

Physical Characteristic and In-Vitro Bioactivity Property of Sintered Glasses made via Sol-Gel and Powder Sintering Process

Syed Nuzul Fadzli Syed Adam^{1*}, Firuz Zainuddin², Azlin Fazlina Osman² and Hasmaliza Mohamad³

¹Faculty of Mechanical Engineering and Technology, Universiti Malaysia Perlis, 01000 Perlis, Malaysia.

²Faculty of Chemical Engineering and Technology, Universiti Malaysia Perlis, 02600, Malaysia.

³School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300, Penang, Malaysia.

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ABSTRACT

In this work, ternary system (SiO₂-CaO-P₂O₅) biocompatible glass with different compositions (CaO/P₂O₅ ratio) were prepared by sol-gel method and sintering process. The physical characteristic and bioactive properties of each different sample composition were analyzed using XRF, particle sizer, N₂ adsorption-desorption, FTIR, XRD, and FESEM-EDX. The sintered glass pellets were subjected to immersion studies in a simulated body fluid (SBF) solution for 14 days. All compositions of gel-glass particulates showed mesoporous-type structures and consisted of very high porosities with nano-pores in size. Different Ca/P ratios in gel-glass composition are affected by different porous characteristics. All compositions of sintered glass showed very good bioactive behavior by significant deposition of the carbonate apatite layer. Sintered glass with the Ca/P ratio = 2.33 showed very significant bioactive properties as it also comprised the highest pore volume and size. However, sintered glass with the lowest Ca/P ratio (Ca:P=1.50) showed a quite significant reduction in the bioactive property as it also consisted of the lowest pore volume and pore sizes. Hence, the in-vitro bioactivity property of sintered glass is significantly influenced by the increase in its porous characteristics due to differences in the Ca/P ratio.

Keywords: Sol-gel synthesis, Bioglass, In vitro bioactivity, Carbonated apatite

1. INTRODUCTION

Bioactive materials are a type of material that has the ability to form a direct chemical bonding with human or animal bone and its soft tissue without having fibrillar connective tissue between them. The interaction between bioactive materials and human body fluid leads to the precipitation of a calcium phosphate-rich layer on the glass surfaces. This calcium phosphate-rich layer is initially amorphous, which subsequently crystallizes into carbonated hydroxyapatite (HCA) structure by the carbonate anions from the solution. A bonding to the bone tissue is only possible when this biologically active HCA is formed on the implant surface, and the rate of tissue bonding correlates with the amount of HCA that is formed [1,2]. Bioactive glass and glass ceramics are multiphase metal oxides composition commonly used as bio-implant and in bone tissue engineering applications. The sol-gel processing method is one of the methods typically used in preparing powder, nanoparticles, thin film, fibers, and bulk forms of glasses and glass ceramics materials. Sol-gel technology was also successfully used to produce glasses with high surface area, high porosity, high purity, and good homogeneity at a practical temperature compared with a conventional high-temperature melt-derived technique [3,4]. These become the factors for glasses bioactivity improvement due to the porous habits of the precursors used. The porous property of glass and glass ceramics become an important factor in determining the bioactive property of glass and glass ceramics [5]. However, no recent established findings relate the

*Corresponding author: syed.nuzul@unimap.edu.my

porous characteristic of sol-gel glass particles and their bioactivity properties. Moreover, because sol-gel procedures are carried out at relatively low temperatures, the addition of some components to lower the melting temperature, such as Na_2O or K_2O , which are used in the conventional melting route, is no longer required. Thus, the use of the sol-gel method in the synthesis of glasses simplified the composition, leading to the first bioactive glasses of the ternary $\text{SiO}_2\text{-CaO-P}_2\text{O}_5$ system [6]. As well as the glass melting process which produces frits during quenching, most of the time, the sol-gel process is also easily produced particles, powders, or fractions due to significant thermal shock and liquid/moisture evaporation pressure during the heating and drying stage. For the bulk form of biomaterials application, the glass particles need to be sintered via the powder metallurgy route. Before sintering, the glass particles made from the sol-gel process will be milled and compacted, then sintered at a temperature below its melting point. In powder sintering technology, the size of particles become an important parameter that affects green body strength and the physical and mechanical property of the sintered bulk. However, data on particle sizes of sol-gel-derived glass prior to the sintering process has not yet been established. Furthermore, scientific studies that utilized the sintering of sol-gel-derived glass particles also are rather unfamiliar compared to the sintering of melt-derived glass particles.

