JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **11** (2000) 609–614

# Development of soluble glasses for biomedical use Part I: *In vitro* solubility measurement

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In this study soluble glasses have been developed for biomedical applications containing $P_2O_5$ as a network former and CaO and $Na_2O$ as modifiers. This study shows that as expected, the glasses have an inverse exponential relationship between solubility and CaO content. Furthermore, there is evidence for compositional related non-linearity in the dissolution of the glasses with time. This is thought to be due to either layer formation on the glass surface hindering ion diffusion, ion exchange process or change of ionic strength of the solution. Bioactivity of these glasses is indicated by the formation of a brushite precipitate, a precursor to apatite formation. Further evidence for bioactivity is also presented in the second part of this paper.

## 1. Introduction

Extensive work has been carried out to produce a bioactive implant material for bone replacement and/or regeneration. This work has centered around non-degradable apatite based materials [1].

Many glasses and glass-ceramics used as surgical implants involve the use of $SiO_4^{4-}$ ions as the network forming anion. Bioglass®, a silica rich glass for biomedical use, has been developed to replace hard or even soft tissue. By regenerating and then forming a stable attachment to the surrounding tissue this material has potential as a long-term device. The biocompatibility of Bioglass® is initiated by a double layer which consists of a calcium phosphate and a silica-rich layer when placing this material in simulated physiological solution or living tissue [2]. The first developed calcium phosphate layer is than transformed to an apatite layer.

However, the long-term reaction, both locally and systemically especially to silica is still unknown. An ideal replacement material would be chemically related to the surrounding tissue, would be biocompatible and bioactive and would be dissolved at the same rate at which the new tissue was regenerated. Furthermore, this would remove the need for a second operation to explant the device as commonly occurs with stainless steel devices [3] and periodontal devices based on PTFE membranes [4]. Advances have been made with periodontal devices, by using degradable polymers based on polyglycolic and polylactic acid polymers [5–7]. However, laboratory [5, 7] and clinical studies [6] indicate that the degradation is extremely non-linear and very unpredictable. This is due to the mechanism by which they degrade [6, 7].

An interesting group of materials that may offer potential for hard tissue surgery are phosphate-based glasses. These are soluble and altering their composition may control their solubility. Preliminary work on stoichiometric crystalline glass-ceramics by Driessen *et al.* showed clearly that rhenanite, a calcium sodium phosphate ($CaNaPO_4$) will transform to apatite within 6 weeks after implantation [8]. According to Videau *et al.* [9] brushite, related to monetite ($CaHPO_4$), octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$) and defective hydroxyapatite one of the most stable precipitations will be transformed into hydroxyapatite as the most stable thermodynamic product at pH 7. This work indicates the potential of sodium-calcium-phosphate based materials for implants, but are limited by their stoichiometry. The work presented here is a study of a basic ternary system, the aim being to correlate composition with solubility and to investigate the ion release and the bioactivity of the glasses. Because these glasses are soluble, ions will leach out into solution to aid the apatite formation. This feature will help to incorporate devices into natural phases of hard tissues. Using different chemical ratios will give different solubility rates to cover the requirements for hard and soft tissue regeneration.

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TABLE I Glass codes and oxide composition in mol %

| Glass Code | CaO content (mol %) | $Na_2O$ content (mol %) | $P_2O_5$ content (mol %) |
|---|---|---|---|
| $Ca_{12}Na_{43}P_{45}$ | 12 | 43 | 45 |
| $Ca_{16}Na_{39}P_{45}$ | 16 | 39 | 45 |
| $Ca_{20}Na_{35}P_{45}$ | 20 | 35 | 45 |
| $Ca_{24}Na_{31}P_{45}$ | 24 | 31 | 45 |
| $Ca_{28}Na_{27}P_{45}$ | 28 | 27 | 45 |
| $Ca_{32}Na_{23}P_{45}$ | 32 | 23 | 45 |
| $Ca_{36}Na_{19}P_{45}$ | 36 | 19 | 45 |

## 2. Materials and methods

### 2.1. Glass preparation

Seven glasses were prepared with the compositions as shown in Table I, using $P_2O_5, NaH_2PO_4, CaHPO_4$ and $CaCO_3$ as starting materials. The precursors were weighed out and then placed in a Pt/10%Rh crucible that was then placed in a furnace at between 1100 and 1200 °C for one hour. This procedure was repeated several times to give three glass rods that have been melted together at temperatures shown in Table II. Upon removal, the glass was poured into a graphite mold preheated to 300–400 °C. The mold was then placed in a furnace at appropriate temperatures and slowly cooled to room temperature to remove any residual stress. The actual processing temperatures are shown in Table II. Having obtained glass rods, these were cut into discs of 15 mm diameter and 2 mm thickness, using a Testbourne diamond saw. Glasses were made with CaO content below 12 mol % CaO, but the solubility was too high and did not allow enough data points to be collected to give statistically meaningful results.

