

CONCENTRATION DEPENDENCE OF BIOACTIVE GLASS DISSOLUTION *IN VITRO*

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Abstract. The effect of glass dosage (0.001gml^{-1} to 0.015gml^{-1}) on the *in vitro* dynamic dissolution behaviour of melt-derived 45S5 and sol-gel-derived 58S bioactive glasses, in simulated body fluid (SBF) at 37°C , was evaluated. These glasses differ significantly in texture, especially the specific surface area and porosity, as a result of differences in manufacturing route. The concentrations of elements (Si, Ca, P and Na) leached from the glasses into the dissolution medium, from 1 to 22 hours, were evaluated using Induced Coupled Plasma analysis (ICP). The reacted powders were analysed using FTIR to observe the formation of a hydroxycarbonate apatite layer on the surface. The results show that the rate of HCA formation on both gel- and melt-derived bioactive glass powders *in vitro* depends on the concentration of the powders in solution. This result must be taken into account when carrying out *in vitro* cell culture studies to simulate conditions *in vivo* and in experiments using extracts of the bioactive glass powders.

Introduction

The ability of bioactive glasses to bond to both soft and hard tissue and promote bone growth has been well documented for melt-derived 45S5 and sol-gel derived 58S bioactive glasses both *in-vitro* and *in-vivo*^[1]. This behaviour has been related to the formation of a biologically active hydroxycarbonate apatite layer (HCA) on the surface of the glasses. The conditions of *in vitro* tests have been shown to be very important in determining the bioactivity and degradation of bioactive glasses^[2]. The rate of glass powder dissolution and the thickness and formation rate of HCA layer depend on the glass composition, glass texture (porosity and surface area), particle size, temperature, pH, agitation rate and the type of medium used^[3]. Specific *in vitro* culture tests, using primary human osteoblasts, revealed that a critical concentration of silicon ions leached from bioactive glasses (16 ppm from 45S5 melt-derived Bioglass[®]) can be used to up regulate growth factor proteins and enhance bone growth^[4]. It is therefore important to be able to predict the release of biologically active ions, from bioactive glass powders that will be placed in the body as either bone filler materials, injectables or incorporated into a scaffold.

The aim of this work is to investigate the effect of glass concentration on the dissolution rates and the rate of formation of the surface HCA layer on melt-derived 45S5 and sol-gel derived 58S bioactive glass powders. The results will be used to define the conditions in which *in vitro* bioactivity and degradation tests should be carried out to characterise new bioactive glasses or scaffolds.

Experimental Procedure

Melt-derived 45S5 (46.1% SiO_2 , 24.4% Na_2O , 26.9% CaO and 2.6% P_2O_5 , in mol) ($<5\mu\text{m}$) and sol-gel-derived 58S (60% SiO_2 , 36% CaO and 4% P_2O_5 , in mol) ($<20\mu\text{m}$) bioactive glass powders were provided by US Biomaterials. The particle size distribution of the powders, specific surface area, porosity, the skeletal density and textural features of the powders were investigated in previous work [1] and are summarised in table 1.

Aliquots of 0.05, 0.075, 0.10, 0.25, 0.50, 0.75 grams of each powder were immersed in 50ml of simulated body fluid (SBF) and placed in an orbital shaker at 37°C, for 1h, 2h, 4h and 22h at an agitation rate of 60Hz. These concentrations correspond to 0.001, 0.0015, 0.002, 0.005, 0.010 and 0.015 gml^{-1} respectively. Three samples were run per test, with the average values reported. A pilot study showed the agitation rate did not significantly affect the dissolution rate.

Extracts obtained by filtration were analysed by ICP (Inductive Coupled Plasma Spectroscopy) and the formation of a hydroxycarbonate apatite (HCA) layer on the dried powders was observed by XRD and Fourier transform infrared (FTIR) spectroscopy.

Results and Discussion

The textural characterisation of the powders used are summarised in table 1. The equivalent particle size at a cumulative percentage of 50%vol powder ($D_{50\%}$) of the 45S5 melt-glass was 3.4 μm , with a specific surface area of 2.7 m^2g^{-1} . The 58S gel-glass exhibited a $D_{50\%}$ value of 6.7 μm and specific surface area two orders of magnitude higher at 126.5 m^2g^{-1} than the 45S5 melt-derived glass powder. This was due to the 58S gel-glass exhibiting a mesoporous texture (average pore size of 6.6 nm), whereas the porosity of the 45S5 melt-glass was negligible. The true density of the two powders was approximately 2.7 gcm^{-3} .

