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Novel Highly Degradable Chloride Containing Bioactive Glasses

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Abstract: Addition of CaF_2 to a silicate bioactive glass favours formation of fluorapatite, which is less soluble in acidic environment than hydroxyapatite. However, excess CaF_2 in the glass is problematic, owing to the formation of crystalline calcium fluoride rather than fluorapatite on immersion. In this paper we investigate chloride as an alternative to fluoride in bioactive silicate glasses and in particular their bioactivity for the first time. Melt-derived bioactive glasses based on SiO_2-P_2O_5-CaO-$CaCl_2$ with varying $CaCl_2$ contents were synthesised and characterised by DSC. Chemical analysis of the chloride content was performed by using an ion selective electrode. Glass density was determined using Helium Pycnometry. The glass bioactivity was investigated in Tris buffer. Ion release measurements were carried out by using ICP-OES. The chemical analysis results indicated that the majority of the chloride is retained in the Q^2 type silicate glasses during synthesis. T_g and glass density reduced with increasing $CaCl_2$ content. Apatite-like phase formation was confirmed by FITR, XRD and ^{31}P MAS-NMR. The results of the in vitro studies demonstrated that the chloride containing bioactive glasses are highly degradable and form apatite-like phase within three hours in Tris buffer and, therefore, are certainly suitable for use in remineralising toothpastes. The dissolution rate of the glass was found to increase with $CaCl_2$ content. Faster dissolving bioactive glasses may be attractive for more resorbable bone grafts and scaffolds.

Keywords: chloride containing silicate glass; sodium free; highly degradable; bioactive glass; apatite

1 Introduction

Bioactive glasses are compelling for extensive use in medical and dental applications, for example bone grafts and toothpastes, due to their ability to degrade in physiological solutions and form a biomimetic apatite-like mineral layer [1–4]. Incorporation of various elements into the glass can tailor and improve the glass properties. For instance, zinc introduces an antibacterial effect [5], strontium stimulates bone regeneration [6, 7], magnesium facilitates processing [8] and fluoride enhances the bioactivity by forming fluorapatite (FAP) [9, 10].

FAP ($Ca_{10}(PO_4)_6F_2$), the fluoride analogue of hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$), is chemically more stable in an acidic environment than HAP [11, 12]. In addition, fluoride is the key agent in preventing dental caries by encouraging remineralisation and inhibiting demineralisation [13, 14]. Moreover, fluoride is able to reduce the glass melting temperature, glass transition temperature and potentially reduces the glass hardness. Softer glasses with lower hardness are potentially less abrasive in toothpastes. The fluoride containing bioactive glasses are therefore particularly suitable for dental applications, especially for remineralising toothpaste.

Recently, Chen *et al.* [15] found that high fluoride contents in bioactive glasses result in the formation of crystalline calcium fluoride on quenching, rather than FAP. A high fluoride content in the toothpaste for children might result in dental fluorosis [16]. The regulatory authorities set a restriction on the fluoride content of toothpastes at 1500 ppm in Europe and 1200 ppm in the US. Hence, an alternative to fluoride component in bioactive glass that also potentially reduces the hardness and abrasivity of the glass is highly desirable.

Chlorine belongs to the same halogen group as fluorine and may have similar effects on the glass properties. However, chloride volatilisation is a significant problem during synthesis [17] and the incorporation of chloride into glasses has rarely been investigated compared to flu-

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Table 1: Glass compositions in mol%. For each glass, the first row is the nominal composition as-designed and the second row is composition re-calculated based on the chloride component analysis.

Glass code	SiO_2	CaO	P_2O_5	$CaCl_2$
GPCl 0.0	38.1	55.5	6.3	0.0
	38.1	55.6	6.3	0.0
GPCl 2.3	37.3	54.3	6.2	2.3
	37.5	54.6	6.2	1.7
GPCl 3.5	36.8	53.6	6.1	3.5
	37.2	54.1	6.2	2.5
GPCl 4.6	36.4	53.0	6.0	4.6
	36.7	53.4	6.1	3.9
GPCl 7.2	35.4	51.6	5.9	7.2
	35.7	52.0	5.9	6.3
GPCl 10.6	34.1	49.6	5.7	10.6
	34.7	50.5	5.8	9.0
GPCl 14.0	32.8	47.7	5.4	14.0
	33.6	48.9	5.6	11.9
GPCl 20.6	30.3	44.1	5.0	20.6
	31.8	46.3	5.3	16.7

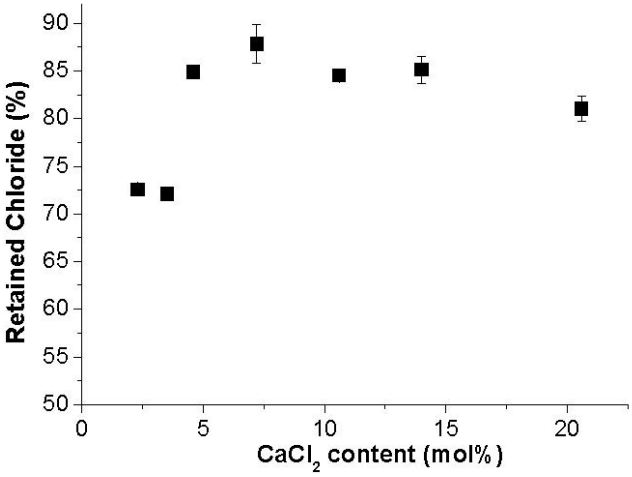

Figure 1: The percentage of the retained chloride in the initial glasses against the as-designed chloride content.

oride. The chloride ion is much larger than the fluoride ion (1.67 Å vs 1.19 Å) and when introduced into a glass, the chloride might expand the glass volume resulting in a more open and less compact glass structure. This could result in softer and less abrasive chloride containing bioactive glasses that can also dissolve faster and form apatite more rapidly. The larger size of the chloride ion compared to fluoride might also hinder crystallisation during synthesis.

