 50 to 1000\(^\circ\)C. The glass transition temperature \(T_\text{g}\) was determined based on the inflection point of the heat flow curve, which was done using Proteus Analysis software (VERSION 5.1.1). Results are reported as the average ± standard deviation (SD).

2.2.4 Helium Pycnometry

Density measurements were retrieved with an AccuPyc 1340 helium pycnometer (Micromeritics, USA) equipped with a 1 cm³ sample insert chamber, which was calibrated and used as per manufacturer instructions. Between 0.7 – 0.8 g specimens of each glass composition (n = 10 per glass, 45-150 \(\mu\)m) was used. The results are reported as the average ± SD. The molar volume of each glass composition was calculated by \(V = M/p\) (M = molecular weight of each individual glass and p = density of the glass).

2.3 Structural Analysis using \(^{11}\)B MAS NMR.

The \(^{11}\)B magic angle spinning (MAS) NMR spectra were acquired on a Bruker Avance NMR spectrometer with a 16.4 T magnet (224.67 MHz \(^{11}\)B Larmor frequency) using a probe head for rotors of 2.5 mm diameter. The samples were spun at 10 and 25 kHz to identify center bands and spinning sidebands. The NaBH₄ resonance served as secondary chemical shift standard at \(-42.1\) ppm relative to BF₃ Et₂O. For the \(^{11}\)B NMR spectra up to 32 scans were accumulated, using a 0.56 s pulse (corresponding to a 15-degree pulse angle in the cubic environment of NaBH₄). The small pulse angle allows the quantitative comparison of sites with different quadrupole couplings. Spin lattice relaxation times, \(T_1\), were determined by a saturation-recovery sequence and found to be between 6 s - 10 s. Pulse repetition times were chosen to be five times of the spin lattice relaxation times. Because of the substantial boron background the spectra of an empty rotor at 10 kHz and 25 kHz spinning were acquired under identical conditions and subtracted after careful phasing and intensity adjustment.

2.4 Quantification of Inorganic Ion Release under Simulated Physiological Conditions

Using 15 mL polypropylene Falcon tubes, 0.1 g of each glass composition (45 - 150 \(\mu\)m) (n=3) was suspended in 10 mL of tissue culture water (Sigma-Aldrich, Canada) [40–43]. This was done for each glass composition at each incubation time point: 24, 72, 168, 336 and 720 hours. Specimens were stored in a shaking water bath (Stuart SB40, Technne Inc., USA), maintained at 37\(^\circ\)C and agitated at 2Hz (longitudinal movement) for the duration of the specified incubation period. On completion of incubation, extracts were decanted and filtered through a sterile 0.20 \(\mu\)m filter.
Table 2: PSA for GB20X composition: <45 and 45 – 150 μm.

<table>
<thead>
<tr>
<th>Sample Designation</th>
<th>Dx 10 (μm)</th>
<th>Dx 50 (μm)</th>
<th>Dx 90 (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;45</td>
<td>45-150</td>
<td>&lt;45</td>
</tr>
<tr>
<td>GB200</td>
<td>11.8</td>
<td>51.8</td>
<td>30.2</td>
</tr>
<tr>
<td>GB201</td>
<td>9.48</td>
<td>61.1</td>
<td>23.4</td>
</tr>
<tr>
<td>GB202</td>
<td>8.35</td>
<td>57.6</td>
<td>24.7</td>
</tr>
<tr>
<td>GB203</td>
<td>6.98</td>
<td>58.2</td>
<td>23.3</td>
</tr>
<tr>
<td>GB204</td>
<td>9.22</td>
<td>58.4</td>
<td>25.9</td>
</tr>
<tr>
<td>GB205</td>
<td>9.13</td>
<td>57.8</td>
<td>25.9</td>
</tr>
</tbody>
</table>

(Sarstedt, Canada) into new clean and sterile 15 mL Falcon tubes. All filtered extracts were subsequently stored at 4°C until required for future testing. The ionic concentrations of B, Na, Sr, and Ga were examined using ICP-OES (Perkin Elmer Optima DV8000, MA, USA). Standard solutions containing B, Na, Sr and Ga (Perkin Elmer, Canada) were prepared and calibration curves were retrieved before and after analysis. Extracts derived from the extraction of GB200-GB205 glasses were diluted (1/10, 1/100 and 1/1000) using 2% HNO₃. The ion concentrations are reported as the average ± SD.