Figure 1 shows plots of the chemical species released from 45S5, after immersion in SBF at 37°C for concentrations ranging from 0.001 gml^{-1} to 0.015 gml^{-1} for 1, 2, 4 and 22 hours. The results show that the silicon, calcium and sodium content in SBF generally increased as glass content in solution increased, whereas phosphorous concentration decreased due to phosphate ions migrating to the glass surface to form a Ca-P rich layer. The plots for 58S gel-glass powders (not shown) showed similar relationship, although calcium release was approximately three times greater. The change in pH of the solution as a function of glass concentration (not shown) also increased as glass concentration increased due to the greater quantities of cations available for ion exchange.

The deposition of calcium-phosphate on the surface of the glass particles was observed by FTIR analyses. Figure 2 shows FTIR spectra for various concentrations of 45S5 powders reacted in SBF for 22 hours. Sharp phosphate peaks at 1060 cm^{-1} (P-O stretch), 571 cm^{-1} and 603 cm^{-1} (P-O bending), indicating HCA formation, were present at glass concentrations between 0.001 gml^{-1} and 0.002 gml^{-1} , but decreased in intensity as glass concentration increased. From glass concentrations of 0.005 gml^{-1} to 0.015 gml^{-1} , the twin P-O bend peaks were not present; instead a single peak corresponding to amorphous calcium phosphate was observed at 571 cm^{-1} and 603 cm^{-1} . The amorphous peak could represent the presence of precursors to hydroxyapatite, such as octocalcium phosphate (OCP) or amorphous calcium phosphate (ACP). The P-O stretch peak was also not present; instead a Si-O-Si stretch was observed, implying that HCA formation was inhibited at high glass concentrations. This was confirmed by the XRD spectra shown in figure 3. An HA peak was observed at all concentrations, but a calcium carbonate (calcite) peak was also observed at glass concentration of 0.015 gml^{-1} . This, combined with the decrease in HCA formation with increasing glass concentration observed from the FTIR, implies that calcite has formed at the expense of HCA formation at high glass concentrations. The reason for this is proposed to be the increase in the ratio of calcium to phosphorous ions in solution as glass concentration increased, from approximately 6 Ca/P at a glass concentration of 0.001 gml^{-1} to 130 Ca/P at a glass concentration of 0.015 gml^{-1} (figure 1). The phosphorous and calcium ions migrate from the solution to the surface of the glass to nucleate HCA precursors. Therefore at high concentrations, when there are excessive calcium ions in solution, calcium carbonate forms at the expense of HCA formation.

Figure 4 shows FTIR spectra for various concentrations of 58S powders reacted in SBF for 22 hours. Phosphate peaks at approximately 1060 cm^{-1} (P-O stretch) 570 and 600 cm^{-1} (P-O bending), corresponding to crystalline HCA formation, were present at all glass concentrations. However the intensity of the P-O bending peaks became less sharp as glass concentration increased. A carbonate peak at 875 cm^{-1} increased in intensity as glass concentration increased. This could demonstrate carbonate being incorporated into the apatite layer to form HCA or some calcite precipitation.

However, the inhibition of HCA formation was less pronounced for the 58S gel-glasses because gel-glasses exhibit a mesoporous texture (pores in the range 2-50nm), in which the mesopores act as nucleation sites for HCA layer formation.

Conclusions

The rate of HCA formation on both gel- and melt-derived bioactive glasses in vitro depends on the concentration of the powders in solution, as well as other factors such as composition, surface texture and particle size. As 45S5 melt-glass concentration increased above a value of 0.002gml^{-1} in SBF the concentration of active ions in solution increased but the rate of HCA layer formation decreased due to calcite forming at the expense of HCA. This result must be taken into account when carrying out in vitro cell culture studies to simulate conditions in vivo and in experiments using extracts of the bioactive glass powders.

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Table 1 Data of powder characterisation: equivalent spherical diameter at the cumulative volume percentage of 50% ($D_{50\%}$), measured by laser spectrometry; specific surface area (SA) and pore size obtained by nitrogen adsorption.

