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Sodium Is Not Essential for High Bioactivity of Glasses

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Abstract

This study aims to demonstrate that excellent bioactivity of glass can be achieved without the presence of an alkali metal component in glass composition.

In vitro bioactivity of two sodium-free glasses based on the quaternary system SiO₂-P₂O₅-CaO-CaF₂ with 0 and 4.5 mol% CaF₂ content was investigated and compared with the sodium containing glasses with equivalent amount of CaF₂. The formation of apatite after immersion in Tris buffer was followed by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), ³¹P and ¹⁹F solid state MAS-NMR. The dissolution study was completed by ion release measurements in Tris buffer.

The results show that sodium free bioactive glasses formed apatite at 3 hours of immersion in Tris buffer, which is as fast as the corresponding sodium containing composition. This signifies that sodium is not an essential component in bioactive glasses and it is possible to make equally degradable bioactive glasses with or without sodium. The results presented here also emphasize the central role of the glass compositions design which is based on understanding of structural role of components and/or predicting the network connectivity of glasses.

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Keywords

Bioactive glass; sodium free; alkali free; fluoride containing; fluorapatite; bioactivity; glass degradation

Introduction

Bioactive glasses degrade in physiological solutions, forming a surface layer of a hydroxycarbonate apatite (HCA) like phase, which allows for the formation of an intimate bond between the glass and living bone. The first bioactive glass (Bioglass® 45S5) was developed by Hench in 1969¹; it has been in clinical use since 1985¹ and is currently used in a range of orthopedic (e.g. NovaBone®), periodontal (PerioGlas®) and toothpaste applications (NovaMin®)². Since then, new bioactive glass compositions have been developed, incorporating strontium³, zinc⁴, cobalt⁵, fluoride⁶, potassium⁷ or magnesium⁸ to combine therapeutic ion release and apatite formation. Bioactive silicate glasses are also of interest for use as bone grafts⁹ or implant coatings¹⁰. Owing to their ability to enhance new bone formation, they are also increasingly used as scaffolds in tissue engineering¹¹. By formation of bioactive glass/polymer composites the mechanical properties can be adjusted for soft tissue¹² or bone fracture fixation¹³ applications or for dental composites⁹.

Bioactive glasses traditionally contain large amounts of sodium oxide (e.g. 26 mol% in Bioglass® 45S5), and according to Hench's original mechanism of bioactivity, sodium is a critical component for glass degradation and apatite formation¹⁴. However, high sodium oxide content bioactive glasses have disadvantages, particularly for applications in bioactive glass/polymer composites: high sodium content usually makes the bioactive glass phase hygroscopic¹⁵, and thereby affects stability, degradation and mechanical performance of the composite materials. This reduces the applicability of conventional high sodium oxide content bioactive glasses as fillers in composites. In addition, according to the mechanism of glass degradation¹⁶, in the first step, sodium ions are exchanged for protons following the glass dissolution and lead to a rapid increase in pH, which favors hydroxyapatite formation but is not favorable for homeostasis¹⁷.

Calcium and sodium oxides are both typical network modifying oxides, though sodium oxide disrupts the glass network much more efficiently as sodium is monovalent cation. However, it is the calcium cation which is required for the apatite formation and therefore keeping high calcium content in glass composition instead of sodium is often more useful for bioactivity. It has been established that the connectivity of the silicate network and the presence of considerable amount of phosphate as amorphous orthophosphate play crucial roles in how fast glass can degrade and form apatite¹⁸. However, the role of sodium presence on the rate of apatite formation is still often pursued as essential; this is perhaps due to significant amount of sodium in the composition of the Bioglass® 45S5. Recent comprehensive structural study combining experimental and computer modelling of the sodium free solgel derived bioactive glasses gave a detailed insight into local environment of simple bioactive glass¹⁹. More complicated glasses with further additional components still present a certain challenge for obtaining such a detailed structural insight. Therefore,

understanding of structural role of the individual components and using it to predict a network connectivity of glasses was found to be useful to design a bioactive glass for a specific application²⁰.

