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Quantification of Anion and Cation Release from a Range of Ternary Phosphate-based Glasses with Fixed 45 mol% P_2O_5

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ABSTRACT: This article reports on the use of ion chromatography (IC) to investigate extensively the release profiles of both cations and anions and characterize the relationship between composition and degradation for a ternary-based Na_2O–CaO–P_2O_5 glass system developed as biomaterials. Studies are carried out on glasses with the formula $45P_2O_5$-55(xCaO–Na_2O) in deionized water, where $x = 30$, 35, and 40 mol%, using a cumulative release method, where the solution is changed at regular intervals.

Degradation behavior is linear with time where the degradation rate shows an initial decrease with increasing CaO content. This rate then increases with a further addition of CaO. Cation release profiles follow similar trends to the degradation rates. Anion release profiles show a decrease for the PO_4 and linear polyphosphate (P_2O_7 and P_3O_{10}) species with increasing CaO content. This decrease is attributed to the cross-linking of the Ca^{2+} ions. In contrast, the cyclic P_3O_9 anion exhibits the highest amount of anionic release, which demonstrates similar trends to the cations. These release patterns suggest that the cyclic P_3O_9 species dominate the degradation rates. The proposed mode of degradation is a hydrolysis reaction, with the cyclic metaphosphate undergoing acid/base catalysis. The pH remains constant for the 30 and 35 mol% CaO glasses, and drops to about 5.5 for the 40 mol% composition. By using a response factor,

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it is possible to semiquantitatively analyze the additional peaks observed in the chromatograms. Suggestions are also put forward as to the identity of some of these unidentified peaks.

KEY WORDS: biodegradable glass, phosphate-based glass, ion chromatography.

INTRODUCTION

Over the past three decades, extensive work has been carried out to produce bioactive implant materials for the tissue regeneration of both hard and soft tissues. An interesting group of materials that show potential in this area are phosphate-based glasses (PBG) [1]. PBG are unique in that they are degradable, and their degradation rate can be tailored to suit the end application [2]. These glasses use phosphorous pentoxide (P_2O_5) as the initial network former and sodium oxide (Na_2O) along with calcium oxide (CaO) as network modifiers [3]. Also, by adding other ions such as iron, further control of the degradation can be achieved [4,5]. Furthermore, Drake and Allen found that PBG with a suitable composition would dissolve in water with zero-order rate constant, and by controlling the composition it was possible to produce glasses which would completely degrade in water from hours to years [6].

Numerous biocompatibility studies have been conducted on PBG [7]. Bitar et al. successfully seeded craniofacial osteoblasts, oral fibroblasts, and hand flexor tendon fibroblasts on PBG. They found that the glasses produced no adverse cell reactions; suggesting they supported both osteoblasts and fibroblasts [8]. Whereas Franks et al. [9] and Salih et al. [10] studied the effects of PBG on MG63 human osteoblast cell lines by carrying out MTT assays. They concluded that the growth of these cells remained largely unaffected in the presence of the glass extracts, and after five days in culture, cell proliferation had increased in some cases. It was also concluded that extracts from the less degradable glass compositions (i.e., ones with greater calcium content) upregulated the proliferation of the cells [9]. Gough et al. on the other hand, investigated the short-term biocompatibility of PBG on a macrophage cell line and primary human craniofacial osteoblasts, and demonstrated minimal macrophage activation with a high osteoblast biocompatibility. They showed that osteoblasts were able to attach, spread, and proliferate in a manner comparable to the positive control along with the formation of a collagen-rich mineralized matrix [11].

A common theme found in the above studies was that glasses with the lowest degradation rates (i.e. high CaO content) appeared to enhance

cell proliferation and attachment. It has been reported that calcium ions (Ca^{2+}) play a key role in cell activation mechanisms, thereby controlling many growth-associated processes and functional activities of cells [10]. This suggests that upon degradation, glass compositions with higher CaO content release higher amounts of Ca^{2+} ions, thus increasing biocompatibility. However, it is well-known that tissue culture media also contain large amounts of Ca^{2+} ions. Therefore, this study proposes that both anions and cations released through the degradation of the glasses would have a positive effect with regard to biocompatibility. For this reason, the aim of this investigation is to fully establish the species and amounts of ions released into solution through degradation using ion chromatography (IC) analysis. Previously IC had successfully been used to characterize polyphosphates and was mainly used on the phosphate manufacturing and food processing industries [12–14]. However, studies thus far on PBG are incomplete [15,16].