Apatite stability follows the order FAP, HAP and chlorapatite (ClAP), with ClAP being the least stable [18]. We therefore would expect HAP to mainly form on dissolution of a chloride containing bioactive glass. Chloride is naturally present in the human body so is biologically acceptable and there is no restriction on the chloride content in toothpastes and biomedical materials unlike with fluoride.

This paper investigates the properties of chloride containing glasses free of sodium based on the SiO_2-P_2O_5-CaO-$CaCl_2$ system. Kiprianov *et al.* [17] proposed that chlorine might volatilise as NaCl. Hence, the absence of sodium would effectively minimise chlorine volatilization during melting. To the best of our knowledge, this study is the first ever investigation of the bioactivity of the chloride containing bioactive silicate glasses.

2 Materials and methods

2.1 Glass synthesis

The studied glasses were designed by incorporating various amounts of $CaCl_2$ into a sodium free calcium-silica-phosphate glass system and the ratio of CaO, SiO_2 and P_2O_5 was kept constant (Table 1). Analytical grade silica (Prince Minerals Ltd., Stoke-on-Trent, UK), calcium carbonate, phosphorus pentoxide and calcium chloride dihydrate (all Sigma-Aldrich, Gillingham, UK) were mixed and melted at high temperature (1550°C for GPCl 0.0 and GPCl 2.3; 1500°C for the rest glasses) for 1 hour in a platinum crucible in an electrical furnace (EHF 17/3, Lenton, UK). A

200 g batch was prepared. The molten glasses were rapidly quenched into deionised water to prevent crystallisation. The collected glass frit was dried overnight, ground into powder by a vibratory mill (Gyro mill, Glen Creston, UK) for 14 minutes and sieved through a 38 μm mesh sieve (Endecotts Ltd, UK).

2.2 Glass characterisation

2.2.1 Compositional analysis

The chloride contents in the initial glasses were quantified using a chloride ion selective electrode (ELIT Cl^- 2844, NICO 2000 UK). Glass powder (75 mg) with particle size smaller than 38 μm was dissolved in the 50 ml solution (48 ml deionized water, 1 ml 69% HNO_3 and 1 ml 5M $NaNO_3$ (ISA solution)). The samples were prepared in triplicate. The 1000 ppm Cl^- stock solution was prepared by dissolving 1.649 g NaCl (Sigma-Aldrich, Gillingham, UK) into 1 L distilled water and diluted to different concentrations to calibrate the electrode.

2.2.2 Differential Scanning Calorimetry (DSC)

The thermal properties of the glasses were evaluated by using a differential scanning calorimeter (DSC 1500 Stanton Redcroft, Rheometric Scientific, UK). The experiment was carried out under Nitrogen (60 ml min^{-1}). Both glass frit and glass powder with particle size less 38 μm (50 ± 0.1 mg) were heated from room temperature to 1100°C at a heating rate of 20°C/min using alumina as a reference. The glass transition temperature (T_g) was obtained from the DSC trace with an accuracy of ± 5°C.

2.2.3 Density Measurement

The density of each glass was determined by Helium Pycnometry (AccuPyc 1330-1000, Micromeritics, GmbH, Aachen, Germany). Two grams of glass powder (< 38 μm) was used and measured with the pressure at 1.6 bar. The density values reported are the mean of ten measurements performed during the experiment.

The experimental molar volume of the glasses is calculated by using the relation [19]:

$$V_m = \frac{M}{D} \quad (1)$$

Where M is the relative molecular mass of glass and D is the experimental density.

2.3 Bioactivity investigation

Glass bioactivity in terms of glass degradation and apatite formation was assessed in Tris buffer solution at pH=7.4 with concentration of tris(hydroxymethyl)aminomethane of about 0.06M. The buffer solution was prepared as described according to Mneimne *et al.* [9]. Glass powder (75 mg) with a particle size smaller than 38 μm was immersed in 50 ml buffer solution for different immersion periods (1, 3, 6, 9 and 24 hours). In order to simulate the human body environment, experiments were performed in a shaking incubator (KS 4000i Control, IKA, Germany) at an agitation rate of 60 rpm at 37°C. The samples were prepared in duplicate.

After the immersion periods, the pH values of the solution were measured immediately using a pH meter (Oakton®, Malaysia). The solution and deposit were separated by filtering using papers with a pore size of 5–13 μm.

An inductively coupled plasma-optical emission spectrometer (ICP-OES; Varian Vista-PRO, UK) was employed to determine the changes in concentration of calcium, silicon and phosphate in the filtered buffer solution after different immersion times. The diluted multi-element stock solutions were used to calibrate the machine.