We have recently shown that it is possible to form sodium-free fluoride containing bioactive glasses^{6a, 21}, which degrade and form fluorapatite (FAP) in simulated physiological solutions. FAP is a significant constituent of tooth enamel and attractive for remineralizing toothpastes and other dental applications, since it is much more resistant to acidic environments than hydroxyapatite (HAP). The presence of fluoride in the bioactive glasses leads to the beneficial formation of FAP and enhanced remineralization and can also alleviate dentine hypersensitivity^{21a} when used in toothpastes.

The aims of this study were to get the insight of designing and development of highly bioactive, though sodium free, fluoride containing bioactive glasses, which are beneficial for FAP formation and which could avoid the potential risks caused by a relatively high pH, and thereby to establish whether sodium is essential for apatite formation of bioactive glasses.

Experimental Section

Glass Synthesis

Two sodium free glass ($\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-CaF}_2$) compositions and two sodium containing glasses ($\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-Na}_2\text{O-CaF}_2$) with equivalent CaF_2 content, both from the previously studied series^{21a, 21c}, were selected for this study (Table 1). All glasses were synthesized by a melt-quench route. Calcium fluoride was added to GPF0.0 and A2, fluoride free formulations (Table 1); this design was chosen against the substitution for CaO in order to keep the network connectivity constant^{6a}. Glass batches of 200 g were produced by mixing analytical grade SiO_2 (Prince Minerals Ltd., Stoke-on-Trent, UK), CaCO_3 , P_2O_5 , CaF_2 (all Sigma-Aldrich) and melting in a Pt/10Rh crucible at 1420-1550°C for 1 hour in an electrical furnace (EHF 17/3 Lenton, Hope Valley, UK). In order to prevent crystallization the melted glass was quickly quenched to room temperature in water. The as-quenched glass frit was dried and ground using a vibratory mill (Gy-Ro mill, Glen Creston, London, UK) for 14 minutes. The obtained glass powder was sieved through a 45 μm mesh analytical sieve (Endecotts Ltd, London, England) to obtain fine powder. The results for the sodium free series were compared to the bioactivity of the sodium containing series $\text{SiO}_2\text{-P}_2\text{O}_5\text{-CaO-Na}_2\text{O-CaF}_2$ of glasses with equivalent CaF_2 contents (Table 1) which has been previously reported^{21a}.

Buffer Solution Preparation

The Tris buffer solution was prepared by first dissolving 15.090 g Tris(hydroxymethyl)aminomethane (Sigma-Aldrich) in 1500 ml de-ionized water. After dissolving, 44.2 ml of 1 M hydrochloric acid (Sigma-Aldrich) was added. The solution was kept in a 37°C incubator for overnight. The pH value was adjusted to 7.3 using 1 M hydrochloric acid before diluting the solution up to total volume of 2 liters with de-ionized water. The solution was stored in a 37°C incubator (KS 4000i control, IKA) before use²¹.

In Vitro Bioactivity Testing

To characterize the bioactivity of glasses the formation of an apatite-like phase was monitored as a function of immersion duration in Tris buffers. Glass powder (75 mg) was dispersed in 50 ml Tris buffer; tests were done in duplicate for each glass composition. The solutions were agitated at a rate of 60 rpm in an incubator (set at 37°C) for various durations (1, 3, 6, 9, 24, 72 and 168 hours). At the end of the immersion period, the pH of the solution was measured using a pH meter (Oakton® pH 11 meter; 35811-71 pH electrode). The solutions were then filtered through filter paper with pore size 5-13 µm. The solid residues from the filter were dried and retained for further characterization by XRD, FTIR and solid state NMR. The filtrate was stored at 4°C for analysis of ionic concentrations.

Analysis of Ionic Concentrations

The filtrate was diluted by a factor of 1:10 and acidified using 69% nitric acid (VWR). The calcium, silicon and phosphorus contents in solution were quantified using inductively coupled plasma-optical emission spectroscopy (ICP-OES; Varian Vista-PRO, UK). Calibration for each of the elements was performed with the solutions prepared by dilution the stock solutions with Tris buffer. The fluoride ion concentration was evaluated using a fluoride ion selective electrode (Orion 9609BN, 710A meter, USA). To establish the linear function of the electrode, a five point calibration was performed on calibration solutions prepared using Tris buffer solution and 1000 ppm fluoride stock solution (Sigma Aldrich). The released concentrations of each element are presented as a percentage of their initial content in the nominal glass composition.

