

# Synthesis of calcium phosphate based marine corals : A comparative study between sol-gel and precipitation method

Siswanto\*, Retna Apsari and Aminatun

Physics Department, Faculty of Sciences and Technology, Universitas Airlangga, Surabaya  
Indonesia

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## ABSTRACT

Research on the effectiveness of calcium phosphate synthesis has been done using the precipitation method and the sol-gel method. The material used is marine coral from the South Coast of Java, namely Banyuwangi, East Java. Indonesia. The results showed that the synthesis of calcium phosphate using the sol-gel method requires a lower sintering temperature compared to the precipitation method. The optimal temperature which produces calcium phosphate is a stable phase of hydroxyapatite using the sol-gel method and precipitation respectively 550 °C and 900 °C. However, the precipitation method produces a greater percentage of hydroxyapatite phase compared to the sol-gel method. In addition, the precipitation method produces optimal calcium phosphate with a Ca/P ratio of 1.66, closer to theoretical calculations.

*Key words* : Calcium phosphate, Marine corals, Sol-Gel, Precipitation method

## Introduction

Calcium phosphate (CaP) is the main mineral that makes up bones and teeth. Calcium phosphate has two atomic structures that make up the amorphous phase and the crystal phase. Synthetic crystalline calcium phosphate compounds have four phases namely  $\text{CaHPO}_2$  (*dicalcium phosphate dehydrate / DCPD*),  $\text{Ca}_8\text{H}_2(\text{PO}_4)_6$  (*octacalcium phosphate / OCP*),  $\text{Ca}_3(\text{PO}_4)_2$  (*tricalcium phosphate / TCP*), and  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  (*hydroxyapatite / HA*). Among the types of CaP salts, hydroxyapatite is the most similar to the mineral portion of the bone. Hydroxyapatite is the most thermodynamically stable crystalline phase of calcium phosphate (Ganachari *et al.*, 2016; Sihotang *et al.*, 2019; Hamidah *et al.*, 2017).

Hydroxyapatite crystal structure can be either monoclinic or hexagonal. Monoclinic hydroxyapa-

tite structures are obtained only in pure conditions with a stoichiometric composition, with a Ca/P ratio of 1.67. Hexagonal structures are generally obtained from non-stoichiometric hydroxyapatite synthesis. The lower the Ca/P molar ratio, the more acidic and the more easily dissolved the calcium orthophosphate compound.

Synthetic hydroxyapatite has long attracted many researchers to be continuously developed, because this material has excellent biocompatibility and has a high affinity with biopolymers (Raya *et al.*, 2015; Musa *et al.*, 2016). Hydroxyapatite is proven to be biocompatible and is very well tolerated by human bones and teeth (Setiawatie, 2015; Kartikasari *et al.*, 2016). In addition it has osteoconductive properties and is proven to be able to stimulate osteoblast differentiation and bone formation. The good characteristics of this biomaterial

cause its use in orthopedics and dentistry, such as bone tissue reconstruction, soft tissue engineering and periodontal defect care, dental implant coatings, filler restoration materials such as composite resins and glass ionomer cement (Mozartha, 2015).

Hydroxyapatite can be synthesized from various natural minerals that have high *calcium carbonate* ( $\text{CaCO}_3$ ) content, such as mammalian bones, shells, corals, or eggshells (Pountos and Giannoudis, 2016). Preliminary research on coral as a candidate for bone scaffold raw material has been conducted by Siswanto *et al.* (2019). XRD (X-Ray Diffraction) test results showed that corals contained 97.69% of  $\text{CaCO}_3$  compounds. The calcium carbonate content in these corals is relatively higher compared to shells (87.12%), eggshells (89.98%), conch shells (68.7%) and mammalian bones (95.7%) (Fleet, 2015). This data provides an overview of the potential of coral as a raw material for the formation of hydroxyapatite compounds.

