Effect of varying phosphate content on the structure and properties of sol-gel derived SiO$_2$–CaO–P$_2$O$_5$ bio-glass

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Abstract. In this work, biocompatible glass (bioglass) particles were prepared by low temperature, acid catalysed sol-gel method. The effect of varying phosphate (P$_2$O$_5$) content (10, 15 and 20 mol %) in the sol-gel derived glass composition were studied. The sol-gel derived bioglass particles were compacted into cylindrical pellets via hydraulic press machine and sintered at 600°C for 3 hours. The bioglass particulates were analysed by x-ray fluorescence (XRF), Fourier Transformed Infrared (FTIR), X-Ray Diffraction (XRD) and nitrogen gas adsorption. Meanwhile, the sintered bioglass pellets were analysed by FTIR, XRD and FESEM-EDX. Furthermore, in vitro bioactivity analysis was performed by immersion in simulated body fluid (SBF) for 14 days. Bioglass particulates with high glassy phase, high surface area and high porosities were obtained for all compositions. Increasing of phosphate content to 20 mol% particularly reduced the porous characteristics of the bioglass particulates. Furthermore, leads to higher bridging oxygen (BO) atoms, higher amorphous silicate networks, lower glass crystallinity and higher number of phosphate crystallites within the amorphous glassy matrix. Increased to 20 mol% of phosphate also reduced the ability of the bioglass surface to induce carbonated apatite formation when immersed in simulated body fluid (SBF) solution.

1 Introduction

Most biocompatible glasses show bioactive properties due to its ability to form bonds formation with bone and soft tissue without having fibrillar connective tissue between them during in vivo test. A common characteristic of bioactive glass is the formation of a carbonated apatite layer on their surface when they are in contact with a physiological fluid,
which is very similar to the apatite in bone thus produced biological fixation between the implant and living bone [1]. Kokubo and Takadama confirmed that in vivo apatite formation can be simulated in vitro using an artificially prepared solution called simulated body fluid (SBF), having ion concentrations being approximately equal to those of human blood plasma. SBF was initially developed by Kokubo for initial prediction about the bioactivity and dissolution behaviour of bioactive materials by examining their apatite-forming ability in the fluid [2]. Recent trend in research studies has been ongoing to explore the application of bioactive glass in cancer treatment [3].

Bioactive glasses are conventionally prepared by melting and quenching process. Melting temperature is usually in range of 1350-1450°C depending on the glass chemical compositions. It was then quench in graphite mold for rods or monoliths [4] or in water for frits [5]. In fact, bioactive glasses with high purity, flexibility and homogeneity can be synthesized at significantly low temperature (below 1000°C) via sol-gel processing technology [6,7]. In additions, sol-gel method is able to produce glass with large surface area and high porosities which have been proven as important factors for its enhanced bioactive property [8]. Chemical composition of bio-glasses was mostly affected their bioactivity including bonding rate, strength and stability of the bond. Until today, various compositions of bioactive glass or glass-ceramics made by sol-gel have been studied by researchers [9]. Most of the studies investigate the influence of composition towards the bioactivity of the glass or glass-ceramics. It has also found that even binary and ternary system which based on SiO$_2$-CaO [10] and SiO$_2$-CaO-P$_2$O$_5$ [11] are biologically active. Meanwhile, the addition of network former oxide like alumina, zirconia and titania increased chemical stability to the structure thus reduces the glass dissolution and affects the glass bioactivity [12-14].