2.4 Characterisation of apatite formation

Multi-techniques involving X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Magic Angle Spinning-Nuclear Magnetic Resonance (MAS-NMR) were employed to characterise the precipitates.

The initial glass powder and the collected precipitates were analysed using an X'Pert Pro X-ray diffractometer (PANalytical, The Netherlands) with a copper (Ni-filtered Cu-Kα) X-ray source. The data were recorded from 5 to 70°2θ at a step size of 0.0334° and a step time of 200.03 s. XRD data were analysed using X'Pert HighScore Plus (v2.0, PANalytical, The Netherlands) in conjunction with the ICDD powder diffraction database.

The glass powders and the collected samples were also characterised using Fourier Transform Infrared Spectroscopy (Spectrum GX, Perkin-Elmer, USA) to study the chemical bonding in the materials. The data were presented from 500 to 1600 cm^{-1} wavenumbers.

In order to understand the evolution of glass after immersion, ^{31}P MAS-NMR was run using a 600 MHz (14.1T) Bruker NMR spectrometer (AV 600 NMR, UK). The spectra were acquired at the resonance frequencies of 242.9 MHz and spinning rate of 10 kHz in a 4 mm rotor. The experiments were set up using 30 s recycling delay and scan

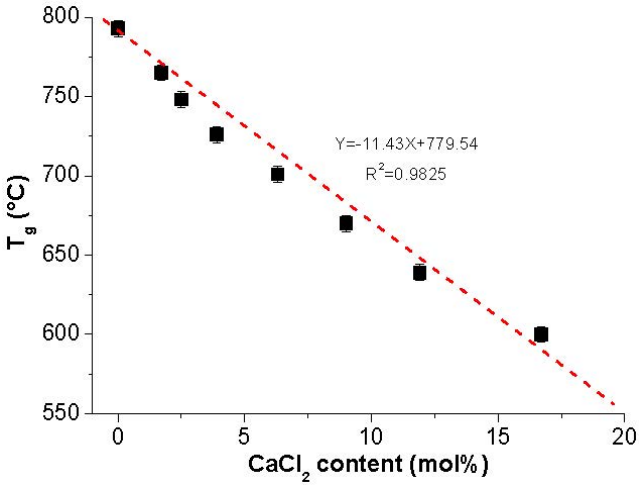

Figure 2: Glass transition temperature (T_g) of fine powder (< 38 µm) plotted against the actual $CaCl_2$ content.

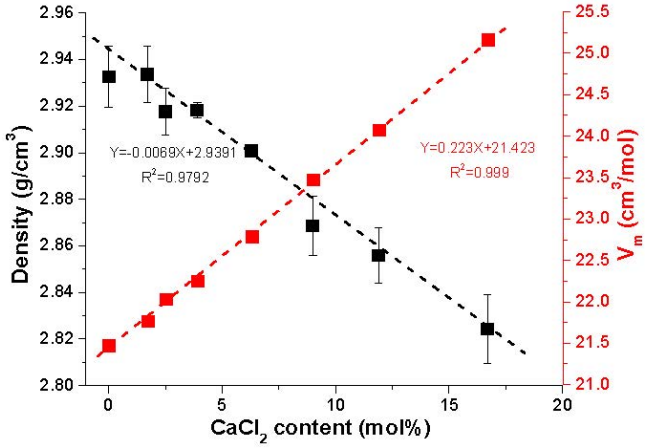

Figure 3: Glass density (T_g (■)) and molar volume of glasses (V_m (■)) profiled as a function of the actual $CaCl_2$ content.

number of 16 or 32. The chemical shift was referenced to the signal of 85% H_3PO_4 at 0 ppm.

3 Results

All the glass frits produced were optically clear. The higher chloride content glasses exhibited a small amount of crystallinity by XRD which is thought to be a result of the fine glass powder surface reacting with atmospheric water to form a mixed hydroxychloroapatite during the collection of the XRD data, since no evidence of crystallinity was found by either ^{31}P MAS-NMR or FTIR [18].

In Figure 1, the percentages of the retained chloride in the initial glasses after volatilization are plotted against the as-designed chloride content. A large fraction 73–87% of chloride has been successfully incorporated into the glasses.

In Figure 2, the glass transition temperature (T_g) of fine powder (< 38 µm) is plotted against $CaCl_2$ content; the data were first presented in [18]. It is clear that T_g values decrease dramatically and linearly (R^2 = 0.9825) with increasing $CaCl_2$ content.

Glass density and molar volume of glasses are also plotted as a function of the actual $CaCl_2$ content (Figure 3). The changes in glass density caused by increasing the $CaCl_2$ content are similar to the change of T_g as a result of increasing $CaCl_2$ content. A continuous reduction of glass density is observed with an increase in $CaCl_2$ content. While the molar volume of glasses increases linearly (R^2 = 0.999) with increasing $CaCl_2$.

Figure 4a shows the pH values measured after 3 and 24 hours immersion in Tris buffer solution for each glass. Interestingly, the glass with a higher $CaCl_2$ content has a higher pH value after 3 hours, while a relatively lower pH value after 24 hours, compared to the compositions with lower $CaCl_2$ content. Overall, a reduction of pH from 3 hours to 24 hours was found; the change was more significant at high $CaCl_2$ content in the glass.