The bioactive glass shows good bioactivity in vivo and in vitro tests due to the function of SiO_2 or silicon in their surface reactions. SiO_2 acts as a matrix as well as a network former that gives chemical stability to its structure. Meanwhile, adding other networks of former oxides like phosphate, titania, and alumina increased the bridging oxygen bonds, reducing glass dissolution and significantly reducing its bioactivity [7]. Furthermore, phosphate in sol-gel glass composition was found to play an important role in increasing its mechanical properties, such as compressive strength [8]. Several previous studies have investigated the effect of Ca/P ratios on the bioactivity properties of sol-gel derived-glass. The properties of bioactive glass in terms of its bioactivity and physical and mechanical properties were influenced by the $\text{CaO/P}_2\text{O}_5$ ratio in the glass system. The results from in vitro studies demonstrated that increasing phosphate concentration in bioglass would promote the crystallization of apatite and increase the carbonate apatite formation. However, above specific percentages, the formation of an apatite layer was impeded due to a substantial pH increase in the medium [8-11]. Hence, in this study, the effect of different composition ratios (Ca/P ratios) on the particle sizes, porous characteristics, and in-vitro bioactive properties of sintered glass will be presented.

2. EXPERIMENTAL

2.1. Preparation of Gel-Glass and Sintered Glass

In this study, all chemicals and precursors were used as received with more than 96.0 % in purity. The calcium oxide (CaO) and phosphate (P_2O_5) compositions were varied to 4.0:1.0, 3.5:1.5, and 3.0:2.0 (in mol %) for differences in the Ca/P ratio. The main precursors used include tetraethyl orthosilicate (TEOS) $\text{Si}(\text{OC}_2\text{H}_5)_4$, triethyl phosphate (TEP), $\text{OP}(\text{OC}_2\text{H}_5)_4$, calcium nitrate tetrahydrate (CNTH), $\text{Ca}(\text{NO}_3)_2\text{OH}$ and nitric acid (HNO_3) as a reaction catalyst. The amount of reagent and precursors used for each sample composition is shown in Table 1. The mixtures were stirred for more than 3 hours to form sols and then cast into the mold for gelation before they were thermally stabilized at 600°C for 2 hours. Furthermore, these gels were grounded to fine powders using agate and mortar. Finally, each sample composition was compacted into 12 mm diameter pellets and sintered for 2 hours at 1000°C in a muffle furnace. More detailed procedures can be referred in our previous paper [12].

2.2. Sample Characterizations and *In Vitro* Bioactivity Evaluations

The chemical composition of each gel-glass sample with different Ca/P ratios was analyzed by wavelength dispersive x-ray fluorescence (WD-XRF) analysis. The particle size of each gel-glass

sample was measured using a Malvern particle size analyzer. The porous characteristic of each gel-glass sample was determined by the nitrogen gas adsorption-desorption method. A simulated body fluid (SBF) solution was prepared prior to the in-vitro test according to the method proposed by Kokubo and Takadama [2]. The detail of the SBF solution preparation, including steps and procedures, also can be found in our previous paper [13]. An in-vitro bioactivity test was carried out by soaking each composition of sintered glass pellets into simulated body fluid (SBF) and kept in an incubator at 37°C for 14 days. Any bone-like apatite deposition on the surface of the sintered glass was analyzed. The sample weight to SBF ratio used during the test was approximately 0.0063 gm/ml, and the solution was replaced every 3 days. Prior to the test, sintered glass pellets were taken out from the solution, washed with acetone, and dried in a desiccator for 48 hours before testing. The structural properties and surface changes for the sintered glass, before and after immersion in simulated body fluid (SBF), were analyzed by Fourier Transformed Infra-red (FTIR), X-Ray Diffraction (XRD), and Field Emission-Scanning Electron Microscope (FE-SEM) together with EDX.