MATERIALS AND METHODS

Glass Preparation

The glass compositions were prepared using NaH_2PO_4, $CaCO_3$, and P_2O_5 (BDH, UK) as starting materials. Table 1 gives a list of the compositions investigated. The precursors were weighed and placed into a 200-mL volume Pt/10%Rh crucible type 71040 (Johnson Matthey, Royston, UK). The crucible was placed in a furnace at temperatures between 1000 and 1050°C for 1 h. The glass melt was then poured into a graphite mold, which had been preheated to 370°C. The mold was placed back into the furnace and left to slowly cool to room temperature, to remove any residual stresses. The 15-mm diameter glass rods obtained from the mold were cut into 2-mm thick disks using a Testbourne diamond saw. See Table 1, for glass codes and melting and annealing temperatures.

Table 1. Glass codes, melting, and annealing temperatures used.

Glass Code	P_2O_5 (mol%)	CaO (mol%)	Na_2O (mol%)	Melt Temp/Time (°C/h)	Temp Annealed
30 mol% Cao	45	30	25	1000/1	370
35 mol% Cao	45	35	20	1000/1	370
40 mol% Cao	45	40	15	1050/1	370

Degradation Studies

The surface area of the glass disks was calculated from the dimensions obtained via Mitutoyo Digimatic Vernier callipers. Three repeat specimens were placed into 25 mL of deionized water and kept in an incubator at 37°C. The degradation studies were conducted using ultrapure water (18.2 MΩ cm^{-1} resistivity) obtained from a PURELAB UHQ-PS (Elga Labwater, UK). At various time points (1, 4 and then every 24 h) the disks were taken out of their respective containers and weighed after the excess moisture was removed by blotting with a tissue. These were then placed in a fresh solution of deionized water and replaced in the 37°C incubator. The experiments were conducted for up to approximately 250 h. The data were presented as weight loss per unit area against time and the weight loss is given by the equation $(M_0 - M_t)/A$, where M_0 is the initial weight of the sample, M_t is the weight at time t, and A is the surface area (mm^2).

Ion Release Studies

The solutions produced through the degradation studies were analyzed using ion chromatography for cation and anion release.

Cation Release

For cation release measurements, an ICS-1000 ion chromatography system (Dionex, UK) was used. A 20 mM MSA (Methanesulfonic acid, BDH, UK) solution was used as the eluent. The ICS-1000 is an integrated and preconfigured system that performs isocratic IC separations using suppressed conductivity detection. In this method, cations were eluted using a 4 × 250 mm IonPac® CS12A separator column, a 25-μL sample loop, and a Cation Atlas® Electrolytic Suppressor (CAES). All results were calculated against a four-point calibration curve using the predefined calibration routine. Calibration solutions were prepared from sodium chloride (Sigma, UK) and calcium chloride ($CaCl_2 \cdot 2H_2O$) (BDH, UK) and a 100 ppm (parts per million) mixed (sodium and calcium) stock solution was prepared, from which serially diluted 50, 20, 10, and 1 ppm standard solutions were obtained. Data analysis was performed using the Chromeleon® software package.

Anion Release

The phosphate anion measurements were carried out using a Dionex ICS-2500 ion chromatography system (Dionex, UK), consisting of a gradient pump with a 25-μL sample loop. In this method,