The presence of phosphate (P$_2$O$_5$) in glass composition is an important factor in determining the glass characteristics for biomedical applications. Besides acted as network former, phosphate was added to the glass composition to simulate calcium phosphate constituents of hydroxyapatite (HA) and tri-calcium phosphate, the inorganic mineral phase of bone [15]. The properties of bio-glass in term of its bioactivity, physical and mechanical properties also will be influenced by the phosphate content in their system. The presence of phosphate content in sol-gel glass assisted in accelerating the crystal growth of apatite and promotes osteogenesis in vivo [16]. The presence of phosphorus in the glass will induce the crystallization of higher number of calcium phosphate phases which increase its bioactivity [17]. Particularly, phosphate content which allows the rapid apatite growth on glass surface was found to be in the range of 2-10%. Glass with lower phosphate content below than 2 % will result in slower rate of apatite layer formation while 10 % of phosphate will inhibit the sign of bioactivity [18]. However, previous studies also showed that sol-gel derived glasses containing of phosphate content up to 15 mol % has showed a good in vitro bioactivity [16] and at the same time significantly enhances its mechanical property [17]. Due to lack of research has been done, the actual effect of phosphate in sol-gel glasses is rather complex to explain and yet has not been fully understood. This leads to gap of knowledge for bio-glass composition with phosphate content higher than 10 mol %. Thus, this study aims to investigate the actual effect of high phosphate content (10, 15 and 20 mol %) in sol-gel derived bio-glass composition on their initial characteristic and in vitro bioactivity property.

## 2 Materials and method

In this study, all the analytical grade chemicals and precurors used were purchased from Merck with more than 99.8 % purity and used as received. The replacement of CaO by P$_2$O$_5$ content were varied according to Ca/P ratio of 40:10, 35:15 and 30:20 (in mol %). The precursors were added into nitric acid aqueous solution while stirring (when the solution maintain their temperature at 50°C and pH below 2) by following the sequence of tetraethyl
orthosilicate (TEOS) $\text{Si(OC}_2\text{H}_5\text{)}_4$, triethyl phosphate (TEP), $\text{OP(OC}_2\text{H}_5\text{)}_3$, and calcium nitrate tetrahydrate, $\text{Ca(NO}_3\text{)}_2$. The mixtures were stirred at least one hour for each chemical to let their reactions completed. The sols then casted into plastic mold prior to gelations. The gels were subjected to aging process for 48 hours at $60^\circ\text{C}$ then dried at $120^\circ\text{C}$ for 48 hours before thermal stabilized at $600^\circ\text{C}$ for 3 hours for the removal of residues. These gels were grounded using agate and mortar. Chemical composition of samples was analysed by X-Ray Fluorescence (XRF). Porous characteristic such as surface area, pore volume and pore size were determined by Berneaur, Elmet and Teller (BET) method. X-Ray Diffraction (XRD) was used to measure crystallinity and phase identification of bioglass. For in vitro bioactivity test, the powders were pressed into disc and heated for another 2 hours at $600^\circ\text{C}$. Bioactivity test was carried out by immersing the glass disc into simulated body fluid (SBF) for 14 days. Carbonate apatite deposition and its formation on the glass surface was observed by Field Emission-Scanning Electron Microscope (FE-SEM) with EDX. The SBF solution was prepared according to established method proposed by Kokubo and Takada [2]. Each different sample of the gel-glass was labelled as SiCaP-10 (10 mol% phosphate), SiCaP-15 (15 mol% phosphate) and SiCaP-20 (20 mol% phosphate).

3 Result and Discussion

3.1 Chemical composition

Table 1 shows the chemical composition (wt. %) of the sol-gel derived glass particles. High purity of gel-glass particles was particularly obtained. $\text{SiO}_2$, $\text{CaO}$ and $\text{P}_2\text{O}_5$ are the major oxide compound for all gel-glass composition with very small traces (ppm) of foreign elements besides below than 10% for loss of ignition (LOI) during test. It indicates that, $\text{CaO}$ was replaced by $\text{P}_2\text{O}_5$ in the sol-gel glass composition where $\text{CaO}$ was directly reduced by the increased in $\text{P}_2\text{O}_5$ content. This also predicts that the role of $\text{CaO}$ as network modifier will be reduced and the role of $\text{P}_2\text{O}_5$ as network stabilizer will increase in the glass networking system. LOI percent were reduced by increased in $\text{P}_2\text{O}_5$ content which reflects the phosphate effect in forming more stabilized glass.