Table 1: Precursors, labels, and ratios used for different gel-glass compositions.

Sample label (Ca/P ratio)	SiCa4P (Ca:P=4.0:1.0)			SiCa4P3 (Ca:P=3.5:1.5)			SiCa4P4 (Ca:P=3.0:2.0)		
	Reagent/ Precursor	Amount used	Moles (%)	Weight (%)	Amount used	Moles (%)	Weight (%)	Amount used	Moles (%)
TEOS	15.0 ml	50	42.45	15.0 ml	50	42.50	15.0 ml	50	43.18
TEP	2.0 ml	10	11.11	3.0 ml	15	15.23	4.0 ml	20	20.98
CNTH	8.0 g	40	36.52	7.5 g	35	34.59	7.0 g	30	30.66
Deionize water	40.0 ml	-		40.0 ml	-		40.0 ml	-	
Nitric acid 1M	1 ml	-		1 ml	-		1 ml	-	
LOI (%)			9.78			7.68			4.40

Note: The chemical composition (in weight %) of prepared gel-glass obtained by XRF. XRF data for SO₃, Fe₂O₃, SrO traces were not shown in this table as their concentration are very small (in ppm).

3. RESULTS AND DISCUSSION

3.1. Physical characteristics of gel-glass

3.1.1. Chemical Composition

The target compositions of gel-glass (in mol %), the amount of chemical precursors used, and the labeling for each glass sample can be found in Table 1. The composition of these powders, identified by XRF analysis by weight %, is also shown in the table. SiO₂, CaO, and P₂O₅ were found to be the major metal oxide compound in all the synthesized gel-glass, and their compositions were in good similarity with the targeted glass composition. The theoretical and experimental compositional percentages were slightly different due to the difference in unit (mol and weight %) calculation and also typically caused by loss of ignition (LOI) during sample preparation. Thus, the compositions of the gel-glasses powder obtained from XRF were found acceptable and in accordance with the targeted composition. The trace of very small concentrations (ppm) of foreign elements such as SrO₂ was also detected in the XRF result, which typically contributed by reagent impurities and slight contamination during the synthesis and processing of gel-glass samples. This XRF data proved that a very high purity of gel-glass particles could be obtained from the sol-gel process used in this study as well as obtained in other studies [7,8].

3.1.2. Particle Size Distribution

The large proportion of gel-glass particulate showed particle sizes in the range of 10-100 nm (below 100 nm), thus confirming their nano-particle-sized range, as shown in Figure 1. The intensity curve of SiCa4P gel glass displayed unimodal distribution, with the major particle size found at 23.62 nm (Figure 1(a)). Meanwhile, SiCa4P3 and SiCa4P4 gel-glass intensity curves showed multimodal distributions with their major particle sizes found around 22.12 nm and 22.02 nm (Figure 1(b) and (c)), respectively. The gel-glass particles obtained for all compositions have good size similarity and can be considered homogeneously in size. The particle sizes obtained in this study are in good agreement with other studies reviewed by Zheng [14], even though in this study, we implement a mechanical grinding process without using the conventional base catalyst. Thus, the differences in Ca/P of gel-glass composition do not significantly affect its particle size characteristic. The higher ranges of particle size present in SiCa4P3 and SiCa4P4 is most commonly due to the agglomeration of fine particles that result from breaking down large particles in the sample powder. Sol-gel parameters used in this study proved able to produce homogenous nano-particulates gel-glass prior to sintering.