Powder Characterization

The glass powders collected from the filter after immersion were characterized by Fourier transform infrared spectroscopy (Spectrum GX, Perkin-Elmer, USA). Untreated glass powder was analyzed for comparison. The data were collected from 1600 to 500 cm⁻¹. X-ray diffraction analysis was carried out using an X'Pert Pro X-ray diffractometer (PANalytical, The Netherlands), with the data collected from 5 to 70° 2θ and an interval of 0.0334°. Phase identification was performed using the PANalytical X'Pert High Score Plus Software (ICDD PDF-4 database).

The solid-state NMR experiments for the sodium free glass compositions were performed on a 600 MHz (14.1T) Bruker NMR spectrometer. ³¹P MAS-NMR was run at the 242.9 MHz resonance frequency using a standard single resonance Bruker probe in a 4 mm rotor at spinning conditions of 8 and 10 kHz. Some ³¹P MAS-NMR measurements were also carried out using a Bruker probe for a 2.5 mm rotor at spinning conditions of 18 and 21 kHz. 16 scans were run with a recycle delay of 60 s for each sample. ³¹P MAS-NMR for the sodium containing glasses was performed on a 200 MHz (4.7T) Bruker solid state NMR spectrometer, at the 81.0 MHz resonance frequency using a 30 s recycle delay and 8 dummy scans. The chemical shift was referenced using the primary reference, 85% H₃PO₄. ¹⁹F MAS-NMR measurements were run at the 564.7 MHz resonance frequency using a standard double resonance Bruker probe with low fluorine background for a 2.5 mm rotor spinning at a speed of about 18 kHz or 21 kHz. Typically 32 or 64 scans were acquired with 8 preliminary dummy scans and 30 s recycling delay. The chemical shift was referenced using

the signal from 1M NaF solution scaled to -120 ppm relative to the CF_3Cl primary standard. The *dmfit* software²² was used for deconvolution of the NMR spectra.

Results and Discussion

Ftir Spectroscopy

Fig 1a presents the FTIR spectra of the solid residues collected after immersion of the sodium free glass GPF4.5 in Tris buffer solution for different time periods. The spectra for glass GPF0.0 were similar to these (Figure S1). The spectra are compared with the results of a sodium containing glass (B2) with the same fluoride content (Fig 1b). Each figure presents the spectra corresponding to relatively short duration times with the bottom spectrum showing the result for the untreated glass (0 h). The identification of the bands is similar to what has been published previously for sodium containing glasses^{21a}. From the comparison of the Figs. 1a-b it is clearly seen that the sodium free glasses degrade and form an apatite-like phase at the same rate or even faster than the sodium containing glasses.

The spectra of the untreated glasses demonstrate broad bands at 1030 cm^{-1} and 920 cm^{-1} , which correspond to Si-O-Si stretch and non-bridging oxygen Si-O⁻ bands, respectively²³. Amorphous calcium phosphate contributes to a peak at about 565 cm^{-1} . Glass degradation and apatite formation occurred rapidly when glasses were immersed in Tris buffer, resulting in significant changes in FTIR spectra, which were similar for both the sodium free and sodium containing glasses. The intensity of non-bridging oxygen Si-O⁻ band at 920 cm^{-1} had decreased dramatically at 3 hour immersion for GPF4.5, indicating rapid degradation in the sodium free glass.

The formation of a crystalline calcium orthophosphate, or apatite-like phase, is clearly seen for the sodium free glasses at 3 hours of immersion. This is evident from appearance of the typical split bands at 613 cm^{-1} and 560 cm^{-1} and several overlapping peaks in the region $1090\text{--}1035\text{ cm}^{-1}$, some of which correspond to $\text{Zr}_3(\text{PO}_4)_4$ ²⁴. The latter region also contains bands for Si-O-Si and carbonate substitution in apatite. The sodium containing glasses showed split bands at 600 cm^{-1} and 560 cm^{-1} at 6 hours of immersion. A sharpening of the absorbance bands at 3 hours immersion for sodium free series and 6 hours for sodium containing glasses is clear evidence for crystals formation. The spectra for glass GPF4.5 at 6 and 9 hours of immersion are nearly identical, while the spectra for glass B2 intensified with an increase in immersion time.