| Sample    | $\text{SiO}_2$ | $\text{P}_2\text{O}_5$ | $\text{CaO}$ | LOI |
|-----------|----------------|------------------------|--------------|-----|
| SiCaP-10  | 42.45          | 11.11                  | 36.52        | 9.92|
| SiCaP-15  | 42.50          | 15.23                  | 34.59        | 7.73|
| SiCaP-20  | 43.18          | 20.98                  | 30.66        | 5.18|

3.2 Porous characteristics

Table 2 showed the surface area, pore volume and average pore size of all gel-glass compositions. The surface area for SiCaP-10, SiCaP-15 and SiCaP-20 gel-glass were at 38.7556 m$^2$/g, 79.0663 m$^2$/g and 62.0557 m$^2$/g respectively. The high surface area obtained by the gel-glass can be explained by the formation of Si-O bonds structure through condensation process which left behind the chemical residue which then evaporates and leaving pores [9]. This large surface area directly reflects the average pore volume (adsorption/desorption) for SiCaP-10, SiCaP-15 and SiCaP-20 gel-glass which were determined at 0.1711 cm$^3$/g, 0.3691 cm$^3$/g and 0.1379 cm$^3$/g respectively. The surface area and pore volume of the gel-glass were increased from sample A to sample B, but then reduces when the phosphate is added to 20 mol % (sample C). From our previous report, it was found that by the increased of phosphate to 20 mol% in bioglass composition has start to increase
vitrification which in turn increased the formation of glassy phase on the surface of bioglass [17]. Phosphate compound is known as one of the common fluxing agents in ceramics. Thus, reduction of surface area and pore volume of bioglass particles in this result can be related to this reaction. The average pore size recorded for SiCaP-10, SiCaP-15 and SiCaP-20 are 23.7092 nm, 21.2512 nm and 14.2411 nm respectively. This indicates, by increase phosphate content in the sol-gel compositions to 20 mol% has significantly reduced the surface area, pore volume and pore size of the prepared gel-glass.

Table 2: Surface area, pore volume and average pore size of different compositions of prepared gel-glass.

| Sample     | BET surface area (m²/g) | Cumulative pore volume BJH adsorption (cm³/g) | Cumulative pore volume BJH desorption (cm³/g) | Average pore size (d.nm) BJH adsorption (nm) | Average pore size (d.nm) BJH desorption (nm) |
|------------|-------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| SiCaP-10   | 38.7556                 | 0.164899                                    | 0.177378                                    | 23.2062                                    | 24.2132                                    |
| SiCaP-15   | 79.0663                 | 0.367021                                    | 0.371158                                    | 22.0517                                    | 20.4507                                    |
| SiCaP-20   | 62.0557                 | 0.129764                                    | 0.146109                                    | 13.3703                                    | 15.1119                                    |

3.3 Chemical structures

Figure 1 displayed the FTIR spectra for all gel-glass compositions. The spectrum bands around 477 cm⁻¹ was observed in all compositions which associated with Si-O-Si bending vibrations and indicates the existence of amorphous silicate. Increased in phosphate has increased this band intensity which correspond to the increased in amorphous Si-O-Si bond.

![FTIR spectra](image)

**Fig. 1.** FTIR spectra of (a) SiCaP-10, (b)SiCaP-15 and (c) SiCaP-20 bioglass

Spectrum bands around 800 cm⁻¹ was observed in all compositions which associated with symmetric stretching of bridging oxygen (BO) atoms between Si-O tetrahedrons. Increased in phosphate content has increased this band intensity which relates to increase in silicate
tetrahedral network structure. Double peaks band found in all the gel-glass composition is associated with crystalline P-O bending. Double spectrum band peaks at 568 cm\(^{-1}\) and 608 cm\(^{-1}\), 565 cm\(^{-1}\) and 600 cm\(^{-1}\) were associated with phosphate group which relates to the presence of crystalline phosphate in the glass. As the phosphate content increased, the band intensity of 565 cm\(^{-1}\) has also increased which indicates the increased in phosphate crystalline structure [6, 11, 17].