### 2.2. Solubility test

Two sets of experiments were carried out to determine the solubility. The first experiments were carried out in distilled water. Glass discs were taken and their surface area was measured prior to placing them in glass containers. Distilled water to a volume of 25 ml was added to the containers and these were then incubated at 37 °C. At various time points, the samples were removed and excess moisture removed with a tissue and then weighed. As the solubility is affected by surface area, the data was presented as weight loss per unit area. To obtain the rate of weight loss, the initial weight $(M_o)$ of each sample was measured as well as the weight loss at time $t(M_t)$ to give a weight loss per unit area thus:

$$\text{Weight loss: } (M_o - M_t)/A \qquad (1)$$

Where $A =$ surface area $(\mathrm{cm}^2)$. The measurements were carried out in triplicate. This data was then plotted as weight loss per unit area against time. The slope of this graph gives solubility in terms of $\mathrm{mg\,cm^{-2}\,hr^{-1}}$. A second set of experiments were carried out under exactly the same conditions except in Hanks Buffered Saline Solution (HBSS) (Gibco BRL, Scotland). For both solubility experiments, the pH of the solution was also measured. All measurements were carried out up to a maximum of 8 weeks.

## 3. Results

Fig. 1a–c shows the measured solubility values of the selected glasses in distilled water. Increasing CaO content in the glasses produces a rapid decrease in solubility as determined by fitting a line to the data and determining the slope. Of further interest is the deviation of the weight loss with time from a linear change to non-linear with increasing CaO content.

Fig. 2a–c shows the measured solubility values of the glasses in HBSS. These are the same glasses as shown in Fig. 1a–c and should be compared. The solubility follows a more linear relationship even for the high CaO containing glasses compared to the tests in distilled water, which were highly non-linear.

Fig. 3 is a comparative graph of the calculated solubility values in distilled water and HBSS. As can be seen, the solubility of the glass shows an inverse exponential relationship with CaO content. Furthermore, the glasses in distilled water show higher solubility for the same glass measured in HBSS.

Fig. 4 shows the change in pH with time of the distilled water in which the specimens were degraded. There are two aspects to this graph. First, there is, as expected, a relationship between solubility and the pH of the solution, when compared at a discrete time point, with the high solubility glasses, i.e. low CaO content, giving high pH values. Secondly, some glasses (low CaO

TABLE II Melting and casting temperatures for glasses studied

| Code with chemical specification in mol % | Melting temperature and time (°C/hours) | Casting temperature (°C) |
|---|---|---|
| $Ca_{12}Na_{43}P_{45}$ | 1050/3 | 300 |
| $Ca_{16}Na_{39}P_{45}$ | 1050/3 | 300 |
| $Ca_{20}Na_{35}P_{45}$ | 1050/3 | 300 |
| $Ca_{24}Na_{31}P_{45}$ | 1050/3 | 380 |
| $Ca_{28}Na_{27}P_{45}$ | 1150/3 | 380 |
| $Ca_{32}Na_{23}P_{45}$ | 1150/3 | 380 |
| $Ca_{36}Na_{19}P_{45}$ | 1150/3 | 380 |