2.5 Mathematically Modeling: Determining Ion Release Mechanisms

Ion release data was fitted to Hopfenberg [44, 45] and Higuchi [44] models and were statistically analyzed to determine model adequacies.

2.6 Statistical Analysis

Linear regression analysis was completed on density data. The two-way analysis of variance (ANOVA) was used to determine statistical significance of component ion release data from each GB composition. A paired T test was used to determine statistical significance of (i) Tₕ and (ii) density data. All statistical analysis was completed with graphPad Prism® Version 8.0.

3 Results

3.1 PSA and XRD

Glasses were confirmed to be amorphous and absent of identifiable crystalline species; the observed broad peaks (Figure 1) occurred due to the variable interplanar spacing (d-spacing) of B[3] and B[4] units that are present within the glass network. For reproducibility and completeness, particle size distribution for each glass are provided in Table 2.

![Figure 1: XRD pattern for each GB20X composition.](image)

3.2 The effect of compositional changes on Tₕ, Density, molar volume

A linear relationship between increasing Ga₂O₃ content versus Tₕ and density was observed (Figure 2 and 3, respectively); Tₕ increased from 552°C to 591°C and density increased from 2.72 g/cm³ to 2.90 g/cm³. Molar volume (Figure 2) initially decreased from 32.73 to 32.58 cm³/mol (GB200 to GB202) and increased thereafter to 33.49 cm³/mol. Each data point, except between GB200
and GB201, were significantly different from the previous
(P value ≤ 0.0001).

Figure 2: $T_g$ versus $\text{Ga}_2\text{O}_3$:$\text{Na}_2\text{O}$ ratio in mol.% ($R^2 = 0.85$).

Figure 3: Density (g/cm$^3$) versus $\text{Ga}_2\text{O}_3$:$\text{Na}_2\text{O}$ ratio in mol.% ($R^2 = 0.96$), and molar volume ($V_m$) versus $\text{Ga}_2\text{O}_3$:$\text{Na}_2\text{O}$ ratio in mol.%.

The stack plot of $\text{^11B} \text{MAS NMR}$ spectra for each composition within the GB20X glass series is illustrated in Figure 4. Two resolved signals were located for each composition; a comparatively narrow signal centered at approximately 0.6 ppm, which is characteristic of tetrahedral B atoms (B[4]) [46] and a second broader signal centered at approximately 14 ppm, which is characteristic of trigonal B atoms (B[3]) [46]. The quadrupole coupling of $\text{^11B}$ (spin I = 3/2),
give rise to a significant broadening of the NMR signals with asymmetrical B environments (i.e., different B[3] structural units) [46, 47], whose distinct lineshapes are broadened due to the amorphous disorder. For each GB20X compositions, the B[4] signal remained in a similar position; minor chemical shifts from 0.62 ppm (GB200) to a more deshielded position of 0.7 ppm (GB205) occurred, however these fractional differences are within experimental error.

The peak position of B[3] signal moved slightly to a more shielded position with increasing $\text{Ga}_2\text{O}_3$:$\text{Na}_2\text{O}$, however much like the B[4] signal, these changes were fractional and within experimental error. The distinction between B[3] groups in rings and non-rings cannot be distinguished from these spectra.

3.3 NMR-MAS

The relative area of the B[3] signal increased, while the B[4] signal relative area decreased progressively as the $\text{Ga}_2\text{O}_3$:$\text{Na}_2\text{O}$ increased. This can be observed in Figure 5.
Table 3: Half-life (h) of release and goodness of fit for B, Sr, Ga, and Na release.