polyphosphates were eluted using a 4×250 mm IonPac® AS16 anion exchange column packed with anion exchange resin. A Dionex ASRS® (Anion Self-Regenerating Suppressor) was used at 242 mA. The Dionex EG40 eluent generator equipped with a KOH (potassium hydroxide) cartridge was used in conjunction with the ASRS®. The newly developed EG40 eluent generator system electrolytically produces high-purity KOH eluents using deionized water as the carrier stream at the point of use. The use of the EG40 hydroxide eluent generator leads to negligible baseline shifts during the hydroxide gradients, along with greater retention time reproducibility. The sample run time was set for 30 min The gradient program started from 30 mM KOH, and after 2 min increased from 30 to 60 mM KOH over 10 min, and remained there for 13 min. After this, it decreased down to 30 mM for 5 min. The Chromeleon® software package was used for data analysis. Only four reagents were commercially available for the preparation of standards – sodium phosphate tribasic (Na_3PO_4), trisodium trimetaphosphate ($Na_3P_3O_9$), pentasodium tripolyphosphate ($Na_5P_3O_{10}$), (Sigma, UK) and tetrasodium pyrophosphate ($Na_4P_2O_7$) (BDH, UK). A 100 ppm working solution containing all of the above four reagents was prepared, from which serially diluted 50, 20, 10, and 1 ppm standard solutions were obtained. Higher phosphate group containing reagents (i.e., P_4 or above) were not commercially available.

pH Measurements

The glass disks were transferred to a fresh solution and pH measurements were taken at every point of time. The pH of the fresh solution (deionized water) was adjusted to neutral before being dispensed and the measurements were taken using a Hanna Instruments pH 211 Microprocessor pH meter (BDH, UK) with an attached glass combination pH electrode (BDH, UK). The pH electrode was calibrated using pH calibration standards (Colourkey Buffer Solutions, BDH, UK).

RESULTS

Degradation Studies

Figure 1 shows that all three compositions exhibited linear degradation behavior against time. There was a decrease in the degradation with an increase in CaO from 30 to 35 mol%, followed by an increase in degradation with an increase in CaO content to 40 mol%.

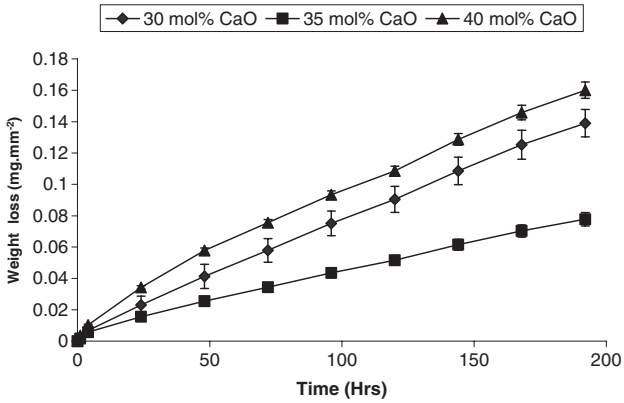

Figure 1. Weight loss vs time for glass compositions investigated.

Cation Release

Figure 2(a) and (b) shows that approximately equal amounts of sodium and calcium ions were released into the solution for all three glass compositions. For the 30 mol% CaO glass, higher amounts of sodium ions were released as compared to the 35 mol% one. The amount of sodium ions released then increased with a further increase of CaO to 40 mol%. The calcium ion release curves showed that the highest release was seen for the CaO to 40 mol% glass, which was expected as this composition contained the highest amount of calcium. However, the lowest calcium ion release was seen for the 35 mol% CaO glass. A correlation was observed between the weight loss behavior and calcium ion release data.

Anion Release

Figure 3(a)–(d) shows the structures of anions investigated. Figure 4(a)–(d) shows their trends of release. It was observed that the trends for the PO_4, and the linear P_2O_7 and P_3O_{10} anions were all similar where the release decreased with an increase in the CaO content, or with a decrease in the Na_2O content. The highest anion release was seen for the cyclic P_3O_9 metaphosphate anion. The release for this anion correlated with the sodium ion release graph.

pH Values

Figure 5 shows that the pH values remained relatively neutral through out the study, with the exception of the glass composition with 40 mol% CaO where the pH value dropped to around 5.5.