The concentration of calcium in solution measured after 6 hours of immersion plotted as percentage of the total amount in the as-designed composition is presented in Figure 4b. There is a clear increase in the percentage of Ca concentration with an increase in $CaCl_2$ content. For example on increasing the $CaCl_2$ content from 0 to 16.7 mol% the percentage of Ca found in solution increases from 58.6% to 83.2%. The trend is practically linear.

The evolution of phosphate concentration measured in solution with increasing immersion time is shown in Figure 4c. In general, all the $CaCl_2$ containing glasses show similar trend of phosphate concentration with respect to time. The phosphate concentration increases in the first 3 hours and drops down afterwards.

The silicon concentration (mg/l) measured in solution is plotted as a function of immersion time in Figure 4d. It is seen that Si concentration increases up to 60–70 mg/l in the first 6 hours, with no significant change observed after longer immersion period. No substantial difference in measured silicon concentration between different glass compositions is observed.

The XRD patterns of each chloride containing bioactive glass on immersion up to 24 hours in Tris buffer are similar. The one for 1.7 mol% $CaCl_2$ containing glass (GPCl 2.3) is selected and presented as an example in Figure 5a. A broad amorphous halo centred at 30°2θ belongs to the untreated glass (0 h). After immersion for 3 hours, the halo broadens out and the peaks at 25.9°(002) and 32°(211) 2θ,

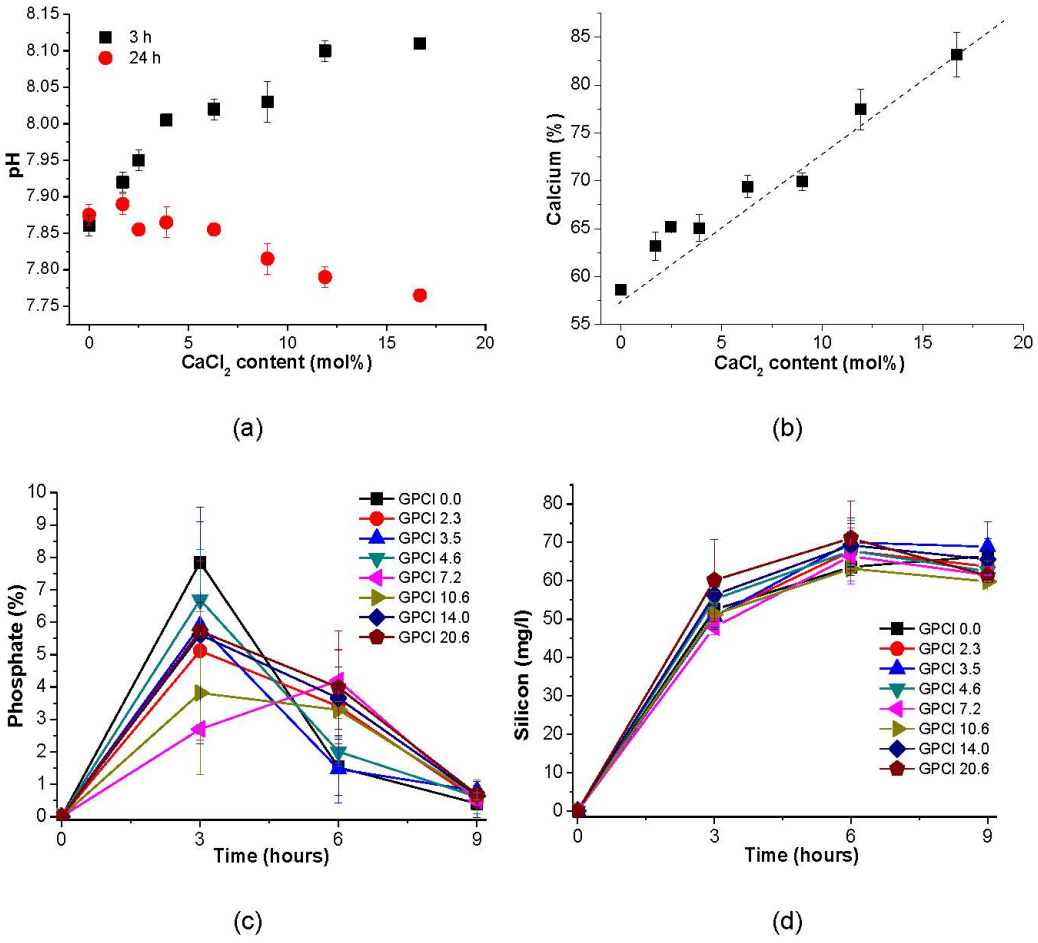

Figure 4: (a) pH value of solution after 3 and 24 hours immersion in Tris; (b) the Ca concentration measured after 6 hours immersion in Tris buffer solution plotted as the percentage of the total calcium content in the as-designed glass composition against the actual $CaCl_2$ content; (c) the concentration of phosphate measured after immersion in Tris buffer presented as the percentage of the total phosphorus content in the as-designed glass composition plotted as a function of immersion time; (d) the concentration in mg/l of silicon measured in Tris buffer plotted as a function of immersion time.