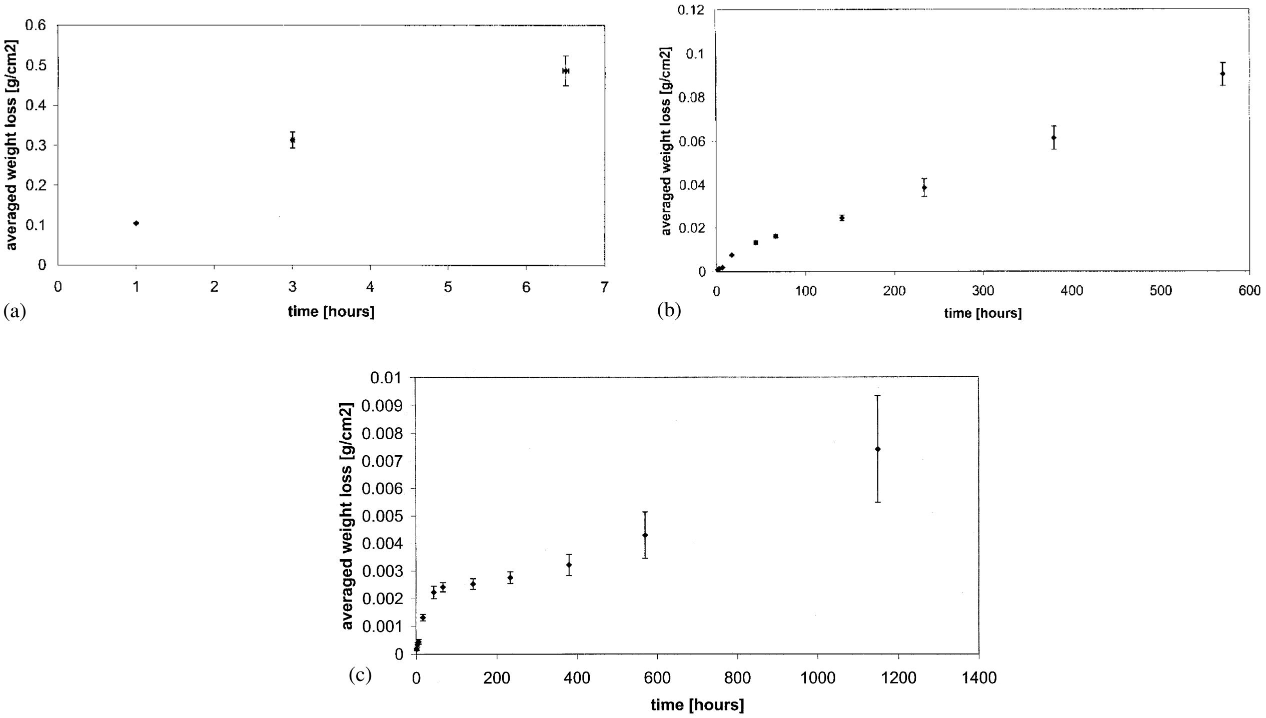

*Figure 1* Weight loss per unit area against time for (a) $Ca_{12}Na_{43}P_{45}$, (b) $Ca_{24}Na_{31}P_{45}$ and (c) $Ca_{36}Na_{19}P_{45}$ measured in distilled water.

content, i.e. high solubility) give non-linear changes in pH with time. Initial values are high, and these drop with time, but the rate of pH change decreases. The low solubility glasses give almost linear changes in pH with time. The pH values are not presented for the HBSS, because the solution is buffered and so the pH changes were minimal.

Fig. 5a and b shows the process of $Ca^{2+}$ ion release over a period of 6 weeks. In the first diagram which plots the $Ca^{2+}$ ion levels against time for the glass with 12 mol % CaO the ion level increases until it reaches its peak point of 0.7 ppm (240 h) at which point, precipitation occurs and this precipitation can be seen in solution. In Fig. 5b, the ion concentration continues to increase and no precipitation occurs to cause a drop in the ion levels.