The bands at 1450 , 1420 or 1413 and 870 cm^{-1} in both series indicate type B carbonate substitution in the apatite phase²⁴. The presence of carbonate in the untreated sodium containing glasses as a result of surface reaction of glass powder with atmospheric moisture is seen from the band at 1450 cm^{-1} and a sharp feature at 870 cm^{-1} (Fig 1b).

X-ray Diffraction

Fig 2 shows the XRD patterns for the glass powders before and after immersion in Tris buffer; the XRD data for the other two compositions are given in the supporting material (Figure S2). The XRD results are consistent with the FTIR data above and show that the

apatite phase in the sodium free glass started emerging no later or perhaps even earlier than in sodium containing glasses.

The XRD patterns for the initial glasses (0 h) showed a typical amorphous halo at about $30^\circ 2\theta$, indicating that the glasses were largely amorphous. A minor presence of apatite crystals in fluoride containing glass GPF4.5 appeared at the detection limit of XRD analysis. This is believed to be a result of surface reaction of glass powder with atmospheric moisture owing to the high reactivity of glass.

Upon immersion in Tris buffer, clear characteristic peaks of apatite were observed at 25.9° and $31.8^\circ 2\theta$ at 3 hours for sodium free glasses and 6 hours for sodium containing glasses, thus, confirming formation of apatite within 3 and 6 hours respectively. With increasing soaking time up to 9 hours, the intensity of the diffraction lines increased from sodium containing glass. However, there was no significant difference in the intensity of the diffraction lines for the sodium free glasses at 6 and 9 hours. For the sodium containing glass B2, a small peak at 28.5° was found after 3 hours immersion, this might suggest the formation of CaCO_3 .

The diffraction peaks for the apatite phase remain broad owing to the small size (typically below 50 nm) and highly disordered character of the crystals formed on soaking of a bioactive glass in a buffer and also the presence of substitutions in the apatite lattice (e.g. carbonate). Unlike sodium containing glasses reported earlier, in the studied sodium free glass no presence of CaF_2 crystalline phases has been detected perhaps owing to relatively small amounts of fluoride in the compositions presented here (Table 1).

Solid State NMR

Fig 3a presents the ^{31}P MAS-NMR spectra for the calcium phospho-silicate glass GPF0.0 (free of fluoride and sodium) before and after immersion in Tris buffer. The spectra for composition GPF4.5 are very similar (Figure S3). It is seen that the changes occur after immersion of the glass powder for 3 hours and then 6 hours. Since during immersion in Tris buffer the glasses were exposed to an environment with only one type of cation, Ca^{2+} (which was released from the glass) the changes in the sodium free compositions were seen only in linewidth but not a ^{31}P chemical shift of the orthophosphate.

The untreated glass (bottom spectrum, Fig 3a) displays a broad signal with the center at 3.0-3.1 ppm that is attributed to an amorphous orthophosphate charge balanced with Ca^{2+} cations. The signal has a slight asymmetry on the right hand side. Deconvolution of the ^{31}P MAS-NMR signal of the GPF0.0 untreated glass using *dmfit* free software suggested the presence of a broad feature centered at about -1 ppm with a detectable intensity (around 10%).

After immersion, the spectra show relatively broad feature at the same position about 3 ppm for all sodium free compositions. At 3 hours immersion the ^{31}P signal centered at 2.9-3.0 ppm narrows down significantly compared to the untreated glass. This signal is typical for the apatite phase formed from the bioactive glasses. A further reduction in the linewidth of the spectra is found at 6 hours of immersion but no significant change between 6 and 9

hours. This reduction in linewidth is due to crystallization of apatite and is consistent with appearance of the apatite crystals seen in the XRD patterns.