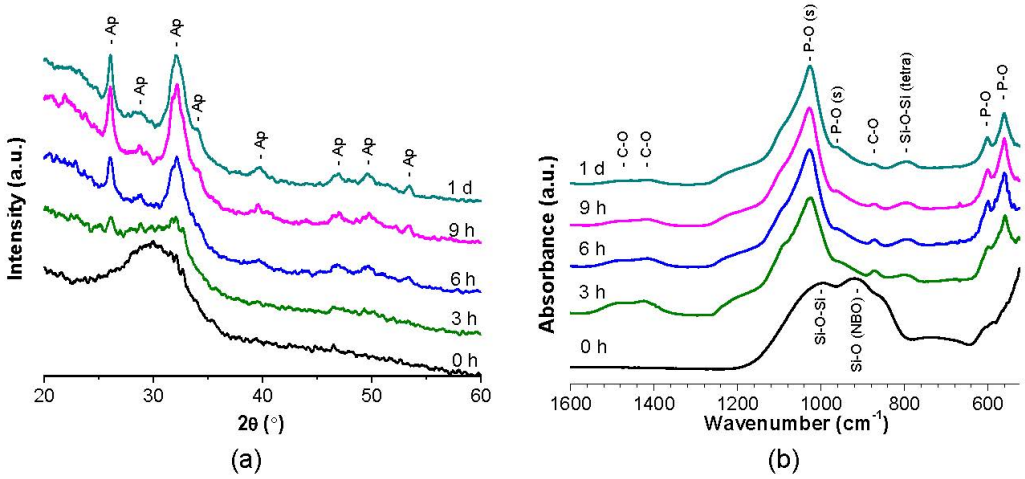

Figure 5: (a) The XRD patterns and (b) FTIR spectra of the 1.7 mol% $CaCl_2$ glass precipitate collected after the immersion in Tris up to 1 day. The numbers are immersion times.

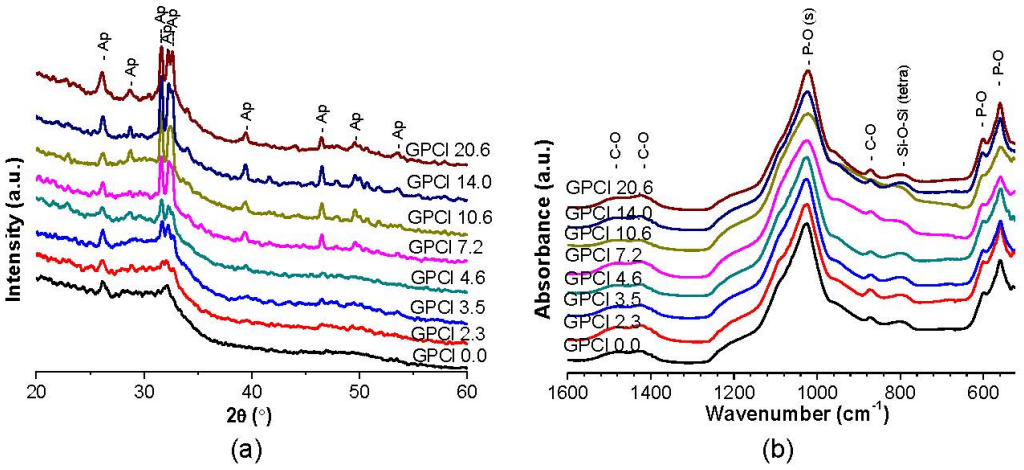

Figure 6: (a) The XRD patterns and (b) FTIR spectra of the glass precipitates collected after 3 hours immersion in Tris buffer solution.

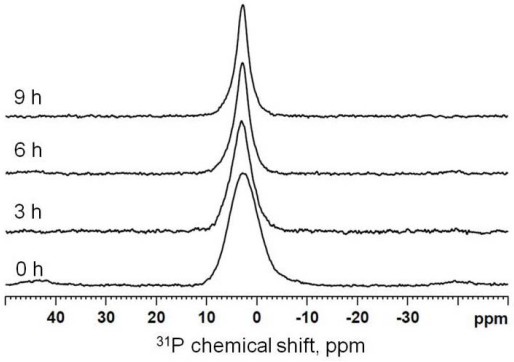

Figure 7: The ^{31}P MAS-NMR spectra of the glass precipitate with 1.7 mol% $CaCl_2$ collected after the immersion in Tris up to 9 hours. The numbers are immersion times.

which match the typical apatite diffraction lines, develop. The intensities of the peaks increase with immersion time up to 9 hours. Longer immersion times do not show any further change in the patterns. After 24 hours, the peak at 25.9°2θ is slightly narrowed down, suggesting the growth of apatite crystals. Similar results were obtained for the rest $CaCl_2$ containing glasses.

In addition, the XRD patterns of glass precipitates after 3 hours immersion are summarised in Figure 6a. It is clear that the peaks at 25.9°, 32° and 32.6°, corresponding to main diffraction lines of apatite appear after 3 hours immersion. The intensities of the diffraction peaks increased with $CaCl_2$ content of the glass. However, the diffraction lines are relatively broad, rather than sharp, indicating the formed apatite crystals are small.