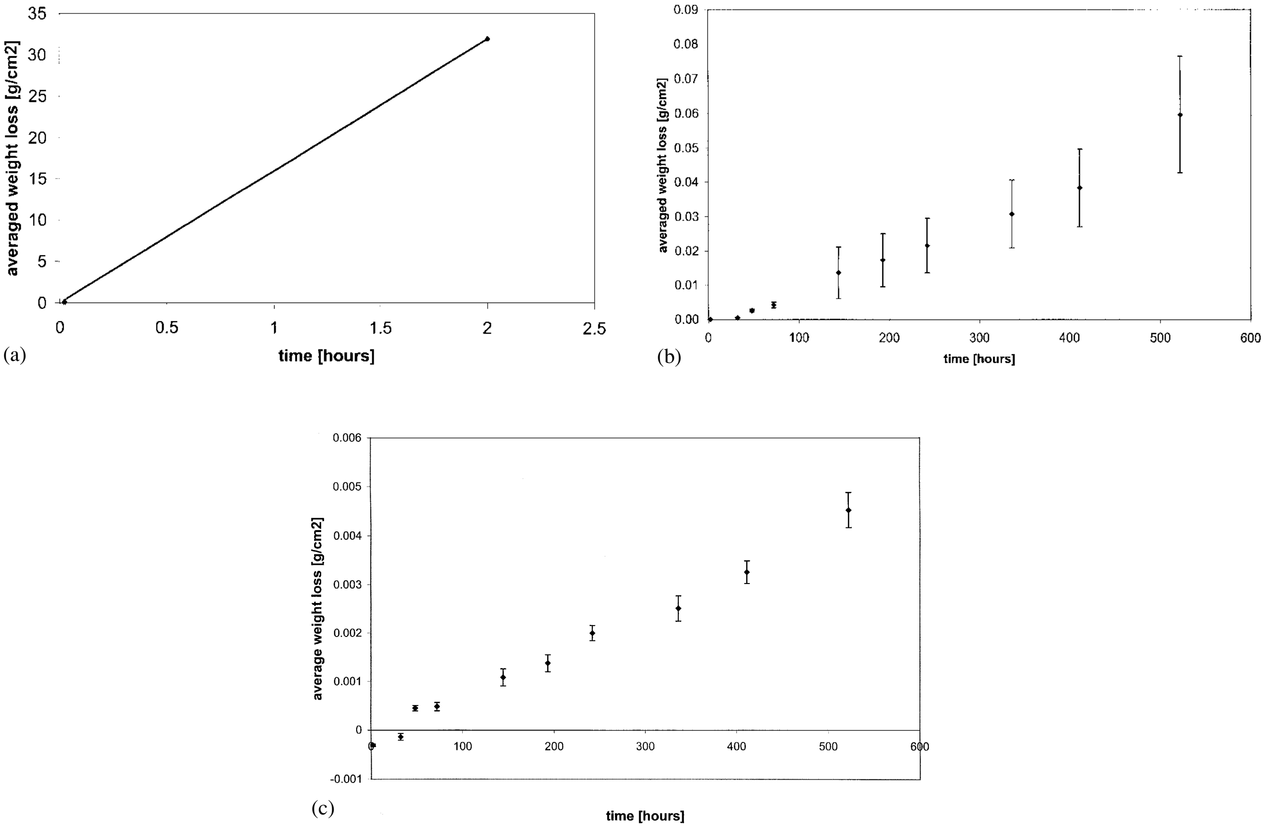

*Figure 2* Weight loss per unit area against time for (a) $Ca_{12}Na_{43}P_{45}$, (b) $Ca_{24}Na_{31}P_{45}$ and (c) $Ca_{36}Na_{19}P_{45}$ measured in HBSS.

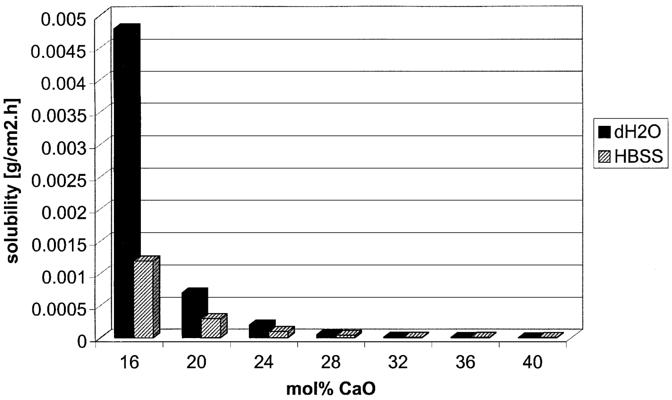

*Figure 3* Solubility values plotted for the different glass compositions and in different aqueous media.

Fig. 6a and b monitors the $Na^+$ release process. Similar to the $Ca^{2+}$ release the $Na^+$ release increases, until at 6500 ppm (240 h), it reaches its peak and the first precipitation takes place. In Fig. 6b, the $Na^+$ ion level is very low and no precipitation took place.

## 4. Discussion

Bunker *et al.* [10] prepared glasses with chemical composition ranging from $xNa_2O$ $(55\text{-}x)CaO$ $50P_2O_5$ (mol %) giving solubility rates of $10^{-5}$–$10^{-6}$ mg $cm^2hr^{-1}$.