<table>
<thead>
<tr>
<th></th>
<th>GB200</th>
<th>GB201</th>
<th>GB202</th>
<th>GB203</th>
<th>GB204</th>
<th>GB205</th>
</tr>
</thead>
<tbody>
<tr>
<td>B release</td>
<td>Half-life</td>
<td>139.9</td>
<td>169.3</td>
<td>220</td>
<td>581.1</td>
<td>579.6</td>
</tr>
<tr>
<td></td>
<td>$r^2$</td>
<td>0.9802</td>
<td>0.9854</td>
<td>0.9898</td>
<td>0.9711</td>
<td>0.9786</td>
</tr>
<tr>
<td>Sr release</td>
<td>Half-life</td>
<td>248.1</td>
<td>317</td>
<td>641</td>
<td>980.3</td>
<td>1028</td>
</tr>
<tr>
<td></td>
<td>$r^2$</td>
<td>0.9911</td>
<td>0.9866</td>
<td>0.9858</td>
<td>0.9988</td>
<td>0.979</td>
</tr>
<tr>
<td>Ga release</td>
<td>Half-life</td>
<td>N/A</td>
<td>221.7</td>
<td>248.5</td>
<td>242.3</td>
<td>118.7</td>
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<tr>
<td></td>
<td>$r^2$</td>
<td>N/A</td>
<td>0.9898</td>
<td>0.9912</td>
<td>0.993</td>
<td>0.9487</td>
</tr>
<tr>
<td>Na release</td>
<td>Half-life</td>
<td>256.3</td>
<td>186.2</td>
<td>220.4</td>
<td>218.1</td>
<td>203.8</td>
</tr>
<tr>
<td></td>
<td>$r^2$</td>
<td>0.9954</td>
<td>0.9859</td>
<td>0.9874</td>
<td>0.9911</td>
<td>0.8752</td>
</tr>
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</table>

Figure 5: Relative populations of B[$3$] ($R^2 = 1.0$) and B[$4$] ($R^2 = 1.0$) structural units versus Ga$_2$O$_3$:Na$_2$O ratio.

3.4 Quantification of Inorganic Ion Release under Simulated Physiological Conditions

The cumulative release of individual constituent elements as a function of Ga$_2$O$_3$:Na$_2$O ratio is provided in Figure 6. Linear release profiles ($R^2 > 0.91$) for B release was observed where the Ga$_2$O$_3$:Na$_2$O ratio within the network was between 4:6 to 8:2 (GB202 and GB204). All other Ga$_2$O$_3$:Na$_2$O ratios reached a plateau at 336 h. B release from each composition was similar until approximately 336 h, thereafter a distinct divergence in release levels was observed. Peak B release for the glass series was observed for glasses GB203 and GB204 (1388 ppm and 1278 ppm respectively) (Figure 6 and 7). No significant difference in B release between GB203 and GB204 was observed up to 720 h. Similarly, no significant difference between GB200, GB201, GB202, and GB205, was observed up to 720 h. The half-life (Figure 8 and Table 3) of release of B increased from 139.9 h to a peak of 581.1 h as we move from Ga$_2$O$_3$:Na$_2$O ratio of 0:1 to 6:4 (i.e. GB200 – GB203). The half-life of release of B remained stable when Ga$_2$O$_3$:Na$_2$O increased to 8:2 (i.e. GB204), then decreased to 143.2 h at a full substitution of Ga$_2$O$_3$ for Na$_2$O (i.e. GB205).