Hydroxyapatite can be made in a laboratory through a chemical process. There are several methods for making hydroxyapatite crystals, including the precipitation method, biomimetic deposition, the sol-gel method, and the electrodeposition method (Azis *et al.*, 2018; Sirait *et al.*, 2018). The precipitation method is part of the best known wet chemical method and the technique most widely used for hydroxyapatite synthesis. This is because the technique can synthesize HA in large quantities without using organic solvents and at a relatively low cost. While the sol-gel method has several advantages, such as being able to control composition precisely, using a low temperature, having high purity and homogeneity, and the granules obtained can reach nano size (Debor, 2018). This article discusses a comparative study of calcium phosphate synthesis made from marine corals using the precipitation and sol-gel method. Characteristics that are comparative indicators are the volume fraction of calcium phosphate formed, and the Ca / P ratio

## Materials and Method

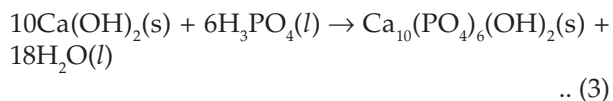
Coral as a hydroxyapatite raw material in this study came from South Coast of Java, namely Banyuwangi, East Java. Also used are 99.8% pure Phosphoric Acid ( $\text{H}_3\text{PO}_4$ ) by Aldrich, distilled water and glycerol. In general, the procedure in this study consisted of three steps, namely the preparation of coral powder, hydroxyapatite synthesis using the

precipitation method and hydroxyapatite synthesis using the sol-gel method.

Powder preparation begins with cleaning the coral using distilled water to remove dirt. Then dried at a temperature of 100 °C to remove water content. Dry corals are manually crushed to a smaller form. Small-sized corals are then crushed using a mortar until smooth. After that the sieving was carried out using a 200 mesh sieve to obtain 70  $\mu\text{m}$  powder. Coral that has become a powder is heated 900 °C for 3 hours to remove impurities. At this stage CaO compounds are formed, according to the reaction stated by Eq. (1).



Calcium phosphate synthesis using precipitation method is done by reacting calcium hydroxide  $\text{Ca}(\text{OH})_2$  with phosphoric acid (equation 3). Calcium hydroxide is obtained through the reaction between calcium oxide and aquades (equation 2). The concentration of phosphoric acid used in this study is 1 M.



The reaction between  $\text{Ca}(\text{OH})_2$  and  $\text{H}_3\text{PO}_4$  was carried out using a stirrer and spin bar for 2 hours at 70 °C. This temperature is to accelerate the occurrence of collisions between ions as the beginning of precipitation. After stirring, the solution is allowed to stand for 24 hours at room temperature to form a precipitate. In this deposition process an ion collection (cluster aggregation) occurs to form amorphous calcium phosphate. Then dehydration is done at a temperature of 110 °C to remove water content and sintering at a temperature variation of 700 °C -1100 °C for 5 hours to form a crystal phase.

The sol-gel method is carried out through various stages including hydrolysis, condensation, aging, and drying. The hydrolysis process is carried out by mixing 50 mL of 1.67 M calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) from marine coral powder with 50 mL of 1M phosphoric acid ( $\text{H}_3\text{PO}_4$ ). The next step is condensation where the transition from sol to gel occurs when heated at 120 °C. At this stage the anatase phase has also formed but is still in an amorphous state. Then the maturation of the gel formed or aging is carried out by settling the gel to change its

nature for 24 hours. In this maturation process a reaction occurs in the formation of gel tissue which is stiffer, stronger, and shrinks in solution. After that, the drying stage is carried out at 120 °C and then calcined at 450 °C to 900 °C each for 6 hours.

## Results and Discussion

There is a change in color and mass of the sample after the calcination and sintering process. Before the calcination process, the color of hydroxyapatite is grayish white, and after the calcination process, the color of hydroxyapatite turns white. The hydroxyapatite mass decreased after the process was carried out by 8.49 percent. This change in mass occurs because of the decomposition of the filling elements and loss of water content from hydroxyapatite. Hydroxyapatite synthesis results were tested using XRD (X-Ray Diffraction) and identification of the peak with the *Search-Match program*. The test using XRD was conducted to determine the phases formed.

The XRD observations of samples synthesized using the precipitation method are shown in Figure 1. With the Search-Match program it can be shown that the sintering temperature greatly affects the type of calcium phosphate formed. Calcium phosphate phases formed include hydroxyapatite (HA), tricalcium phosphate (TCP) and tetracalcium phosphate (TTCP) as shown in Table 1. At 700 °C-900 °C, calcium phosphate formed in HA and TCP phases. If the sintering temperature is increased, the TCP phase changes to TTCP. Calcium phosphate synthesis for biomaterial applications is called optimal if the percentage of HA formed is high. The optimal sintering temperature for the formation of calcium

phosphate occurs at 900 °C, with HA formed at 96.6% and the remaining calcium phosphate in the TCP phase. This compound is more easily absorbed by bone than HA and TTCP. However HA is more thermodynamically stable compared to TCP and TTCP. Therefore for bone graft applications, the number of HA phases determines the success rate.