3.1.3. Porosities

Nitrogen (N₂) adsorption-desorption isotherm (in Figure 2) for all Ca/P ratios of gel-glass nanoparticles obtained were corresponding to the type IV isotherm, which exhibited a multilayer formation as a result of pore walls surface coverage in the mesoporous range followed by pore filling [15]. The H1 hysteresis loop could be observed in all nanoparticle cases of these isotherms, which occurred due to the unsimilar path of adsorption and desorption branch as the p/p_0 value increases. This H1 hysteresis loop is usually related to the porous materials, indicating the presence of cylindrical pores at both ends in the samples. This H1 hysteresis loop also consists of agglomerates or compacts of almost uniform spheres shaped in a fairly regular array, and therefore, it has narrow pore size distribution [16]. Based on the N₂ adsorption-desorption result, all gel-glass compositions showed highly mesoporous characteristics. The surface area measured for SiCaP, SiCaP3, and SiCa4P4 gel-glass were at 62.0557 m²/g, 79.0663 m²/g, and 38.7556 m²/g, respectively. However, the surface area of these gel-glass samples is relatively much lower compared to other mesoporous silica [14]. Furthermore, the SiCa4P3 gel glass indicates the highest porosity structures when it adsorbs and desorbs at 238.7239 cm³g⁻¹ of nitrogen gas compared to the other two gel glasses. The pore size distribution determined from N₂ isotherm, measured for each composition, was plotted and shown in Figure 3. The maximum pore size obtained for all gel-glass compositions were at 126.9641 nm, 156.7135 nm and 42.9485 nm for SiCa4P, SiCa4P3 and SiCa4P4 respectively. The pore size of these gel-glass samples was relatively larger compared to other mesoporous silica [17]. Again, the SiCa4P3 gel-glass particles also showed the highest value of pores size (in width), and this trend should be related to the above N₂ adsorption-desorption isotherms, where SiCa4P3 gel-glass also indicates the highest porosity structures. Hence, the differences in Ca/P of gel-glass composition affected its porous characteristic. The SiCa4P3 sample showed the highest porous characteristics in terms of surface area, pore volume, and pore size.

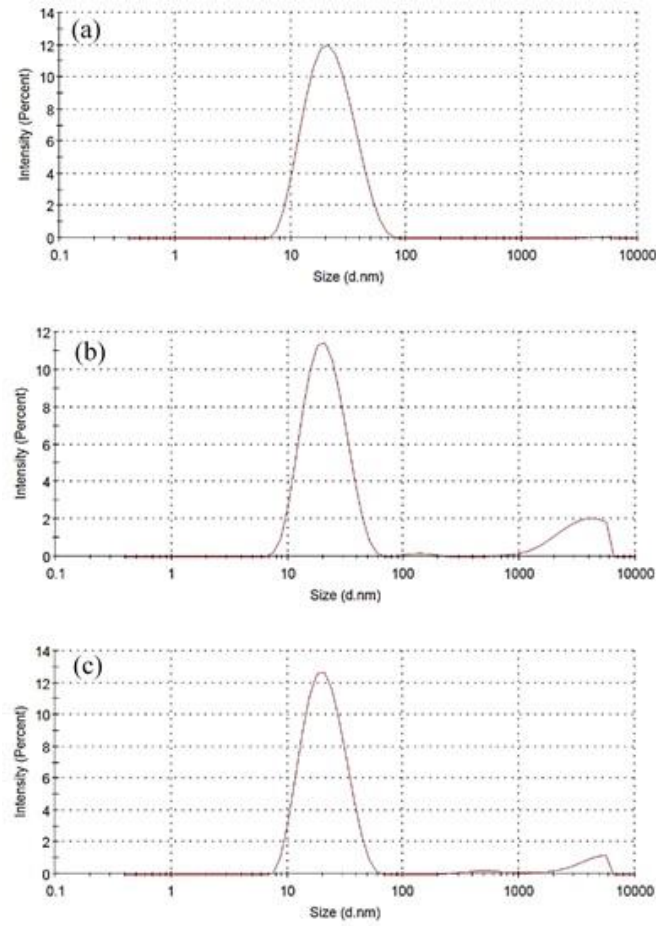


Figure 1: Particle size distribution for (a) SiCa4P (b) SiCa4P3, and (c) SiCa4P4 gel-glass.