Rather than plateau within 720 h, the release profiles observed for Sr appear broadly linear over the extraction period and were between 92 – 1233 ppm. Sr release from GB200 - GB203 was similar at all time points <336 h (Figure 6). However interesting observations occurred at 720 h (Figure 6 and 7), where it was observed that a Ga$_2$O$_3$:Na$_2$O ratio of 4:6 to 6:4 (GB202 and GB203) resulted in the highest levels Sr release. Increases the Ga$_2$O$_3$:Na$_2$O ratio to >6:4 caused a significant reduction in total levels of Sr release. This paralleled with the half-life release of Sr (Figure 8 and Table 3), which increased from 248.1 h to a peak of 1028 h for Ga2O3:Na2O ratio of 0:1 to 8:2 (i.e. GB200 – GB204), and subsequently decreased to 746.2 h for a full substitution of Ga$_2$O$_3$ for Na$_2$O (i.e. GB205).

Interestingly, linear release profiles ($R^2 = 0.93$) for Ga release was observed where the Ga$_2$O$_3$:Na$_2$O ratio within the network was between 4:6 to 6:4 (GB202 and GB203). All other Ga$_2$O$_3$:Na$_2$O ratios reached a plateau at 336 h (Figure 6). The corresponding half-life (Figure 8 and Table 3) for GB202 and GB203 remained similar at 248 h and 242 h respectively. The shortest half-life of release was observed at GB204 and GB205 (118 h and 111 h respectively). At 720 h it is obvious that there is a predictable effect of Ga$_2$O$_3$:Na$_2$O ratio on peak Ga release up to 6:4, for ratios >6:4 peak Ga release is decreased (Figure 7).

A constant release profile of Na was observed for GB200 – GB203 up to 720 h ($R^2 > 0.87$). GB204 attained peak release at 336 h (Figure 6). Peak Na release was observed at 303.8 ppm for GB200 (highest loading of Na$_2$O). Ion release decreased sequentially, with the lowest Na release observed at 105.4 ppm from GB204 (lowest loading of Na$_2$O) (Figure 6 and 7).
3.5 Modelling Component Ion Release

Component ion release was subjected to the Hopfenberg model and the Higuchi model to determine the mechanism of release from each composition (Figure 9). An excellent fit ($r^2 \geq 0.93$) to the Hopfenberg model [44, 45] was observed for each ion in each composition. A good fit ($r^2 \geq 0.85$) to the Higuchi model [44, 48] was also observed for each ion from each composition.
Figure 9: (i – ii) Sr and Ga release data applied to the Hopfenberg Model, \( r^2 > 0.98 \) for Sr, \( r^2 > 0.93 \) for Ga. (iii – iv) Sr and Ga release data applied to the Higuchi Model, \( R^2 > 0.96 \) for Sr, \( R^2 > 0.86 \) for Ga.

4 Discussion

With appropriate compositional modifications, the unique structural nature of vitreous Boron oxide may provide an innovative degradable platform for the controlled localized release of TMIs, Ga and Sr [3, 9, 12]. This is specifically related to the cation’s concentration dependent ability to modulate the molecular structure of the network (i.e. conversion between B[3] and B[4] structural units) [13, 14]. This structural mechanism combined with the inclusion of biologically interesting metal ions provides an opportunity to engineer glass-based biomaterials, where the therapeutic component assists in controlling dissolution kinetics and network degradation.

Substituting Ga\(_2\)O\(_3\) with Na\(_2\)O caused substantial structural changes within the borate network and consequently glass properties were affected. Specifically, increases of the Ga\(_2\)O\(_3\):Na\(_2\)O ratio caused a decrease in the relative population of B[4] structural units and an increase in the B[3] structural units (Figure 5). During the initial
substitutions (i.e. 0:1 – 6:4 Ga$_2$O$_3$:Na$_2$O) both the half-life of release and ion release at 720 h increased for B, Sr, and Ga, where peak release for each ion was observed at 6:4 Ga$_2$O$_3$:Na$_2$O.