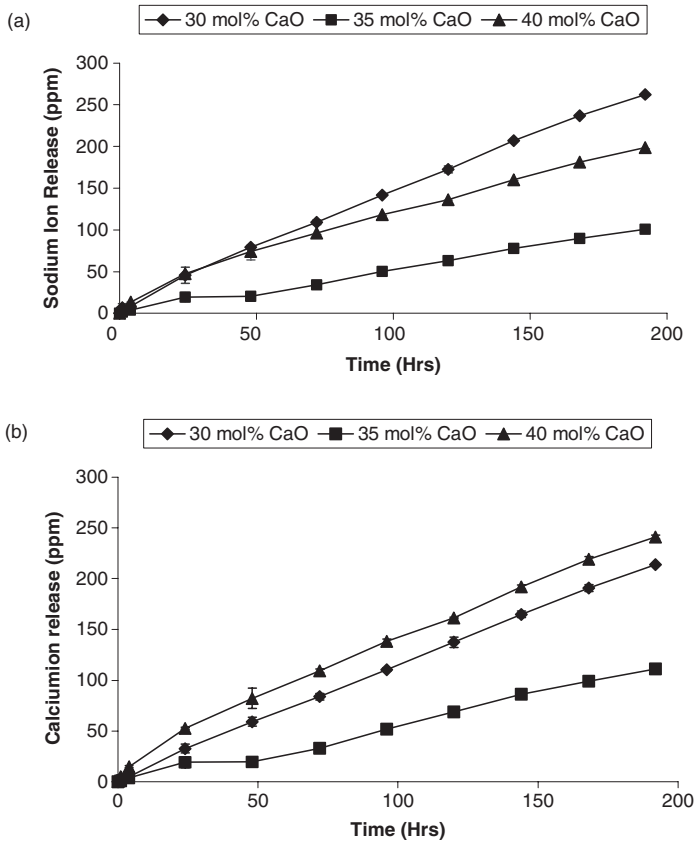

Figure 2. (a) Sodium ion release vs time for glass compositions investigated and (b) calcium ion release vs time for glass compositions investigated.

DISCUSSION

This study investigated the relationship between degradation and ion release for a group of ternary phosphate-based glass compositions with increasing CaO content. A cumulative degradation and release analysis was carried out, where the solution was replaced at 1, 4 and then every 24 h for up to approximately 250 h and the data obtained showed a linear relationship with time. Therefore, it was possible to calculate the rate of degradation, and the rate of ion release for both the cationic and the anionic species. Figure 6 shows the relationship between these rates and the CaO content. As can be seen, the cation rates of release data showed similar trends to the weight loss data. However, the Ca^{2+} ion release

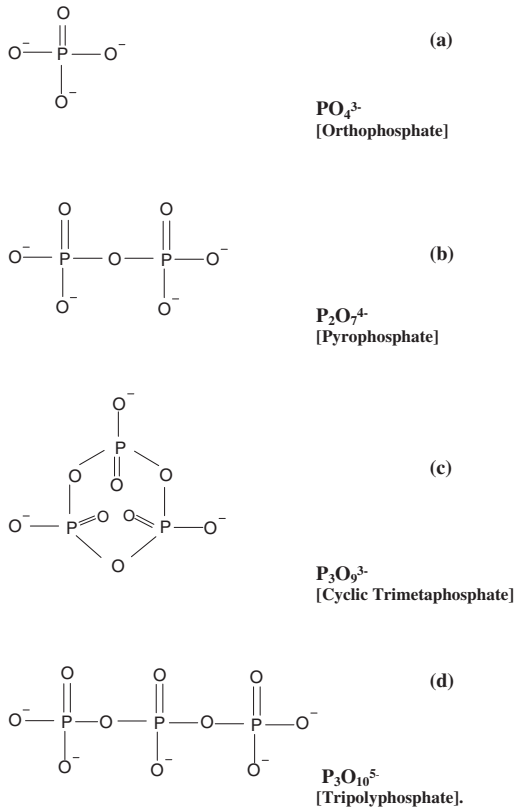

Figure 3. Structures of anions investigated.

profile showed an identical trend with the weight loss data, whereas the Na^+ ion release profile showed a trend identical to the P_3O_9 anion release. This cumulative study was found to be a more accurate form of analysis compared to a previous study [2], (carried out in a static environment) as no precipitation buildup was experienced. However, in the previous study, a decrease in the rate of weight loss was seen with increasing CaO content for the same compositions, and in the present one a decrease was seen with an initial increase in the CaO content, after which the rate of weight loss increased again for the 40 mol% CaO glass. The patterns observed in the degradation rates were attributed to structural differences in the compositions, as two phases were identified from the XRD analysis [3] for the fixed 40 mol% CaO composition, which were $NaCa(PO_3)_3$ and CaP_2O_7. Furthermore, phase diagrams showed that this composition lay very close to a eutectic point [17–19].