3.4 Crystallinity and phase analysis

Low intensities and broader diffraction peaks can be observed in all gel-glass compositions (Fig. 2), thus indicate the dominance of amorphous structure of these compounds. Indeed, sol-gel derived bioglass exhibits low sintering ability [23]. As the phosphate content increased, the percent of crystallinity has decreased from 35.5% to 31.6% respectively (obtained from the software). It can also be observed, increased to 20 mol% phosphate increased the silicate amorphous peaks which situated at 2\(\theta\)=20-32\(^{\circ}\). This is because, increasing of phosphate to 20 mol% in gel-glass composition promoting gel networking structure of Si-O-Si. This can also relate with FTIR result, where increased in phosphate has also increased the amorphous silicate (Si-O-Si) band intensity which responsible for the reduction of the gel-glass crystallinity. Several diffraction peaks were found which corresponded to (i) dicalcium silicate (Ca\(_2\)(SiO\(_4\)), (ii) tricalcium silicate oxide (Ca\(_3\)(SiO\(_4\))), (iii) hydroxylapatite (Ca\(_5\)(PO\(_4\))\(_3\)(OH)) and (iv) apatite (Ca\(_{10}\)(PO\(_4\))\(_6\)((CO\(_3\))\(_0\).75((CO\(_3\))\(_0\).75((OH))\(_0\).5\)). Increased of phosphate content (SiCaP\(_{20}\)) has increased the diffraction intensities of hydroxylapatite (HA) phase. This indicates that increased of phosphate content to 20 mol% has increased the formation calcium phosphate phase (HAp), which also observed in FTIR result (at band 565 cm\(^{-1}\)).

![Fig 2. XRD analysis of (a) SiCaP-10, (b) SiCaP-15 and (c) SiCaP-20 gel-glass](image-url)
3.5 In vitro bioactivity

Bioactivity property of gel-glass disc can be determined by observing the carbonate apatite formed on the surface and their elemental composition changes after 14 days of immersion in SBF. The results are shown in Fig. 3. It can be seen that highly porous layer structure (carbonated apatite with micron-sized particles) were formed on top of each bioglass surface. For SiCaP-10 and SiCaP-15, the layers of carbonated apatite were fully covered the entire surface of them however only certain part of the SiCaP-20 has covered by the carbonate apatite (refer to 2,000X magnification image).

![Fig 3](image)

Fig 3. Surface morphology with EDS spectrum for (A) SiCaP-10, (B) SiCaP-15 dan (C) SiCaP-20 after immersed in SBF for 14 days. At left are images 2K magnification meanwhile at right are images 50K magnification. The circle shows the bioglass surface without apatite.
Furthermore, these layers were sufficiently thick which depleted the Si spectrum and consisted of C, Ca, P, O spectrum elements which verification of carbonated apatite structure. Larger images (50,000X) showed the typical images of ‘cauli-flower’ structures which frequently related to biological carbonated apatite. The carbonated apatite’s Ca/P ratios recorded for each sample after the immersion were 1.78, 1.60 and 1.27 respectively, where the first two were closed to Ca/P ratio of stoichiometric HA at 1.67 [1]. This result showed that all samples were basically bioactive especially for SiCaP-10 and SiCaP-15 but become less bioactive for SiCaP-20 based on the apatite layer structure and Ca/P ratio of the apatite formed.

4 Conclusion

Three different Ca/P ratios (phosphate composition) of gel-glass particles and pellets were successfully prepared through low temperature, acid-catalysed sol-gel technique. SiCaP-15 indicates the highest porosity structure, pore size, surface area, and pore volume compared to other sample and also very bioactive indicated by well formation of carbonated apatite crystallites on its surface. Increased in phosphate content in gel-glass composition has increased the amorphous silicate (Si-O-Si) structure, increased bridging oxygen atoms between Si-O tetrahedrons, increased the crystalline phosphate structure and decreased the pores which in turn reducing the rate of bioactivity property. These bio glasses especially SiCaP-15 were suggested to be used in bone defect treatment and also as filler in polymer based bioactive nanocomposite materials.

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