Looking first at the boron coordination’s: for the chosen borate-to-cation composition range of this study, the oxygen-to-boron ratios for all glasses are below 2, i.e. below the range, where conversion of B[4]’s to B[3]’s with NBO would dominate with increased oxygen concentration. Here, if all the cations were network modifiers, one would expect an increase in B[4] concentration, since per Na$_2$O replacement with Ga$_2$O$_3$two additional oxygens are introduced, in principle available to modify the B[3] groups into B[4] borons. Instead, the solid state NMR results show a decrease in the B[4] concentration with increased Ga$_2$O$_3$ introduction. This observation may be explained by understanding that Ga may act as a network former. In the starting material Ga$_2$O$_3$, Ga may exist in tetrahedral and octahedral co-ordination states. Ga accomplishes this by corner and edge sharing polyhedra, with some oxygens linked to as many as four Ga atoms [49]. To obtain tetrahedral coordination with only corner shared polyhedra, Ga sequesters bridging oxygens away from the boron network, hence reducing the B[4] and increasing the B[3] concentration. In fact, statistically, if Ga would attract only doubly bonded oxygens, the B[4] reduction should be even stronger than experimentally observed in this study, pointing to oxygens, which are triply coordinated, hence also contributing to the network connectivity. The formation of octahedrally coordinated Ga should decrease the B[4] concentration even further.

Depending on the glass composition, Ga has been shown to exhibit two different coordination states; a fourfold coordinate state (GaO$_6$) and an octahedral coordination (GaO$_6$) [26]. The former, which has been proposed to alternate with tetrahedral structural units (e.g. B[4] and PO$_4$), is generally associated with an increase in network stability. While alternatively, GaO$_6$ is generally assumed to exhibit a disruptive role within the glass network [29, 50]. Based on the crystal structure data, Ga enters the glass network in highly interconnected corner and edge sharing GaO$_4$ and GaO$_6$ polyhedra, de-stabilizing the network by sequestering oxygen away from the borate structures and causing the formation of hydrolysable B[3] units.

Generally it is assumed that increasing the concentration of B[3] and minimizing B[4] units causes an increasingly cross-linked network, a Ga$_2$O$_3$:Na$_2$O of ≥ 6:4 resulted in a plateau and subsequent reduction in the release half-life and ion release levels at 720 h, for B, Sr, and Ga [13, 14, 51]. This is consistent with our hypothesis, that above this ratio the increased removal of Na is counteracted by the stabilizing role of GaO$_6$ tetrahedra within the network, which contributes to the continuing decline of the relative population of B[4] structural units.

Reddy et al. [26] attributed borate network stabilization and destabilization to the presence of Ga in the form of GaO$_6$ and GaO$_4$, respectively. The presence of GaO$_6$ was confirmed by Infrared spectroscopy, and was observed to decrease with increasing network destabilization. A similar compositionally induced response, was observed by Subbalakshmi and Veeraiah [50] in a phosphate based glass. Valappil et al. [52] attributed stabilization of a phosphate network to increasing covalency in the P – O – Ga bonding interactions, which replaced more ionic P – O – Na associations, on Q$^3$ chain terminating groups. However, contrary to other studies, Ga was determined to primarily be in the form of GaO$_4$. Possibly indicating, that the increased stabilization observed ≥ 6:4 Ga$_2$O$_3$:Na$_2$O with the GB20X series may not be solely due to the increased presence of GaO$_4$.

Contrary to conventional phosphosilicate glasses, studies have shown that borate glasses dissolve congruently (where congruent implies that the ratios of the constituent elements in solution is the same as that in the dissolving solid) via the simultaneous hydration of ions and hydrolysis of the network [53, 54]. During this dissolution, the formation of a dissolution limiting barrier/layer does not tend to form, which in theory may permit the constant zero-order release of constituent ions. This is dependent upon a number of factors, such as the chemical composition and structure of the glass-based material, and the extraction media [54].