Sample	$D_{50\%}$ μm	SA m^2g^{-1}	Pore Size \AA
45S5	3.4	2.70	--
58S	6.7	126.5	65.5

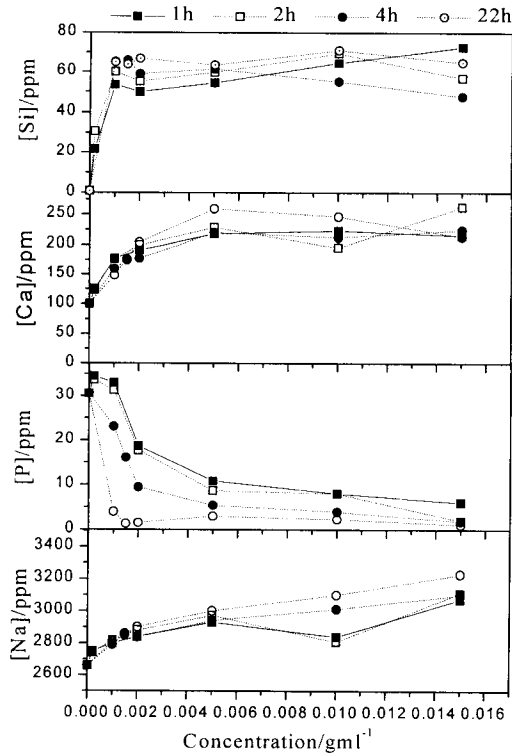


Figure 1. ICP plots of elemental concentration in solution against glass concentration for 45S5 melt-glass powders reacted in SBF at 37°C for 1h, 2h, 4h and 22h.

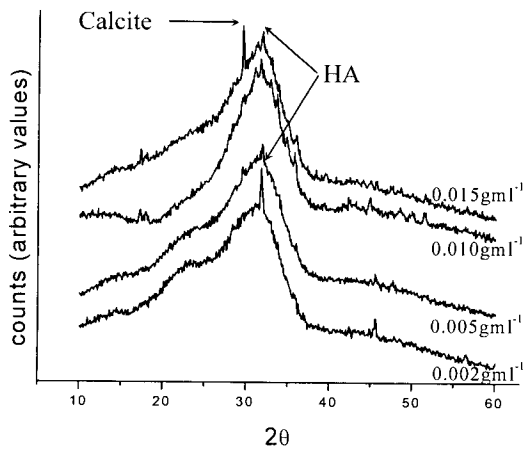


Figure 3. XRD spectra of 45S5 powders immersed in SBF at various concentrations for 2 hours at 37°C.

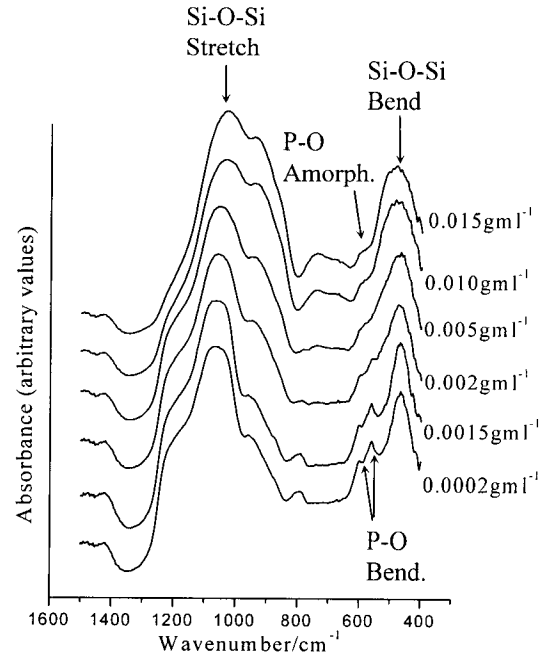


Figure 2. FTIR spectra of 45S5 powders immersed in SBF at various concentrations for 22h at 37°C.

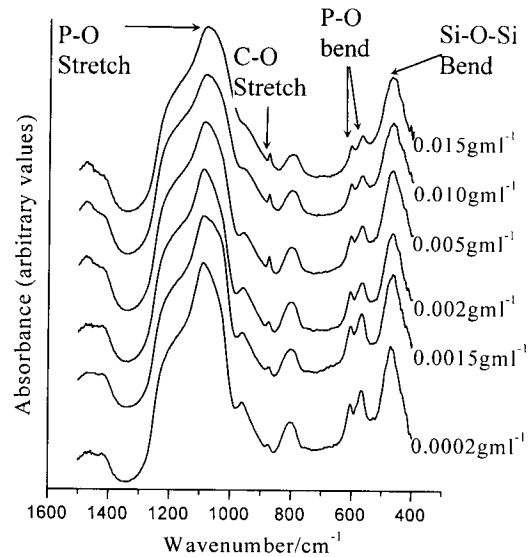


Figure 4. FTIR Spectra of 58S powders immersed in SBF at various concentrations for 22h at 37°C.

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