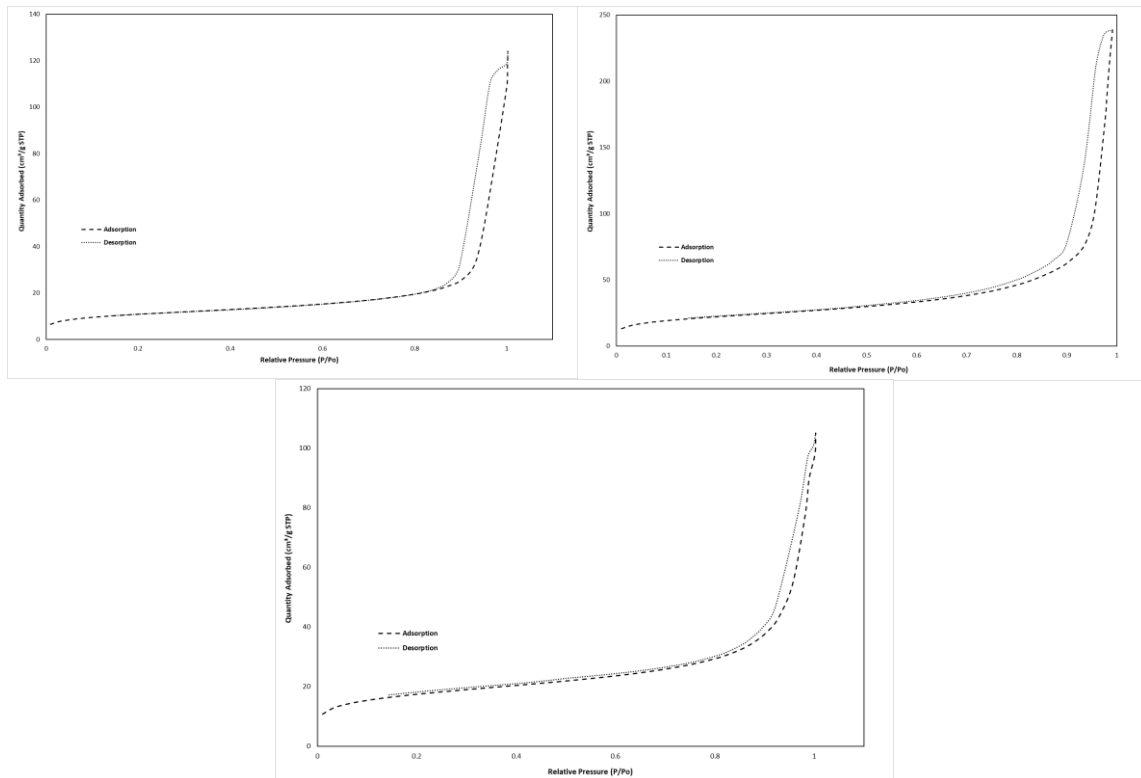


Figure 2: Nitrogen physisorption linear isotherms of (a) SiCa4P (b) SiCa4P3, and (c) SiCa4P4 gel-glass.