Generally, research pertaining to a controlled delivery systems aim to provide sustained zero-order release kinetics (i.e. a constant release rate over time), as it is thought to minimize fluctuations in local concentrations [55]. This is important as fluctuations may lead to periods of under and over-dosing, a reduced time within the therapeutic window, and consequently a reduction in the lifespan of the therapeutic effect [56]. This is particularly relevant to TMs, which are eliminated or metabolized quickly in the body.

Within the GB20X series constant release profiles were obtained for (i) Sr from each composition, irrespective of Ga$_2$O$_3$:Na$_2$O, and (ii) Ga from GB202 and GB203 (i.e. 4:6 and 6:4 Ga$_2$O$_3$:Na$_2$O). Both of these metals ions, posses considerable therapeutic potential [30, 57, 58]. Sr, which has a dual ability to increases bone formation and decrease bone resorption is used as an anti-osteoporotic agent for post-menopausal women. The minimum in vitro therapeutic concentration required for this effect is 87.62 ppm (0.1mM), which was obtained from each
GB20X composition between 155 – 190 h of extraction [59]. Contrary to Sr, Ga has displayed therapeutic potential in a wide spectrum of indications, with the most prominent being anticancer therapy. Preclinical investigations of the cytotoxicity of gallium nitrate on four hepatocellular carcinoma cell lines have shown that the IC\textsubscript{50} value for Ga is 1743 ppm. This was obtained in from GB202 and GB203 after 40 – 45 h of extraction [33].

The approach of using a borate glass networks, as a platform for the delivery of the TMIs, should be expanded beyond those elements considered in this work (i.e. Sr and Ga) so as to include other TMIs and non-metallic therapeutic inorganic ions; specifically, in the context of optimizing efficacy and selectivity, while minimizing systemic toxicity. In this regard, borate networks offer an opportunity to expand the compositional palette for new bioactive glasses, which may have clinical applications in and, possibly beyond, tissue repair and regeneration.

5 Conclusion

The initial increase in Ga\textsubscript{2}O\textsubscript{3}:Na\textsubscript{2}O ratio (i.e. 0:1 – 6:4) caused destabilization of the borate network to occur. This was observable by increases in release half-life and ion release (up to 720 h) of the constituent ions B, Sr, and Ga. This behavior is consistent with the observed structural responses; continuous increases (i.e. to full substitution of Ga\textsubscript{2}O\textsubscript{3}:Na\textsubscript{2}O (i.e. 0:1 – 1:0)) in the relative population of hydrolysable B[3] units and decreases in B[4] units. Irrespectively, at ≥ 6:4 Ga\textsubscript{2}O\textsubscript{3}:Na\textsubscript{2}O ratios, network stabilization appeared to occur, with decreases in the release half-life and ion release of B, Sr, and Ga at 720 h of extraction. While the current data set is unable to definitively determine the exact coordinate state of Ga, our statistical analysis supports the formation of GaO\textsubscript{4} tetrahedra, which is primarily associated with assisting in network stabilization. The release profiles are consistent with two competing processes: a decrease of networks through the reduction of B[4] and increase of B[3] groups, but an additional increase in network connectivity through the GaO\textsubscript{4} tetrahedra, possibly linked to triple coordinated oxygens. A peak in component ion release (B, Ga, and Sr) and a subsequent decrease thereafter appeared to be occurring at ≥ 6:4 Ga\textsubscript{2}O\textsubscript{3}:Na\textsubscript{2}O ratio, where Ga may be increasingly present as GaO\textsubscript{4} coordinate state, which is primarily associated with assisting in network stabilization. In addition to this, constant release profiles were obtained for (i) Ga from GB202 and GB203 (i.e. 4:6 and 6:4 Ga\textsubscript{2}O\textsubscript{3}:Na\textsubscript{2}O ratio), both of which displayed a similar half-life of release, and (ii) Sr from each composition (i.e. 0:1 – 1:0 Ga\textsubscript{2}O\textsubscript{3}:Na\textsubscript{2}O ratio). Demonstrating further that with appropriate compositional modifications borate glass networks may be utilized for the controlled deliver of TMIs.

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