In this work, glasses were prepared in the range $xNa_2O\text{-}(55\text{-}x)CaO\text{-}45P_2O_5$ and these gave glasses in the same solubility range in distilled water as expected from the study of Bunker. However, this work reports that the dissolution of metaphosphates is independent of glass composition, which is not the case in our study. It should be noted that as the CaO content increases and hence the solubility decreases, the dissolution with time appears to become more non-linear. Delahaye *et al.* [11] investigated dissolution kinetics on phosphate glasses and postulated the decrease of dissolution rate as due to an increase in ionic strength of the solution. The $Ca^{2+}$ ion measurement curves seem to mirror the weight loss process. Glasses with higher CaO content release more $Ca^{2+}$ ions with time into the solution and therefore show a higher decline in the dissolution rate because they can increase the ionic strength. Hence the composition of the glass influences the dissolution rate. It is also possible to be caused by an ion exchange process, however this would have a $t^{1/2}$ kinetic, which was not observed. The possibility of a crystalline layer formation on the glass surface which would slow the process down can only be verified for glasses with a CaO content of ∼ 20–28 mol %. High soluble glasses dissolve too quickly to form a layer and high CaO containing glasses do not form this layer at all [11].

Of interest is the effect that the HBSS has on the solubility of the same glass. The HBSS reduces the solubility and this is expected, as the ion concentration in solution will slow down the diffusion of ions from the glass. However, it should be noted that for glasses with a CaO content below 16 mol %, the glasses appear to have a higher solubility in HBSS compared to distilled water. The mechanism for this has been proposed as being due to the $Cl^-$ ions in the HBSS strongly reacting with the $Na^+$ from the glass, thus significantly increasing the solubility.

The pH value varies between 5–9 depending on the composition (see Fig. 4). All glasses show an increase in pH in the first few hours followed by a decrease towards neutral. High solubility glasses show a higher increase than the lower soluble glasses and show overall higher pH values for the whole duration of the study.

All the glasses gave a precipitate into solution. However, the precipitate was highly amorphous and thus the phases could not be determined. However, by annealing at 400 °C the precipitate may be unambiguously identified as $Ca_2P_2O_7$ (Fig. 7). This may indicate the formation of brushite, as on heating, the following reaction occurs:

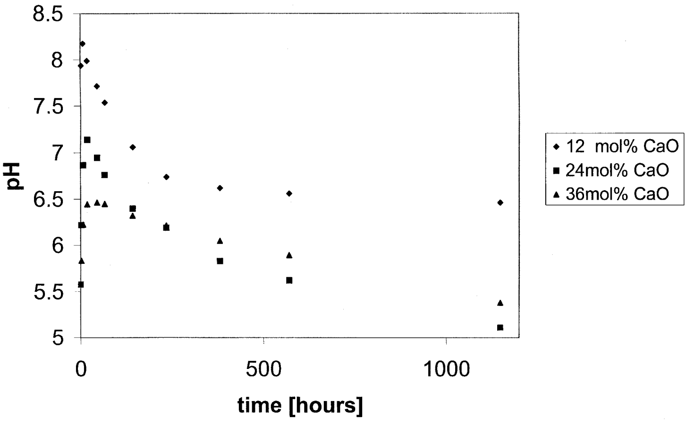

*Figure 4* Change in pH of the distilled water with time plotted for the three glasses $Ca_{12}Na_{43}P_{45}$, $Ca_{24}Na_{31}P_{45}$ and $Ca_{36}Na_{19}P_{45}$.

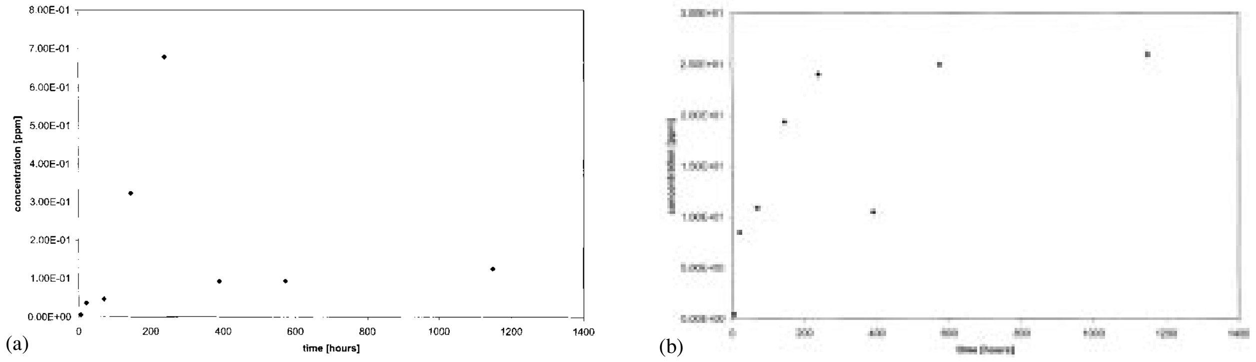

*Figure 5* Change in $Ca^{2+}$ ion concentration in distilled water with time for (a) $Ca_{12}Na_{43}P_{45}$ and (b) $Ca_{36}Na_{19}P_{45}$.