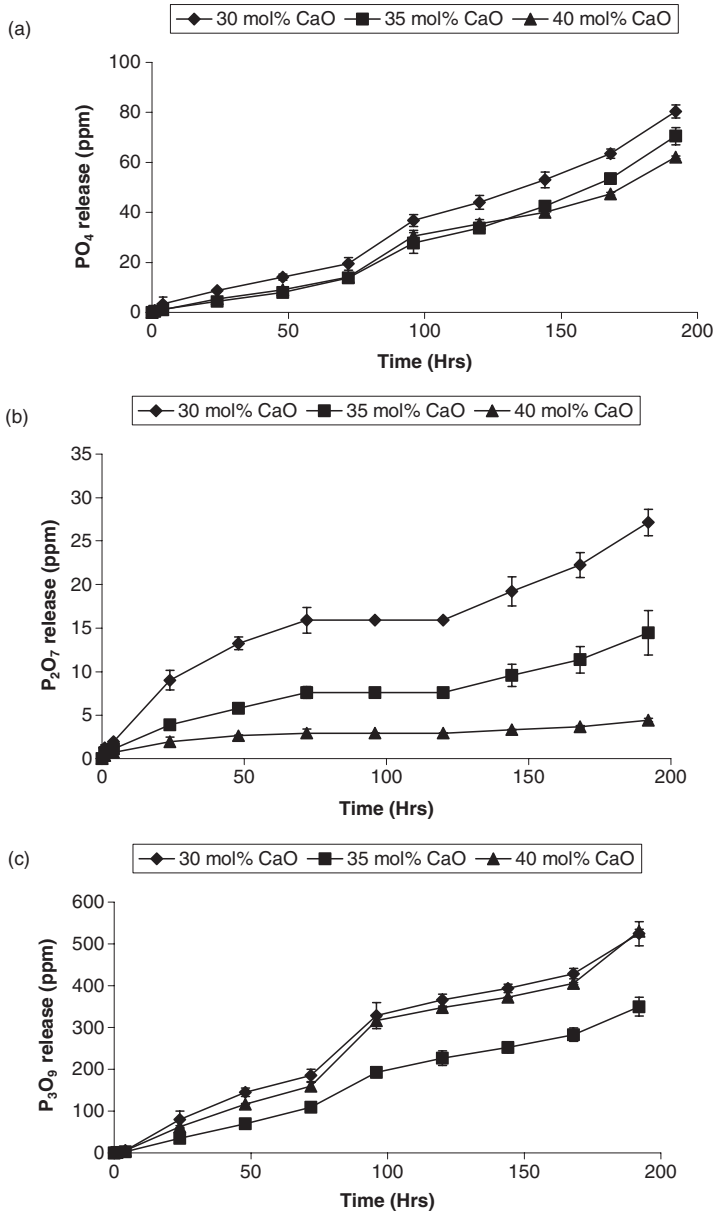

Figure 4. (a) Orthophosphate (PO_4) release vs time for glass compositions investigated; (b) Pyrophosphate (P_2O_7) release vs time for glass compositions investigated; (c) Cyclic (P_3O_9) metaphosphate release vs time for glass compositions investigated; and (d) Linear (P_3O_{10}) polyphosphate release vs time for glass compositions investigated.