Fig 3b shows the ^{31}P MAS-NMR spectra for the sodium containing composition (A2) without fluoride before and after immersion in Tris buffer. Unlike the sodium free glasses, the spectra display distinct changes in the ^{31}P peak positions owing to the presence of sodium. The spectra of the untreated glass showed a broad signal at 9.0 ppm assigned to an amorphous orthophosphate phase charge balanced by a mixture of Ca^{2+} and Na^+ , with the ratio of the cations close to a random arrangement according to composition. The position of the signal for the B2 was at 8.8 ppm (Figure S3) since it contains slightly less sodium oxide 26 (Table 1). A slight asymmetry at the low frequency is still present in the sodium containing compositions; the intensity is shifted to a higher frequency side, which is consistent with the effect of sodium cations on the ^{31}P chemical shift.

At 3 hours immersion the ^{31}P signal broadens out significantly for the sodium containing series and shifts towards 7-6 ppm, as shown in Fig 3b. At 6 hours the main signal shifts further to a region between 3.8 and 3.4 ppm in both fluoride free and fluoride containing compositions, and finally at 9 hours the position becomes close to an apatite like phase at 2.9-3.0 ppm. The shifting of the peaks with immersion time indicates glass degradation and apatite-like phase formation, which is reflected by a reduction in the proportion of orthophosphate charge balanced with Na^+ ions and the fact that orthophosphate is predominately charge balanced with Ca^{2+} cations for apatite crystallization.

Fig 4 presents ^{19}F MAS-NMR spectra of the GPF4.5 and B2 glasses immersed in Tris for 6 hours compared to the untreated glasses. The difference in the appearance of the ^{19}F MAS-NMR spectra between the two glasses is explained by the presence of sodium in B2 composition that creates a mixed cation environment of the fluorine atoms. The broad signal at -96 ppm of the sodium free glass (bottom, Fig 4a) is assigned to an amorphous F-Ca(n) environment^{21c}. Consistently with the ^{31}P MAS-NMR data, the ^{19}F signal narrows down after immersion. A relatively sharp feature at about -102 ppm can be seen for the powders immersed for 6 hours, which is attributed to a crystalline fluorapatite environment of fluorine²⁷.

The ^{19}F MAS-NMR spectrum for the sodium containing composition B2 is more featured. This is owing to the formation of the mixed Ca/Na environment around fluorine atoms and is similar to what was seen for the glasses with a lower phosphorus content elsewhere^{26a} and in a study on the fluorine environment in mixed Ca/Na glasses published recently²⁸. Three main features at -134, -175 and -226 ppm are seen in the spectrum for the B2 glass corresponding to strongly overlapping signals from the mixed environments. The most negative position is close to F-Na(n) with mostly sodium cations around the fluorine atoms, with the signal -134 ppm corresponding to a higher fraction of calcium than sodium cations around fluorine. However, a relatively sharp feature at -99 ppm, which was clearly absent in the untreated glass is found in the ^{19}F NMR spectrum of B2 glass at 6 hours immersion. This position is close to the chemical shift of fluorine in the fluoride-substituted apatite environment and therefore was assigned to it.

pH Changes

Fig 5 shows the time profiles of pH in Tris buffer measured after immersion of glass powders. The pH becomes more alkaline with time. An effect of fluoride on lowering pH rise was noticeable in both sodium free and sodium containing compositions. It is attributed to the presence of a less amount of network modifying oxides in the glasses with a higher fluoride content. Additionally, the pH rise was more pronounced in sodium free glasses than in sodium containing ones, which was not an anticipated result as the opposite was expected based on the earlier studies¹⁷.

The rise in pH is a consequence of ion exchange between $\text{Na}^+/\text{Ca}^{2+}$ from glasses and proton ion from buffer solution and is typically observed on immersion of bioactive glasses in physiological fluid during the first 6 hours; after that the pH does not change significantly up to 6-9 hours. This trend was observed for a number of glass series and slight differences in time points depend on composition and glass dissolution rate^{21, 29}.

Ion Release

Fig 6 presents ion release data on the calcium, phosphorus, fluoride and silicon for the sodium free and sodium containing glasses. In general, different glass compositions from the same glass series show nearly identical ion release trends for each ion.