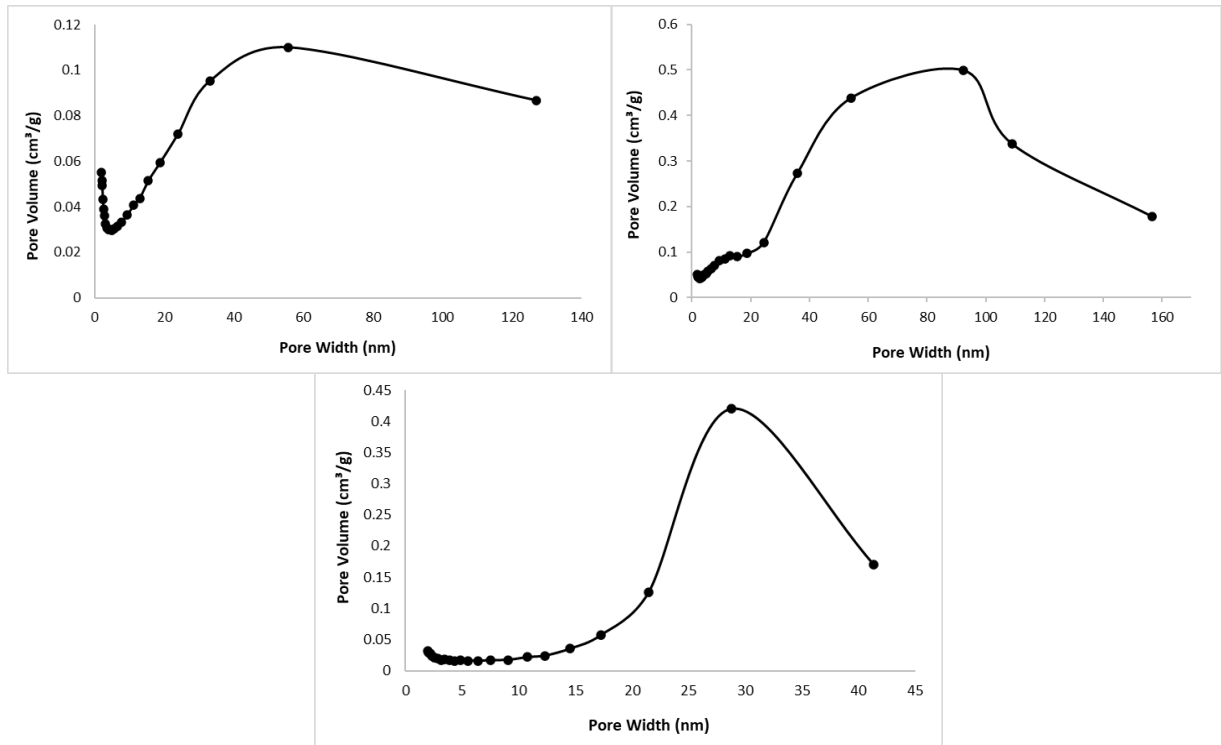


Figure 3: Pore size distribution of (a) SiCa4P, (b) SiCa4P3, and (c) SiCa4P4 gel-glass.

3.2. In Vitro Bioactivity of Synthesized Gel-Glass

3.2.1. Fourier Transformed Infra-Red analysis (FTIR)

FTIR spectrum of each sintered glass composition after soaking in SBF for 14 days is shown in Figure 4. Typical silicate spectrum bands were observed approximately at 477, 800, and 1100 cm^{-1} . An increase in the Ca/P ratio of gel-glass commonly increased these bands due to the increase in silicate glass network structure [8]. Double peaks bands at 604 cm^{-1} and 567 cm^{-1} were found in all sintered glass compositions, indicating the existence of crystallized calcium phosphate on the glass surface [18]. Meanwhile, higher absorption of carbonate (C-O bending of carbonate ion) band at spectrum band around 1480 cm^{-1} and 1430 cm^{-1} were shown by all sintered glass compositions; hence, indicative of high carbonate ions was found on the sintered glass surface. The band at 875 cm^{-1} (C-O bending vibration from the carbonate group in carbonated apatite) was also observed after immersion, indicating carbonated apatite formation on the surface [19]. This C-O spectrum intensity was decreased when the Ca/P ratio decreased. Thus, it indicates the formation of carbonated apatite structure on the sintered glass surface specifically for SiCa4P and SiCa4P3.