Figure 5b shows the FTIR spectra of 1.7 mol% $CaCl_2$ containing glass precipitate after immersion up to 9 hours in Tris. The bands at 1028 and 920 cm^{-1}, which correspond to Si-O-Si stretching bands and non-bridging oxygen bands (Si-NBO$^-$) respectively, were seen in the spectrum of the untreated glass at the bottom. After 3 hours immersion the spectra change markedly, including the elimination of band at 920 cm^{-1}, the sharpening of band at 1030 cm^{-1}, the development of split peaks at 560 and 613 cm^{-1} and twin peaks at 1420 and 1450 cm^{-1} [9]. These changes suggest rapid glass degradation and apatite-like phase formation. Similar changes to the spectra were found for the rest of the $CaCl_2$ containing glasses as shown in Figure 6b.

^{31}P MAS-NMR was used to investigate phosphorus environment and identify apatite-like phase formation after immersion. The spectra of glass GPCl 2.3 before and after immersion are shown in Figure 7. In the spectrum of the untreated glass, a single peak at about 3 ppm, corresponding to amorphous calcium orthophosphate is visible. Upon immersion, the peaks narrow down, first after 3 hours and then further after 6 hours of immersion. However, the chemical shift remains constant. Similar changes in the spectra of the rest of the glass compositions were observed.

4 Discussion

The results of the chemical analysis of the chloride content indicate that between 70 and 90% of the chlorine is retained in the glasses after melting. Thus, the majority of the chloride is retained in the silicate glass during melting. In general, the higher amount of $CaCl_2$ losses is found in the lower $CaCl_2$ containing glasses. This can be attributed to a higher melting temperature of 1550°C for synthesis of the lower $CaCl_2$ containing glasses (GPCl 2.3 and GPCl 3.5).

Volatilisation of chloride is probable by two routes; direct volatilisation as $CaCl_2$ and loss as HCl by reaction with water vapour in the furnace atmosphere. If chloride volatilises as HCl, the CaO content in the actual glass composition would increase and the SiO_2 and P_2O_5 contents would decrease. Therefore, the glass network connectivity (NC) would decrease with an increase in $CaCl_2$ content and also the glasses would tend to crystallise. If chloride volatilises as $CaCl_2$, the CaO, P_2O_5 and SiO_2 would increase in proportion and the glass NC would remain constant.

The XRD patterns of the initial glasses suggest a very small fraction of glass crystallisation, which is probably caused by reaction with water on the glass surface during data collection rather than crystallisation on quenching [18]. In addition, the ^{29}Si MAS-NMR spectra (Figure S.1 (Supplemental material)) suggest that the obtained glasses have mainly the Q^2 structure and not a mixture of Q^1 and Q^2. Thereby, the chloride loss is likely to occur as $CaCl_2$ rather than HCl. The actual glass compositions have been calculated and summarised in Table 1.

It is important to note here that most chlorides with the notable exception of $CaCl_2$ have low boiling points and this has generally resulted in either no chloride remaining in the silicate glass or very little. Consequently chloride containing glasses have rarely been studied and those that have $CaCl_2$ content typically less than 2 mol%. This contrasts with the glasses here the highest of which contains 16.7 mol% $CaCl_2$.

This work reveals that the incorporation of $CaCl_2$ results in a very marked reduction in the glass transition temperature and density. It is proposed that in this $CaCl_2$ containing glass system, the Cl-Ca(n) species form in an analogous fashion to the F-Ca(n) species in the CaF_2 containing glasses [18, 20, 21]. The formation of Cl-Ca(n) species has been found in very low $CaCl_2$ (< 2 mol%) containing glasses by Sandland *et al.* [22].

The formation of hypothetical "$CaCl^+$" species, which has same field strength as Na^+ but lower than Ca^{2+}, will weaken the electrostatic force between two NBOs in the glass structure. Therefore, the more $CaCl_2$ is introduced into the glass composition, the weaker electrostatic forces between the two NBOs form and the lower is the glass transition temperature. T_g is a significant parameter, which can be used to indirectly predict glass solubility, degradability and hardness of glasses within certain compositional ranges [23–25]. The hardness measurement of bioactive glasses is challenging, because of their tendency to surface react during sample preparation. However, both T_g and glass hardness are determined by the average bond strength in the glass structure, thus hardness and T_g might be expected to exhibit a strong correlation. Farooq *et al.* found that the hardness of the CaF_2 containing bioactive glasses with different amounts of Na_2O reduces with decreasing T_g [25].

Additionally, the packing density which was used to mirror the compactness of the glass can be also used to predict glass hardness [26]. As shown in Figure 3, the glass density decreases with an increase in $CaCl_2$ content. This is due to the fact that the chloride ion is substantially large. Incorporation of chloride therefore increases the glass molar volume and results in a more open and less compact glass structure. Hence, to some extent the presence of chloride dilutes the glass network and expands the glass volume, which leads to a decrease in density and packing density of the glass. Moreover, the formation of large "$CaCl^+$" species in the glasses results in a weakening of the silicate glass network. The effect shown in this study is comparable with the effect on the replacement of Na_2O for CaO (replaced one Ca^{2+} ion by two Na^+ ions) and K_2O for Na_2O (K^+ is larger than Na^+, ion radius: 1.52 Vs 1.16 Å) in the glass. On incorporation of $CaCl_2$, the glass hardness would be expected to decrease, as a consequence of a reduced compactness of the glass. Therefore, the incorporation of $CaCl_2$ results in a reduction in T_g and probably the glass hardness.