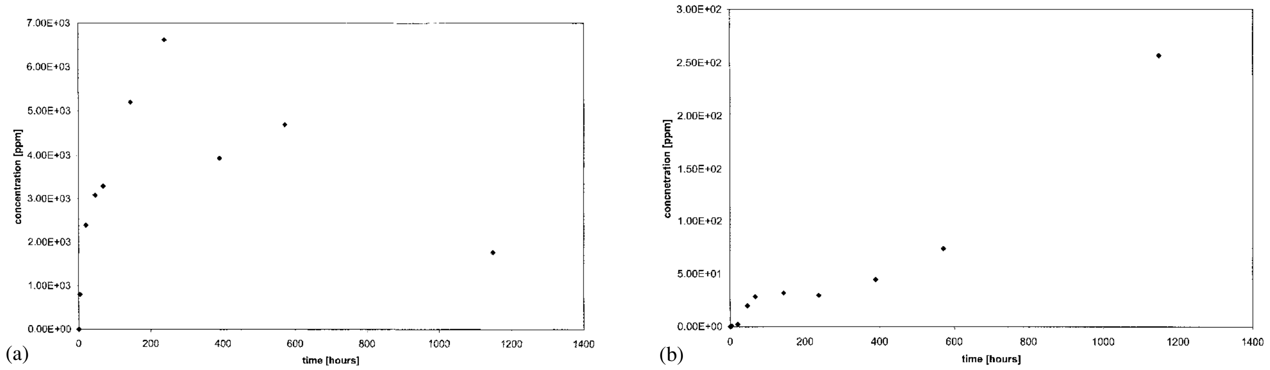

*Figure 6* Change in $Na^{2+}$ ion concentration in distilled water with time for (a) $Ca_{12}Na_{43}P_{45}$ and (b) $Ca_{36}Na_{19}P_{45}$.