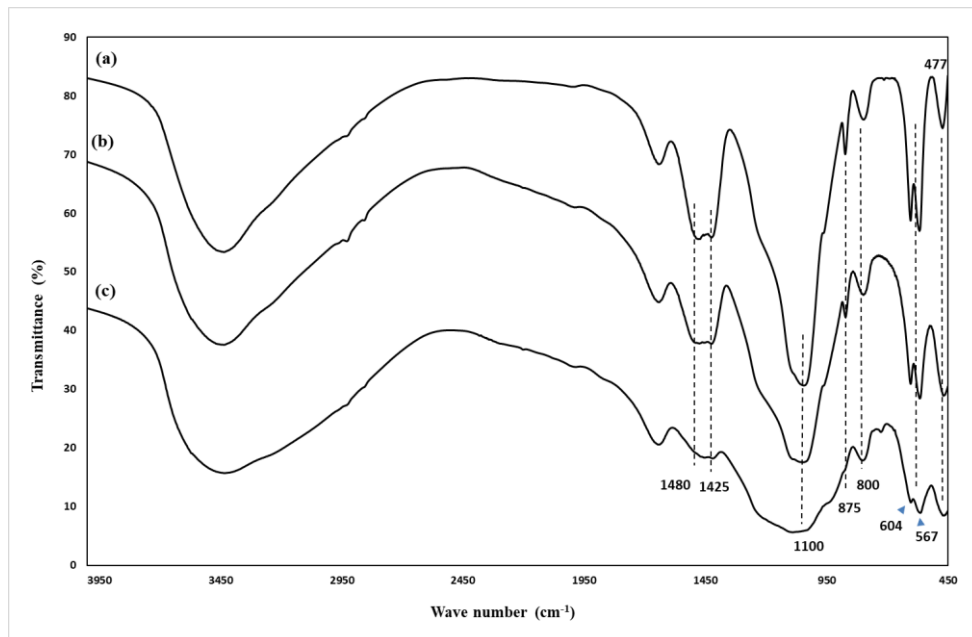


Figure 4: FTIR spectrum for (a) SiCa4P, (b) SiCa4P3, and (c) SiCa4P4 after immersed in SBF for 14 days.

3.2.2. Field Emission-Scanning Electron Microscopy (FE-SEM) analysis

The surface morphology of sintered glass and its elemental compositions, before and after soaked for 14 days in SBF solution, are shown in Figure 5. The morphology of each sintered glass before soaking was the prominence of amorphous and glassy phases with significant nanopores structure (Figure 5 A1–C1). These images prove the nano-porosities structure of the sintered glasses shown by BET testing result (subtopic 3.1.3). After soaking, the surface of all sintered glasses was completely covered with an apatite-like structure that appeared on the entire morphology. The growth of these apatite-like structures was significant in SiCa4P and SiCa4P3, which formed coral-like structures; other reviews frequently related it to carbonated apatite structures [20]. The deposition and growth rate of this carbonated apatite are crucial to allow rapid bone bonding during biomaterial implantation in a living tissue environment. The increased concentration of Ca, P, and O elements also are indicative of apatite layer structure as measured by the EDX spectrum. The Ca/P ratios of the morphology obtained for SiCa4P, SiCa4P3, and SiCa4P4 were 1.78, 1.60, and 1.27, respectively, where the SiCa4P3 was close to the Ca/P ratio of stoichiometric HA at 1.67 [21]. Hence, the decrease in the Ca/P ratio of gel-glass composition was implicated in decreased bioactivity property, where SiCa4P and SiCa4P3 showed significant growth of apatite than SiCa4P4.