The glasses with lower hardness are attractive for the desensitizing toothpastes for treating dentine hypersensitivity. A contributory cause of dentine hypersensitivity is loss of enamel at the cervical margins due to brushing with an abrasive toothpaste. Bioglass® 45S5 has been used in commercial toothpastes as NovaMin® technology for the remineralisation of teeth and for treating dentine hypersensitivity [27]. The hardness of the 45S5 composition is 4.68 GPa, whilst the enamel hardness is about 3.5 GPa only. In order to avoid enamel wear, a glass with a hardness value less than 3.5 GPa is attractive. Chloride containing bioactive glasses with their low T_g are one potential route to achieve this, since the hardness of a glass is expected to correlate to the glass transition temperature. The lower is the T_g, the softer the glass is likely to be. Thus chloride containing bioactive glasses could potentially reduce the abrasivity and enamel loss caused by tooth brushing. Moreover, rapid glass degradation and fast apatite formation found in this study make these chloride containing bioactive glasses also attractive for use in the remineralls-ing toothpastes.

According to the mechanism of dissolution of bioactive glasses proposed by Hench *et al.* [28, 29], the first step is the ion exchange of alkali ions (glass modifier cations) with protons (H^+) from body fluids. In the studied glasses, Ca^{2+} will be exchanged for protons in the Tris buffer during

glass degradation. The removal of protons from solution causes an accumulation of hydroxyl groups; hence, an increase in both Ca^{2+} release and the pH of the Tris buffer in the early period of glass immersion can be explained.

Previous work on CaF_2-containing bioactive glasses by D.S. Brauer *et al.* [20] showed that this pH rise was less pronounced with increasing CaF_2 content in the glass, and we originally interpreted this as caused by another type of ion exchange occurring: hydroxyls (OH^-) from the solution for F^- from the glass in addition to H^+ for modifier cations. If this was the case, however, we would observe the same effect here, with an exchange of OH^- for Cl^- resulting in a lower pH with increasing $CaCl_2$ content in the glass, but we do, in fact, observe the opposite trend. This suggests that halides, such as CaF_2 or $CaCl_2$, present in and released from bioactive glasses contribute indirectly only to pH changes in the dissolution medium. More detailed study [14] suggests that the pH rise typically observed for bioactive glasses is caused by the silicate part (i.e., by ion exchange between modifiers ionically connected to non-bridging oxygens and H^+) only, with contributions from the CaF_2 part being negligible.

The ion exchange process between modifier cations and protons in the present study is aided by incorporation of large chloride ions, which have the capacity to expand the glass network. This more open and expanded glass structure allows ions to diffuse much more easily into and out of the glass and therefore facilitates ion exchange, particularly for short immersion times. As a consequence there is a more rapid release of Ca^{2+} ions and a more rapid rise in pH in the glasses with higher $CaCl_2$ content during the first few hours of immersion. However after a longer immersion period (24 hours), a less pronounced rise in pH of the glasses with more $CaCl_2$ content was found. This can be attributed to a decrease of glass modifier content (here: CaO only), with an increase in $CaCl_2$ content.

The studied glasses were designed by adding $CaCl_2$ to glass, while reducing the amounts of all other components proportionally so as to keep glass structure constant (Q^2 glass structure). In a fixed weight of glass (75 mg) used for the bioactivity study in Tris buffer, the amount of the silicate part of the glass (i.e. $Si\text{-}O^-$ $1/2Ca^{2+}$ bonds) allowing for exchange of modifier ions decreased with an increase in $CaCl_2$ content in the glass. This could result in a reduction of the total amount of ion exchange occurring, therefore a less pronounced pH rise was observed for glasses with higher $CaCl_2$ contents. Additionally, the consumption of hydroxyl ions during the formation of hydroxycarbonated apatite and probably also the dissolution of atmospheric CO_2 lead to a reduction of pH after 24 hours immersion compared to 3 hours.

Rapid glass degradation and release of Ca^{2+} and PO_4^{3-} ions facilitate apatite-like phase formation. Apatite-like phase formation of these $CaCl_2$ containing bioactive glasses was evaluated in Tris Buffer. Unlike simulated body fluid (SBF) and artificial saliva (AS), Tris buffer contains no Ca^{2+} or PO_4^{3-} ions. The Ca and P detected in solution and any apatite detected therefore originate entirely from the glass composition. This considerably simplifies the analysis and interpretation of the degradation/dissolution of the glass. As discussed above, chloride promotes glass degradation as a result of expanding glass volume and the rapid glass degradation provides ions, such as Ca^{2+}, PO_4^{3-} and OH^- for apatite-like phase formation.

From the ion release data, the highest amount of phosphate was present in solution is around 7.85% after 3 hours immersion, this indicates a consumption of phosphate for apatite-like phase formation at an even earlier immersion time than 3 hours. The tests are done under phosphate deficient condition, as with the glass being only source of Ca^{2+} and PO_4^{3-}. The ratio of Ca:P ratio in glasses are 4.4:1, while the Ca:P ratio in apatite is 1.67:1. Therefore, the amount of apatite-like phase formation depends mainly on the phosphate content and high phosphate contents in bioactive glass are favourable for apatite-like phase formation, as also suggested by the earlier finding in paper by Mneimne *et al.* [9].