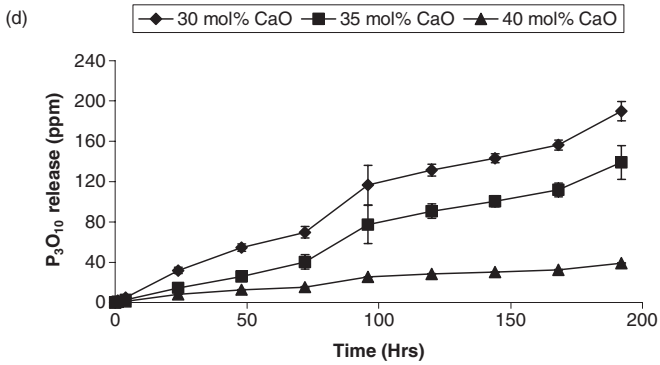

Figure 4. Continued.

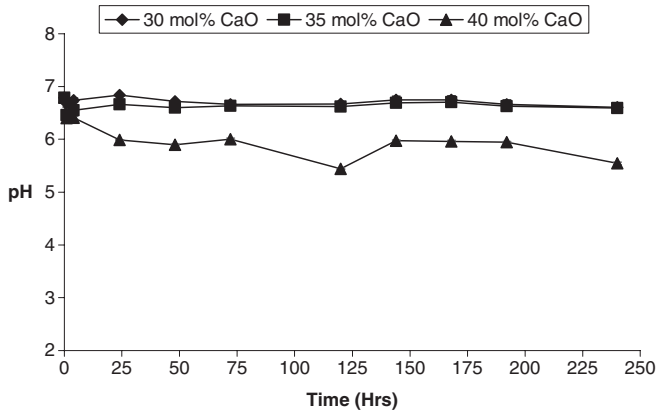

Figure 5. pH change vs time for glass compositions investigated.

The anion release curves showed that all three compositions indicated a decrease in the rates of release for the orthophosphate PO_4 and the linear (P_2O_7 and P_3O_{10}) phosphate species with increasing CaO content. This observation suggested that this was due to the fact that these anions were branched, and thus cross-linked with the Ca^{2+} ions. This has previously been observed, and attributed to the Ca^{2+} ions forming chelates that have strong complexing abilities with the linear polyphosphates [20]. However, the highest rate of release was seen for the P_3O_9 anionic species as shown in Figure 6. This suggested that the P_3O_9 anion dominated the degradation process and that there was a significant proportion of these fragments present in the original glass structure with most, if not all, of the P_3O_9 unbranched, with the remaining

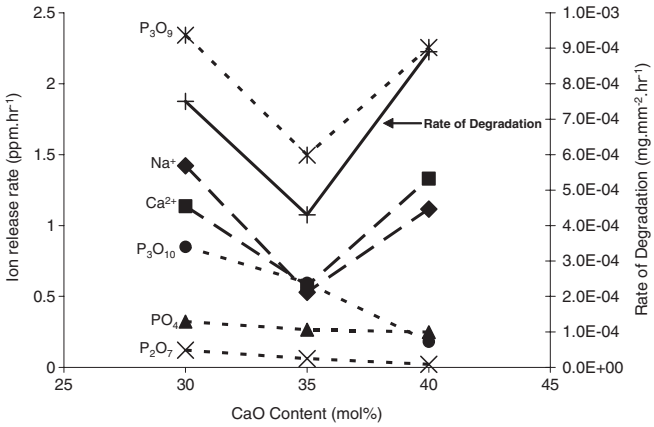

Figure 6. The relationship between the cation and anion and rates of release, and rate of degradation as a function of CaO content.

negative charges on the anions ionically bonded with the Na^+ ions. The correlation seen with the rate of release for the Na^+ ions and the P_3O_9 anion release, seemed to confirm this. Studies conducted by Wazer and Holst [21] showed that straight chain and unbranched ring phosphate anions could be found in aqueous solutions of phosphoric acids.

It has been stated that three types of reactions can occur in the degradation mechanisms of PBG [22], which are acid/base, hydrolysis, and hydration reactions. Hydrolysis reactions are the cleaving of P–O–P bonds and this mechanism exhibits clear pH dependence. Furthermore, Watanabe et al. stated that the rate of hydrolysis of small ring cyclic trimeta- and tetrametaphosphates decreased in acidic solutions, and increased in basic solutions with an increase in the pH value for all solvents. Therefore, the hydrolysis of these small-ring metaphosphates was an acid/base catalyzed reaction in all solvents [23]. Also Wen and Jijian [24] stated that for a solution with a $pH \geq 5$, the degradation of PBG is controlled by the hydrolysis process of the phosphate chain. As is seen from Figure 5, the pH for compositions with 30 and 35 mol% CaO remained neutral for the duration of the study, whereas for the composition with 40 mol% CaO, the pH dropped to about 5.5. Therefore, the proposed mode of degradation for the compositions investigated in this study was the hydrolysis reaction with the P_3O_9 anionic species undergoing acid/base catalysis.