In the case of sodium free glass series (Fig 6a), the relative concentrations of calcium in solution were around 55% at 3 hour, reached over 60% at 6 hours and was nearly the same at 9 hours. Release of silicon into Tris buffer solution increased up to 40-50% at 3 hours of immersion and remained nearly constant up to 9 hours. The concentration of fluoride ions in solution reached a maximum at 3 hours and then decreased at 6 hours. No further reduction was found between 6 and 9 hours. The amount of phosphorus released in Tris buffer solution after 3 hours remained quite low, below 5%. The absence of significant phosphorus released at the early time points might indicate a rapid consumption of phosphate for apatite. In the absence of other ionic species in Tris buffer solution it is calcium and phosphate from the glass are to be used for apatite formation. However, bioactive glass compositions are phosphate deficient in terms of apatite formation. Therefore, potentially all phosphate can be fully consumed to form apatite phase.

In the case of sodium containing glass series (Fig 6b), the concentrations of calcium, phosphorus and fluoride ions increased rapidly in the first 3 hours and decreased with a further immersion up to 9 hours, suggesting a continuous apatite formation. These coincide with the apatite formation manifested in FTIR (Fig 1b), XRD (Fig 2b) and ^{31}P NMR (Fig 3b) data.

Since the amount of apatite formed is limited by nearly constant phosphate content in glasses (Table 1), a similar amount of calcium from both series was required for combining phosphate to complete apatite formation. In addition, sodium containing glasses only contain half amount of calcium compare to the equivalent sodium free glasses. Hence, a lower calcium concentration was seen in sodium containing glasses.

Final Discussion

The presence of significant amounts of sodium oxide in the first bioactive glass, Bioglass® 45S5 (46.1SiO₂-2.6P₂O₅-26.9CaO-24.4Na₂O, mol%), incited the scientists to ascribe to sodium cations an essential role in the mechanism of bioactivity^{16b}. Within the limitations of this study, the results on sodium free glasses presented here show that excellent bioactivity can be achieved in glasses without sodium present in the composition. The bioactivity of a glass is considered here in terms of their rate of glass dissolution and apatite formation under physiological conditions, specifically in Tris buffer.

The results of FTIR (Fig 1a), XRD (Fig 2a) and ³¹P MAS-NMR (Fig 3a) experiments performed on the solid phase remaining after immersion of sodium free glasses in Tris buffer solution consistently indicate the formation of apatite-like phase within 3 hours. This rate of apatite formation is much higher than that possible to achieve for the 45S5 composition³⁰. Bioglass® contains nearly half amount of P₂O₅ than the glasses studied here and requires up to 24 hours of immersion in Tris buffer for an apatite to form³¹. The results presented here demonstrate that an enhanced bioactivity can be achieved with a high phosphate content in glass, this is consistent with the finding by Mneimne *et al.*^{21a}. Furthermore, it is also essential that the phosphate remains largely as amorphous orthophosphate charge balanced with available cations in glass structure²⁵, which was confirmed for the glasses here from ³¹P MAS-NMR.

Owing to such a high rate of apatite formation only short term reactions in Tris buffer (up to 9 hours) have been considered here. Moreover, the FTIR and ³¹P MAS-NMR spectra as well as XRD patterns were practically identical at 6 and 9 hours of immersion for the same sodium free composition, which suggests that the reaction had been completed by 6 hours. This is also consistent with nearly constant concentrations of silicon, calcium and fluoride ions measured in solution between 6 and 9 hours.

The presence of fluoride is known to stimulate fluoapatite formation^{21a}. It was important to obtain evidence for a formation of fluorapatite in this study using ¹⁹F MAS-NMR as XRD or FTIR alone are not capable of detecting fluoride substitution in the poorly crystalline apatite. It is interesting that the ¹⁹F MAS-NMR spectra show a characteristic sharp appearance of the fluorapatite at about -102 ppm at 6 hours of immersion. This is consistent with the decrease in fluoride concentration in solution between 3 and 6 hours (Fig 6a) suggesting consumption of fluoride for a fluorapatite formation only at 6 hours; however, XRD patterns along with FTIR and ³¹P MAS-NMR spectra showed presence of an apatite-like phase earlier than that, at 3 hours of immersion. This suggests that the apatite-like phase formed at 3 hours does not contain fluorapatite or any fluoride substituted apatite yet. It is possible that this transient apatite-like phase can actually be assigned to octacalcium phosphate, which is often considered to be a precursor to an apatite phase.