Brauer *et al.* [20] suggested that the formation of silica gel contributes to the nucleation of apatite, since phosphate concentration decreased when silicon concentration reached the solubility limit. However, as shown in Figure 3d, the silicon concentration reached the solubility limit (60–70 mg/l) after 6 hours immersion and was almost constant there afterwards, while phosphate decreased after 3 hours. This suggests that apatite-like phases might not be necessarily forming only on the surface of silica gel.

All the XRD, FTIR and NMR results suggest rapid apatite-like phase formation within 3 hours immersion in Tris, which is comparable to the equivalent CaF_2 containing bioactive glasses [30]. From the XRD patterns, the diffraction lines developed after 3 hours immersion matched those for the typical diffraction lines of hydroxyapatite, however, the intensities of the three main peaks between 31° to 33° 2θ are not completely identical with those for hydroxyapatite. This suggests the possibility of the presence of CO_3^{2-} or Cl^- in the apatite lattice.

In the apatite crystal lattice, the hydroxyl ion is too large to fit in the space at the centre of Ca(II) triangle and is displaced above the plane of the triangle. The chloride ion is larger than the hydroxyl ion, it displaces even further above the plane of the Ca(II) triangle. Therefore, chlorap-

atite is less stable than hydroxyapatite and is unlikely to form after immersion of the bioactive glass. Furthermore, chlorapatite is known to convert to hydroxyapatite with time in the presence of water [31].

The constant chemical shift (3 ppm) of the glass powders before and after immersion in Tris in the spectra of ^{31}P MAS-NMR indicates that almost all phosphorus is present as orthophosphate charge balanced by Ca^{2+} cations [20]. In the case of before immersion, the amorphous calcium orthophosphate results in a broad amorphous peak, since there are local variations in the local environment around the phosphorus. In the case of after immersion, the calcium orthophosphate peak gradually becomes sharper as the result of the formation of a crystalline calcium orthophosphate (apatite) and the amorphous calcium phosphate species in the glass dissolving. Thus, a progressive reduction in width of the ^{31}P peak was seen with an increase in immersion time.

It is worth comparing the chloride containing bioactive glasses studied here with the closely related fluoride containing glasses [30], both glass series show rapid apatite-like phase formation within 3 hours immersion in Tris. In the case of CaF_2 series, apart from forming fluorapatite on immersion, an additional phase of CaF_2 was also detected in the high CaF_2 containing glasses (over 9.3 mol%). Furthermore, CaF_2 is present as the main fluoride containing phase in the glass with 25.5 mol% CaF_2. The formation of CaF_2 might reduce the bioactivity in terms of the ability to bond to bone. However, in the case of $CaCl_2$ series, the apatite-like phase is the only phase formed after immersion for all $CaCl_2$ contents. In addition, the increase of $CaCl_2$ content stimulates glass degradation, which therefore facilitates apatite-like phase formation. The high bioactivity of the chloride containing glasses with their rapid dissolution rate are attractive for use in toothpastes and resorbable materials, especially bone grafts and scaffolds.

5 Conclusion

In this paper, the bioactive silicate glasses with chloride have been synthesised and studied for the first time. These novel chloride containing bioactive glasses contain up to 16.7 mol% of the chloride thus it is demonstrated that a significant retention of chloride is possible to achieve in this type of the silicate glass. This study has revealed that the chloride containing bioactive glasses are highly degradable and form an apatite-like phase within 3 hours immersion in Tris buffer. Glass degradation rate has been found to increase with $CaCl_2$ content. A reduction of density and an increasing of glass molar volume on increasing $CaCl_2$ suggest that chloride expands the glass volume and dilutes glass network, therefore, promoting ion exchange and glass degradation. The DSC results demonstrated a distinct reduction in the glass transition temperature (T_g) correlating linearly with an increase in chloride content. The decrease of T_g indicates a potential decrease in the glass hardness. These novel chloride containing bioactive glasses have outstanding potential for applications in remineralising toothpastes and resorbable bone substitutions.

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Supplementary Material

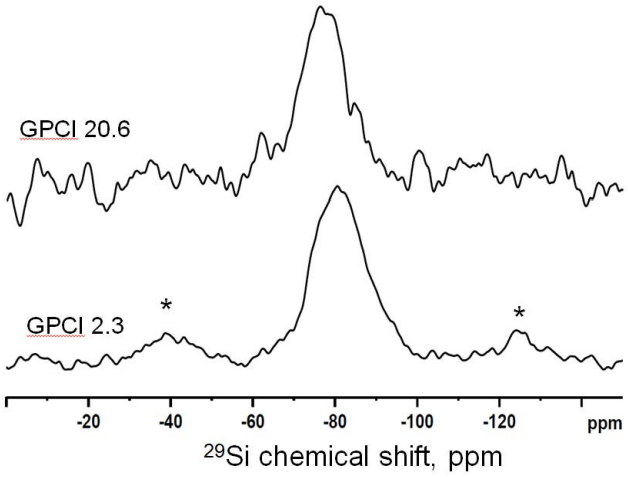

Figure S.1: ^{29}Si MAS-NMR spectra of the as-quenched 1.7 mol% and 16.7 mol% $CaCl_2$ containing glasses.