In this study, an attempt was made to quantify the previously unidentified longer chain polyphosphates detected in solution as a result of glass degradation. Figure 7 shows a typical chromatogram obtained

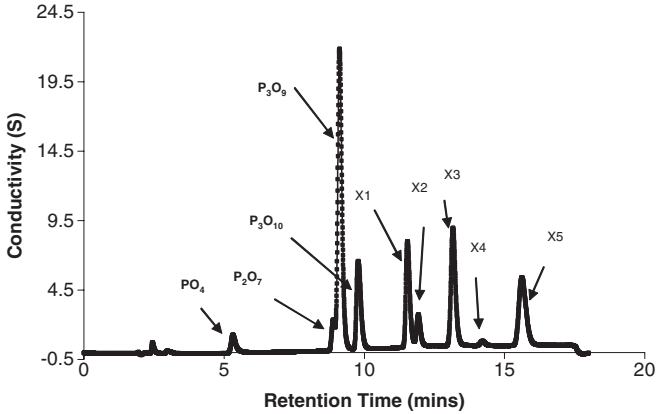

Figure 7. An example of a chromatogram obtained for anion release measurements.

from the ICS-2500. In general, up to nine peaks were seen in the chromatograms, however, only four were identifiable, and the extra peaks observed were labeled as peaks X1–X5. Since the majority of the peaks were eluted before the 30 min time point, this was set as the sample run time. In turn, this minimized the possibility of hydrolytic degradation of the phosphate species present in the solution. Stover et al. [25] suggested that by using the area under the peaks, it was possible to semiquantify the additional phosphate peaks. They compared the results obtained from peak area-average chain lengths obtained via IC analysis, with ^{31}P NMR, and potentiometric pH titrations. The IC results correlated with average chain lengths determined by the other methods for up to $\check{n} = 15$, where $\check{n}$ is the number average chain length. This gave an indication of the accuracy to be expected at different $\check{n}$ values. Stover et al. also suggested that the methods used to obtain chain length numbers above, yielded number-average rather than mass-average chain lengths. Polyphosphates with $\check{n} < 5$ (P_2O_7 and P_3O_{10}) showed approximately equal conductivity: mass response factors determined by injecting known amounts of standard compounds. Therefore, by using a Response Factor (RF) given by the gradient of peak area (A) versus concentration (C) obtained from the P_3O_{10} calibration (Figure 8), it was possible to quantify the additional eluting polyphosphate species. However, if there were any cyclic metaphosphates present, these could also be quantified via the RF value obtained from the cyclic trimetaphosphate (P_3O_9) calibration. Therefore, through the use of RF, peaks labeled $X1$–$X5$ were quantified and Figures 9 and 10 show the release of $X1$ and $X4$ respectively. It is reasonable to assume

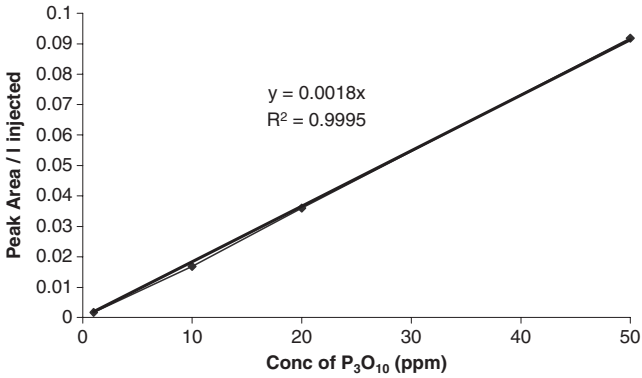

Figure 8. An example of Response Factor obtained for a P_3O_{10} calibration.