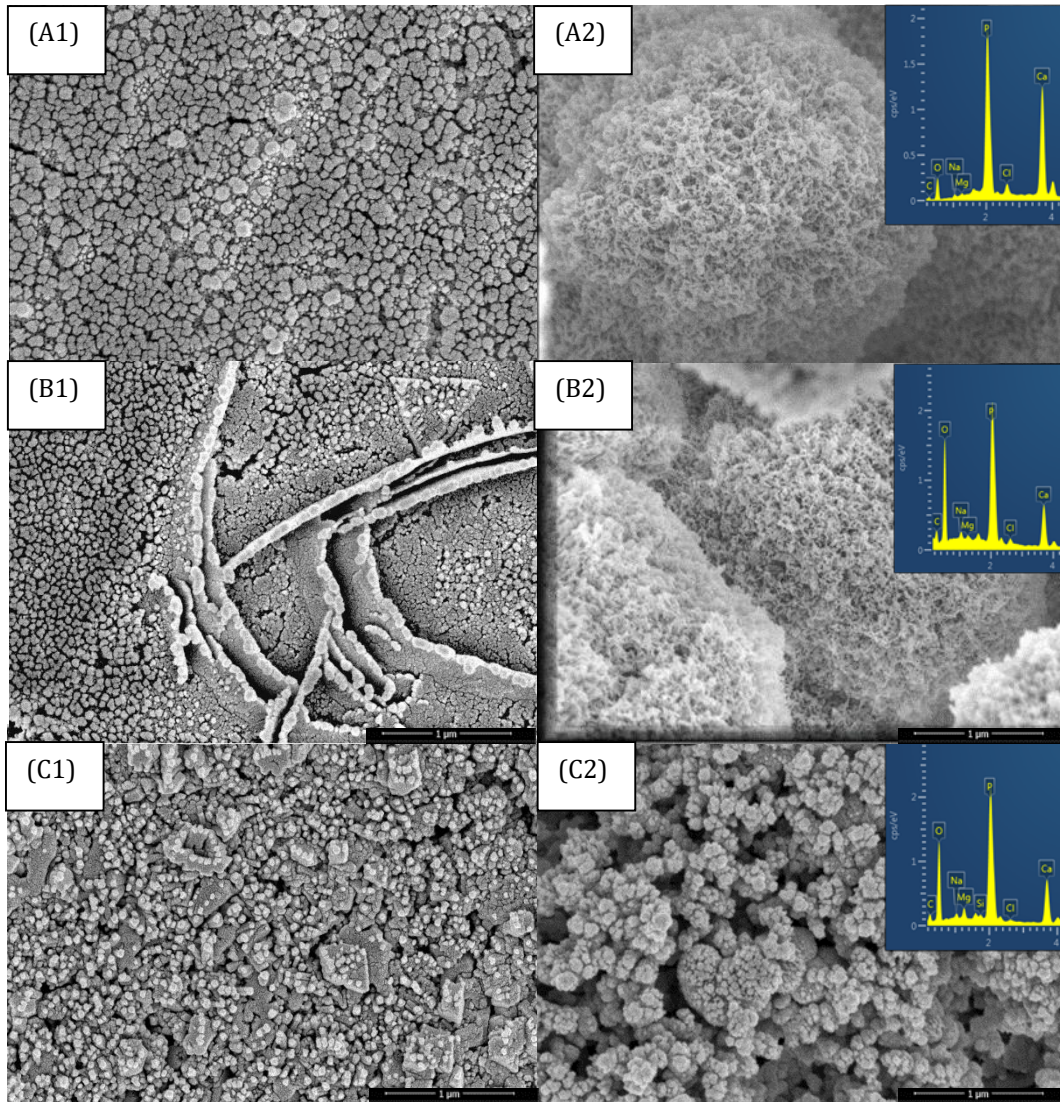


Figure 5: Surface and EDX spectrum for (A1) SiCa₄P, (B1) SiCa₄P₃, and (C1) SiCa₄P₄ prior to soaking and (A2) SiCa₄P, (B2) SiCa₄P₃ and (C2) SiCa₄P₄ after soaking in SBF for 14 days (image at 100,000x).

4. CONCLUSION

Sol-gel route and powder sintering process used in this study succeeded in preparing particulates and bioactive sintered glasses with different calcium and phosphate compositions. Different in the Ca/P ratio of gel-glass compositions have affected its porous characteristics hence determining the bioactivity performance of sintered glass. In this study, decreased Ca/P ratio tends to decrease their porous characteristics and increase the stability of sintered glass structure, reducing the in-vitro bioactive property of sintered glass. This study proved that the higher surface area, pore volume, and pore size of gel-glass particles relatively leads to the higher bioactive property for the sintered glass due to higher rate and faster ionic dissolutions and apatite depositions during immersion in body fluid. The particulates and sintered glasses prepared in this study have excellent potential as bioactive scaffolding implant material in biomedical applications.

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