# Effect of temperature on hydroxyl carbonated apatite from ash bagasse

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## Abstract:

Silica–based bioactive material can promote the nucleation of hydroxyl carbonated apatite (HCA) which roles on bone mineralization processes. The sol–gel synthesis produces nanoparticle. The small particle increases the surface area of particle that is known has high bioactivity. Sintering temperature affect properties and characteristics of the glass. The aims of the research was to know the effect of sintering temperature on HCA formation of nano bioactive silica from ash bagasse. The study was a laboratory experimental with control group post–test only design. ilica derived from sugarcane bagasse was used as a source of silica to synthesize bioactive glass and sodium silicate as a precursor. Sintering temperatures are 1 000 ºC and 1 500 ºC. Polysaccharide from seaweed was used as a binder to make plates from the bioactive glass. The plates were immersed in simulated body fluids (SBF) for 2 h, 12 h and 24 h. HCA formation was observed with Scanning Electron Microscope (SEM) and Forier Transform Infra–Red (FTIR). Two way anova and LSD test. The result of research shows that there were significantly different on HCA formation with variety of sintering temperature. Soaking time affects number and velocity of HCA formation. High sintering temperature cause increase HCA formation, but its formation is slower. The condition caused by crystal formation which produce bond more stable.

**Keywords:** Bioactive glass, mineralization, sintering, sodium silica, waste utilization, zero waste

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### Introduction

Bioactive glasses are materials which is able to create chemical bonds with surrounding tissue without intermedium fibrous layer [(1)]. Bioactive glasses consist of calcium and phosphate which shows the same proportion as bone hydroxyapatite. Its reaction with artificial body fluids will form a layer of hydroxycarbonate apatite (HCA) [(2)]. The HCA layer promotes the adsorption of proteins that play an important role in anchoring, proliferation and subsequently causing cell differentiation. Differentiated cells become the appropriate phenotype, so cells are able to synthesize extracellular matrices that will be mineralized [(3)]. Because of these properties, bioactive glass (BAG) is widely used as a regeneration of soft and hard tissue, wound healing and antibacterial applications [(4)]. The advantages of bioactive glass nano–particles play a role in remineralization and dentinal repair to overcome sensitivity problems [(5)]. The purpose of this study was to determine the effect of sintering temperature on HCA formation on bioactive silica from bagasse ask

The composition of bioactive glass consists of silicon dioxide ($SiO_2$) (46.1 mol %), calcium oxide (CaO) (26.9 mol %), sodium oxide ($Na_2O$) (22.4 mol %), and diphosphorus pentaoxide ($P_2O_5$) (2.6 mol %) which able to form HCA in less than 2 h and bind tissue [(2)]. Bioactive glass nano–particles (BGNs) were developed to increase the potential for clinical applications of BAG. Nano–particles, will increase a larger surface area, higher bioactivity, and also facilitate controlling its size homogeneity [(4)]. The sol–gel–based method is used to synthesize nano–particles [(5)]. Ungureanu *et al*. [(6)] bioactive glass obtained from the sol-gel process has higher bioactivity than bioactive which is processed by melting even though the composition is the same. This method will increase its

bioactivity [2]. The purpose of this study was to analize the effect of sintering temperature on the formation of HCA on bioactive nano silica from bagasse ash.