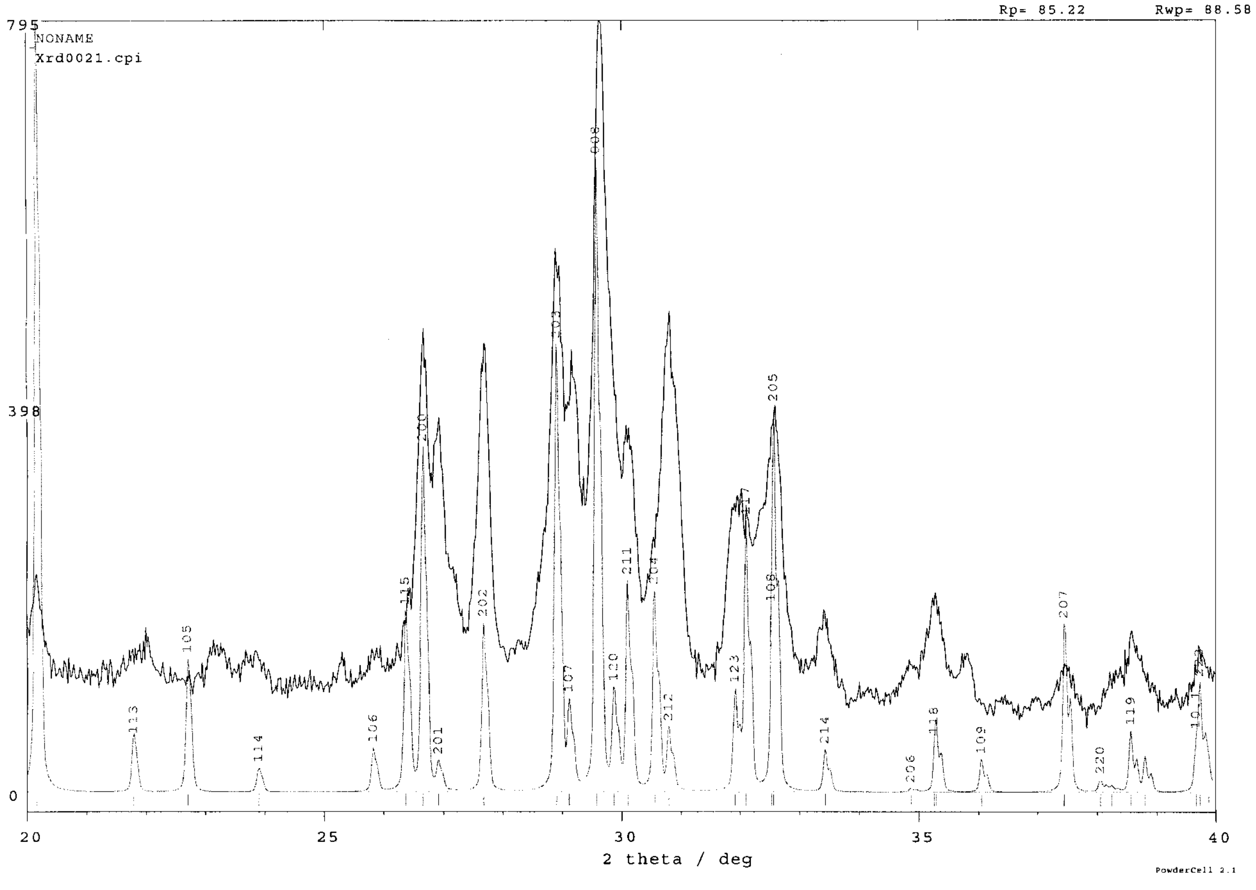

*Figure 7* X-ray diffraction data from annealed precipitate from solution for glass $Ca_{16}$ $Na_{39}$ $P_{45}$.

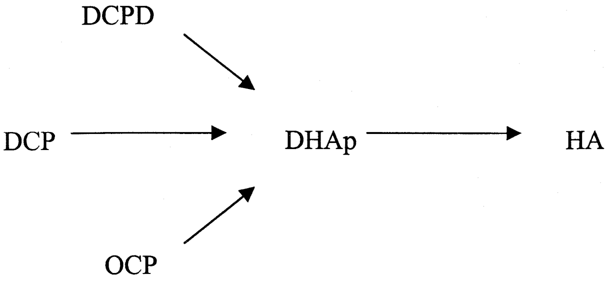

*Figure 8* Maturing pathways for formation of HA, where DCPD is dicalcium phosphate dihydrate (brushite), DCP is dicalcium phosphate (monetite), OCP is octacalcium phosphate, DHAp is defect apatite and HA is hydroxyapatite.

$$CaHPO_4 \cdot 2H_2O \text{ (brushite) } \rightarrow CaHPO_4\text{(monetite)} + H_2O \qquad (2)$$

and

$$2CaHPO_4 \rightarrow Ca_2P_2O_7 + H_2O \qquad (3)$$

According to Videau *et al.* [9] various pathways of maturing calcium phosphate precipitation have shown that hydroxyapatite (HA) is the end product. They studied a system prepared of mixed $Ca^{2+}$ and $PO_4{}^{3-}$ ions in solution at RT and pH of 4–5. They found a calcium phosphate ($CaHPO_4 \cdot 2H_2O$, brushite) formed. Other pathways were found and are shown in Fig. 8.

In order for this to be formed, both $H^+$ and $OH^-$ ions need to be consumed from the solution, which explains the initial high pH values found. With time these drop to around neutral values or slightly below and this is explained by the inversion of the $CaHPO_4$ to HA to decrease the pH within a period of 8 weeks.

If there is only $CaHPO_4$ to be formed then one should expect a precipitation occurring in the high CaO containing glasses, which is not the case. The explanation might be found in the low $Na^+$ ion level, which is present in this solution. In order to precipitate material, both ions must be present. Under this assumption the X-ray analysis should reveal a sodium-calcium-phosphate which was not the case. Therefore the first preliminary X-ray analysis has to be verified in future studies.

## 5. Conclusions

The solubility of these glasses is dependent on the composition and the medium on which the test has been carried out. Glasses with a higher calcium content show a slower solubility than glasses with higher sodium content. The pH measurements reveal the same compositional dependence. This is an important factor considering further *in vitro* studies where cells are sensitive to the pH of the medium.

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*Received 21 June 1999*
*and accepted 17 November 1999*