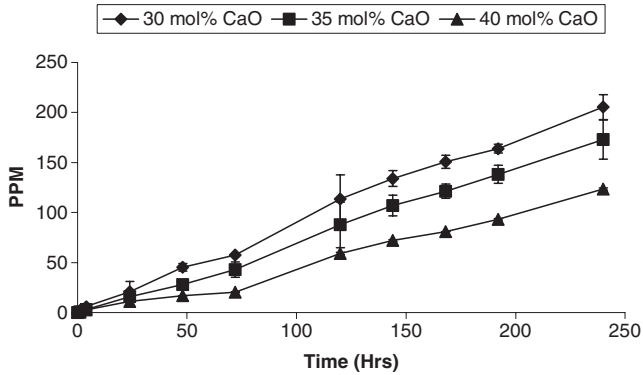

Figure 9. Anion release vs time measured from peak labeled *X*1.

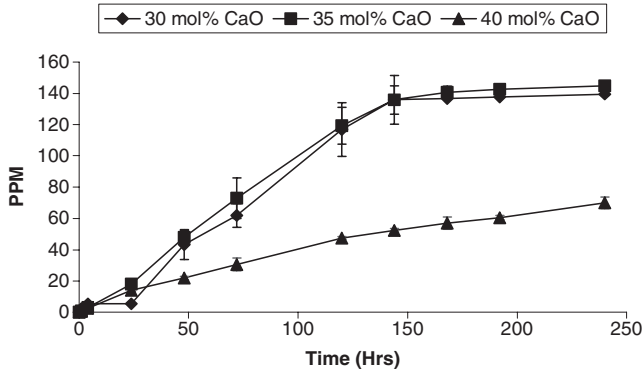

Figure 10. Anion release vs time measured from peak labeled *X*4.

that $X1$ is a linear anionic chain species (P_4O_{13}), due to the correlation seen from the rates of release, which show a decrease with increasing CaO content, as was observed with the linear phosphate species, earlier in this study (see Figure 9). The same correlation was also observed for $X3$ and $X5$. The $X4$ peak appears to be a cyclic species, due to exhibiting rates of release patterns similar to the P_3O_9 species suggesting that this could be a cyclic P_6O_{18} species (see Figure 10). XRD data conducted in a previous study [2] showed that the main phase identified from the compositions with fixed 30 and 35 mol% CaO content, was a $Na_4Ca(PO_3)_6$. This phase was not identified for the 40 mol% CaO glass, hence the difference seen in rates of release. The same trend was seen for $X2$, however, it cannot be unequivocally stated whether this peak was a cyclic tetrametaphosphate (P_4O_{12}) species or a P_5 anionic species.

CONCLUSIONS

- The degradation rates for a ternary-based Na_2O–CaO–P_2O_5 glass showed an initial decrease with increasing CaO content, which then increased with a further addition in CaO. The cation release profiles exhibited similar trends to the degradation rates obtained.
- A decrease in the rate of release was seen for the PO_4 and the linear (P_2O_7 and P_3O_{10}) polyphosphate species with increasing CaCO content. It was suggested that these anions were branched and cross-linked with the Ca^{2+} ions.
- The P_3O_9 anionic species exhibited the highest rates of release which also correlated with the trends seen in the cation release profiles, suggesting that this anionic species dominated the degradation rates and that there was a significant proportion of this fragment present in the original glass structure. It was also suggested that the P_3O_9 was unbranched, with the Na^+ ions taking up the residual charge on this anion, due to the correlation seen between sodium ion release and P_3O_9 anion release.
- The proposed mode of degradation was the hydrolysis reaction, with the small cyclic metaphosphates undergoing acid/base catalysis.
- The pH of the degradation solution remained fairly neutral for the fixed 30 and 35 mol% CaO compositions, and dropped to about 5.5 for the fixed 40 mol% compositions.
- By using Response Factors it was possible to quantify the additional peaks observed in the chromatograms. Suggestions were also put forward as to the identity of some of the unidentified peaks. However,

these suggestions can only be verified via obtaining the appropriate standards.

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