Overall, the presence or absence of sodium in both series did not significantly affect the bioactivity of the glasses in this study. Both fluoride containing glasses regardless sodium presence or absence showed formation of fluoride substituted apatite. This emphasizes the significance of a structural factor taken into account here for designing the bioactive glass compositions, as the NC was kept constant at about 2.1 for the both series. Either one of two

concepts, the network connectivity^{16a} or split network model¹⁸, can be used for predicting the bioactivity for a glass composition, although with certain limitations.

The mechanism of bioactivity re-cited widely ^{16b} involves two processes, glass degradation and apatite formation. The role of sodium cations originally appeared to be essential particular for the glass degradation part. That is why the first step in Hench's mechanism assumed an ion exchange between sodium cations from glass and protons from solution which then enables the next step, the alkaline hydrolysis of the Si-O-Si linkages with the formation of the silanol Si-OH groups at the interface between glass and solution.

The results obtained here validate the view of congruent dissolution of entire silicate chains as proposed earlier^{6a} without Si-O-Si hydrolysis necessarily occurring. Thus, the low degree of polymerization of the silicate network (or low network connectivity) for this type of glasses controls the rate of glass degradation rather than an ion exchange of the highly mobile alkali cations followed by hydrolysis of Si-O-Si linkages. Only for glasses with a higher network connectivity Si-O-Si bond hydrolysis is a key step in the degradation process.

Conclusion

The results of a bioactivity study on sodium free glasses are presented. The study of the solid residues recovered after immersion in Tris buffer and analysis of the ion release in solution revealed that sodium free glasses are capable of high bioactivity and can form apatite on immersion in physiological fluids as early as 3 hours. A high phosphate content is essential for high bioactivity, i.e. early apatite formation. Although an apatite-like phase was seen to form within 3 hours, fluorapatite was identified in the ¹⁹F MAS-NMR spectra only at 6 hours. This suggests initial formation of an apatite-like precursor phase free of fluoride. Overall, the results of this study showed that the presence of sodium is not essential for glass bioactivity. These results question the accepted mechanism of glass bioactivity where the first step is assigned to an ion exchange between sodium ions in glass and protons in solution. The structural factor of silicate chains and the role of phosphate are emphasized for delivering a high and predictable bioactivity of a glass composition.

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Fig. 1. FTIR spectra of the solid residues recovered from the filter after immersion the glasses (a) sodium free glass GPF4.5 and (b) sodium containing glass B2 in Tris buffer for duration times indicated.

Fig. 2. XRD patterns of the solid residues recovered from the filter after immersion of (a) sodium-free glass GPF4.5 and (b) sodium-containing glass B2 in Tris buffer for durations indicated.

Fig. 3. ^{31}P MAS-NMR spectra of (a) sodium free glass GPF0.0 and (b) sodium containing glass A2 immersed in Tris buffer for durations indicated. The bottom spectrum is for the untreated glass powder (0 h).

Fig. 4. ^{19}F MAS-NMR spectra of (a) sodium free glass GPF4.5 and (b) sodium containing glass B2 immersed in Tris buffer for durations indicated. The bottom spectra are for the untreated glass powders (0 h). Asterisks mark the spinning side bands.

Fig. 5.
pH of Tris buffer solution after immersion of sodium free and sodium containing glasses.
Note where error bars are not seen, they are smaller than the data point.

Fig. 6. Concentration of calcium, phosphorous, silicon and fluoride ion released from (a) sodium free bioactive glasses and (b) sodium containing bioactive glasses into Tris buffer plotted as a percentage of the content of each of those elements in the batched glass composition. Note where error bars are not seen, they are smaller than the data point. The solid lines are used to demonstrate the change trend of ion concentrations.

Table 1

Glass compositions in Mol%

Glass Code	SiO ₂	CaO	Na ₂ O	P ₂ O ₅	CaF ₂
GPF0.0	38.1	55.5	-	6.3	-
GPF4.5	36.4	53.0	-	6.0	4.5
A2	38.1	25.9	29.6	6.3	-
B2	36.4	24.7	28.3	6.0	4.5