## Materials and Methods

### Synthesis of bioactive glass nano silica from sugarcane bagasse:

Bagasse which has been dried under the sun, burned with fire for 2 h. The ash was divided into two to be burned using 1 000 ºC and 1 500 ºC furnaces for 2 d, then sifted using a 200 mesh sieve. 25 g of the obtained powder was inserted into the erlenmayer tube, added HCL 150 mL 0.1 M solution, and stirred for 1 h (aiming to eliminate) the content of other metals other than silica found in ash and then was left at room temperature for 24 h. Then the solution was filtered and rinsed with distilled water to a normal pH of 7, dried at 100 ºC using an oven for 2 h. Amount 10 g of the solution was put into an erlenmayer tube, mixed with a solution of 60 mL 2 N NaOH, and stirred until the solution boils for 60 min, cooled at room temperature, filtered with Whatman filter paper no. 42. The obtained gel is the filtrate in the form of wet sodium silica. Sodium silica was dried in an oven with a temperature of 110 ºC for 2 h to produce sodium silica solid [7, 8]. Amount 5 g of sodium silicate were mixed with 15 mL of dionished water in an erlenmeyer tube and stirred using a magnetic stirrer, added 2.5 mL of ethanol, stirring until the solution appeared clear. Then added 2 M HNO3 drops and keep stirring for 1 h, added 0.5 g $P_2O_5$ (phosporus pentoxide), stirring for 45 min. 4.1 g of Ca ($NO_3$) $2.4H_2O$ (calcium nytrat tetrahydrat) was added and remained stirred for 45 min. Finally the mixture was stirred for 1 h until a gel is formed. The gel was then allowed to stand for 5 d in room temperature. After 5 d, the gel was dried at 700 ºC for 5 h. Bioactive glass was formed, crushed and sifted using a 200 mesh sieve and placed into a small bottle [9].

### Production of artificial body fluids:

Artificial body fluids were made by mixing 700 mL of aquades, KCL 0.2 g $L^{-1}$, NaCL 0.7 g $L^{-1}$, $NaHCO_3$ 0.3 g $L^{-1}$, $Na_2HPO_4$ 0.07 g $L^{-1}$, $K_2PO_4.3H_2O$ 0.2 g $L^{-1}$, 13 g $L^{-1}$, HCL 40 mL , MgCl. $6H_2O$ 0.3 g $L^{-1}$ until dissolved, add distilled water until it reaches 1 000 mL volume and pH was set to 7.43 [10].

### BAG disc preparation:

BAG powder was mixed with seaweed polysaccharide powder. The dough was printed in metal molds with a diameter of 5 mm × 4 mm × 2 mm using an agate spatula, compacted using a cement stopper and covered with a metal plate. The mold was pressed with the same strength, then removed from the mold and difurnace at a temperature of 700 ºC for 5 h.

### Sample treatment:

Samples of size 3 mm × 5 mm were inserted into the petri dish filled with artificial body fluids, incubators at room temperature for 2 h, 12 h and 24 h. Samples were taken to be analyzed by Scanning Electron Microscope (SEM) and FTIR to observe the hydroxycarbonate apatite formation.

### Image Processing:

Image processing obtained from SEM was analyzed using ImageJ 1.49v software. By setting the threshold level, the formation of the HCA is covered in the red area [11]. The data obtained were tested for normality using the Shapiro–Wilk test and homogeneity test using the levene test, then the Two–Way Anova test was carried out followed by the LSD test.

## Results

Photographs from scanning electron microscope (SEM) on hydroxycarbonate apatite (HCA) formation were analyzed using ImageJ 1.49v software. Each sample was photographed at four visual fields, which were then counted as percent area of hydroxycarbonate apatite (HCA). The area of hydroxycarbonate apatite (HCA) formation can be seen in table 1.

**Table 1.** Average hydroxycarbonate apatite (HCA) formation

| Group | Duration soaking (h) | HCA formation (%) |
|---|---|---|
| Sintering 1 000 | 2 | 33.28 |
| Sintering 1 000 | 12 | 34.19 |
| Sintering 1 000 | 24 | 35.37 |
| Sintering 1 500 | 2 | 45.872 |
| Sintering 1 500 | 12 | 46.112 |
| Sintering 1 500 | 24 | 47.007 |

Bioactive glass based silica from sugarcane baggase with 1 500 ºC sintering has a higher average HCA formation compared to 1 000 ºC sintering. BAG with a sintering temperature of 1 500 ºC after soaking for 2 h, 12 h and 24 h showed the formation of HCA was 45.87 %, 46.11 % and 47.07 % respectively. These results are greater than BAG which are sintered at a temperature of 1 000 ºC with the same immersion time. Histogram description can be seen in figure 1.

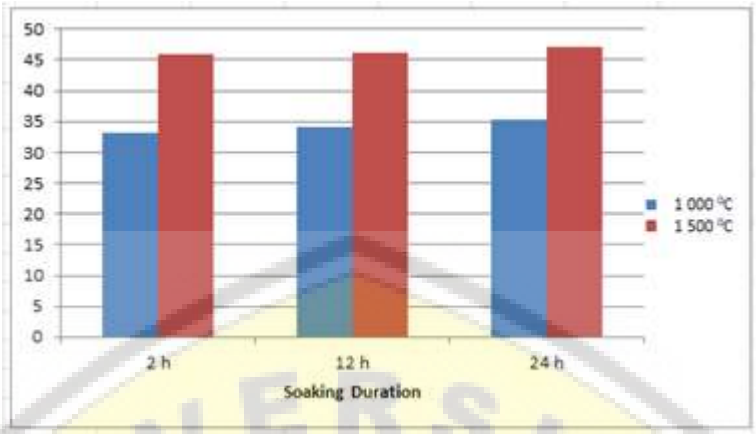


**Figure 1.** Histogram description of HCA formation

The description of SEM shows the difference in the area of hydroxycarbonate apatite (HCA) formation in ImageJ 1.49v software with the threshold menu. The area of hydroxycarbonate apatite (HCA) formation will show red. The percentage of the HCA formation area is shown in the red area compared to the whole image in one field of view. The area with low HCA formation will show a little red in the image that has been processed. Conversely, areas with high levels of HCA formation will show a broad red color. Image processing results are seen and compared between groups which can be seen in figure 2.

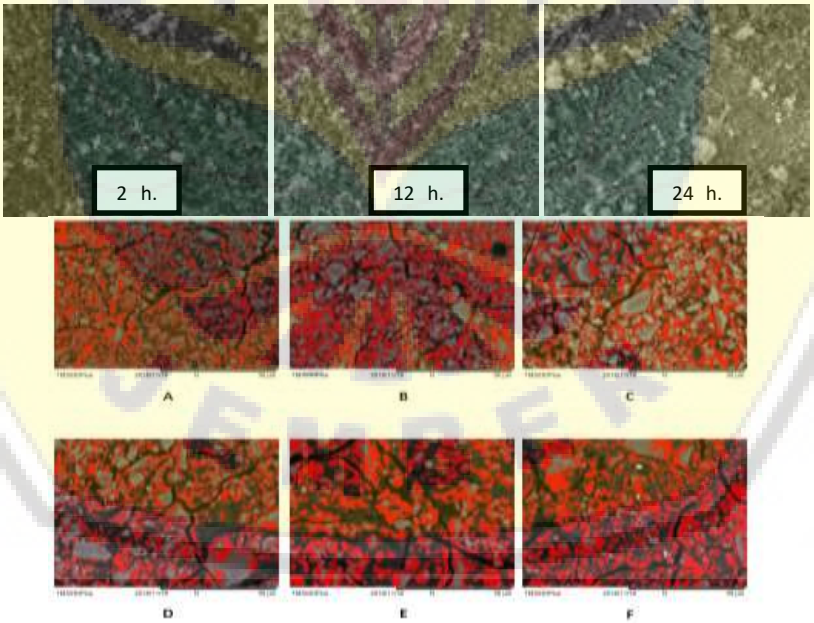


**Figure 2.** Photo SEM and imageJ analysis

The results of the two way Annova test showed a significant difference ($p < 0.05$) in the formation of hydroxycarbonate apatite (HCA) between bag based bagasse and sintering at 1 000 °C and 1 500 °C. The BAG group with sintering 1 500 °C shows a greater percentage of HCA formation compared to the BAG group with 1 000 °C sintering. Post hoc LSD testing for the formation of hydroxycarbonate apatite (HCA) showed a significant difference ($p < 0.05$) between groups.

**Discussion**

The general characteristic of bioactive glass is that its surface can form HCA which is almost the same as bone mineral and can interact with collagen fibrils from damaged bone [12]. Bioactive glass based on silica from bagasse ash soaked in body fluids will make ion exchange with ions where phosphate ions, calcium ions, and other ions will continue to migrate through the silica gel layer which will form a new layer called amorphous calcium phosphate (calcium layer phosphate) then after the growth of this layer and silica gel will combine OH– and CO32– which form the crystallization of HCA [13]. The exchange is due to the process of breaking the ion bonds. The process is influenced by the shape and size of the particles. Higher surface area and smaller particle size will cause faster dissolution compared to larger particles [14]. Therefore the sintering temperature greatly influences the solubility process. The higher the sintering temperature, causing the bioactive glass surface microstructure to become denser and fused (solid and compact), the size of the pores decreases and results in shrinkage in the sample [15]. At a temperature of 1 000 ºC the structure of the silica atom in bagasse ash is a combination of amorphous and crystalline atomic arrangements, where the arrangement of amorphous atoms has an irregular arrangement of atoms, ions, and molecules so that when combined with crystalline atomic structures which has a regular polymer arrangement it will produce strength that can be controlled properly. In addition, at the temperature resulting silica is able to attract and bind all the compounds around it. Silica contained in bagasse ash will react with ions in body fluids to form hydroxycarbonate apatite (HCA). Bioactive glass nano silica synthesized at 1 500 ºC has a crystobalite crystal structure, namely α–tetrahedral. This crystobalite crystal has a tetragonal structure [16]. *C*rystobalite has a polar and non–polar surface. Compounds that have the same properties can bind to the two surfaces. The surface of crystobalite can bind polar and non–polar compounds. Bonds with polar compounds are dominated by electrostatic bonds and dipole–dipole bonds. Whereas the non–polar part can bind to the non–polar crystobalite surface by means of Van Den Walls bonding [17].

The mechanism forming the HCA layer begins with the presence of bioactive glass material which is exposed to body fluids. The alkaline ions will be released and replaced by $H^+$ or $H3O^+$ cations from body fluids. After that there will be an increase in local pH which causes damage to the Si–O–Si bond and the release of silicon, so that the silanol group is formed. If the local pH is less than 9.5, the silanol group polymerizes and condenses on the bioactive glass surface, forming a sillica gel layer. The sillica structure will continue to experience ion exchange. Calcium and phosphate ions and ions in body fluids will migrate through the sillica gel layer, then form an amorphous calcium phosphate layer above the sillica gel layer. After the growth of sillica gel and calcium phosphate layer, there will be a combination of $OH^-$ and $CO3^{-2}$ which causes crystallization of HCA [18].

The formation of hydroxycarbonate apatite (HCA) layer is influenced by several factors including dissolution rate, immersion time, ion exchange and size of bioactive glass. In accordance with the statement of Macon, *et al*. [19], that there will be differences in the nucleation of apatite hydroxyapatite in different glass compositions and also in glass with the same composition but different sizes. In addition to the dissolution rate, the formation of HCA is also influenced by the length of immersion, the longer the immersion process the more the HCA layer is formed [20]. The weak bond between bioactive glass causes the dissolution of bioactive glass material in body fluids and causes microporus to the material so that it causes body fluids to enter the microporus to increase ion exchange. In addition, because the size of nanometer–sized bioactive glass nano silica causes more contact surface area and causes more dissolved ions so that the formation of HCA will increase [21].

**Conclusions**

Concluded that high temperature on sintering cause crystalization that affects HCA formation.

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