The results of XRD observations on samples synthesized using the sol-gel method are shown in Figure 2. Like the analysis conducted on the precipitation method, the *Search-Match program* can be shown that the sintering temperature greatly affects the type of calcium phosphate formed. Calcium phosphate phases formed include hydroxyapatite (HA), tricalcium phosphate (TCP) and tetracalcium phosphate (TTCP) (Table 1). In contrast to the synthesis

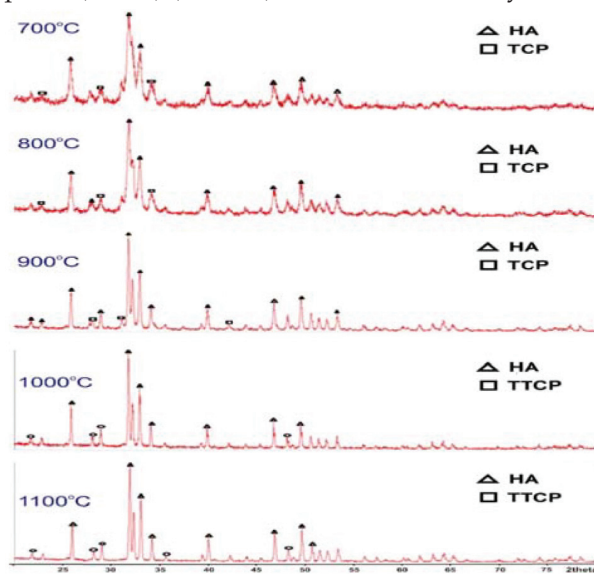


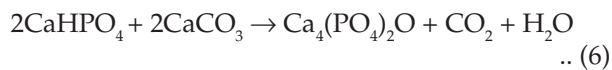
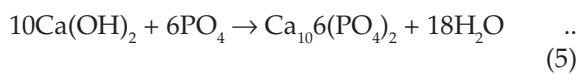
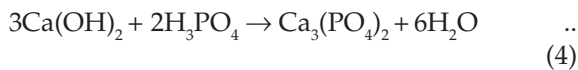
Fig. 1. XRD samples use the precipitation method for temperature variations (700 °C - 1100 °C)

**Table 1.** Content Identification The samples were synthesized using the precipitation method and the sol-gel method

Sintering temperature	Precipitation method Volume fraction (%)			Sol-gel method Volume fraction (%)		
	HA	TCP	TTCP	HA	TCP	TTCP
450°C	-	-	-	86.4	13.6	-
550°C	-	-	-	90.8	9.2	-
650°C	-	-	-	42.4	-	57.2
700°C	81.1	18.9	-	27.7	-	72.3
750°C	-	-	-	27.6	-	72.4
800°C	81.4	19.6	-	17.9	-	82.1
850°C	-	-	-	12.9	-	87.1
900°C	96.6	3.4	-	12.4	-	87.6
1000°C	77.1	-	22.9	-	-	-
1100°C	73.1	-	26.9	-	-	-

of the precipitation method, the sintering temperature of the crystalline phase formation in the sol-gel method occurs at a lower temperature of 450 °C-550 °C. The optimal sintering temperature of HA phase formation occurs at 550 °C with a volume fraction of 90.8%. If the sintering temperature is increased, it will reduce HA phase and eliminate TCP phase and TTCP will be formed.

The process of TCP, HA and TTCP can be explained by chemical reactions such as equation (4), (5), and (6).



Based on the processing temperature of the for-

mation of calcium compounds, especially the HA phase, the sol gel method is more advantageous because it requires a relatively lower sintering temperature than the precipitation method. For optimal HA phase formation, the sintering temperature in the sol gel method is only 550 °C, while the precipitation method is 900 °C. However, based on the percentage of HA formed, the precipitation method is more effective than the sol-gel method because it can produce 96.6% HA. The ratio of Ca / P to the optimum HA which was formed using the sol-gel and precipitation methods were 1.65 and 1.66, respectively. The ratio of Ca/P to pure HA is 1.67. The increase in sintering temperature, both using the sol-gel method and the precipitation method causes the Ca/P ratio to be greater than 1.67. The greater the Ca/P ratio, the more difficult it is to absorb by the bone, making it less good for bone graft applications. This is because it can weaken the osteoconduction and osteoinduction properties of

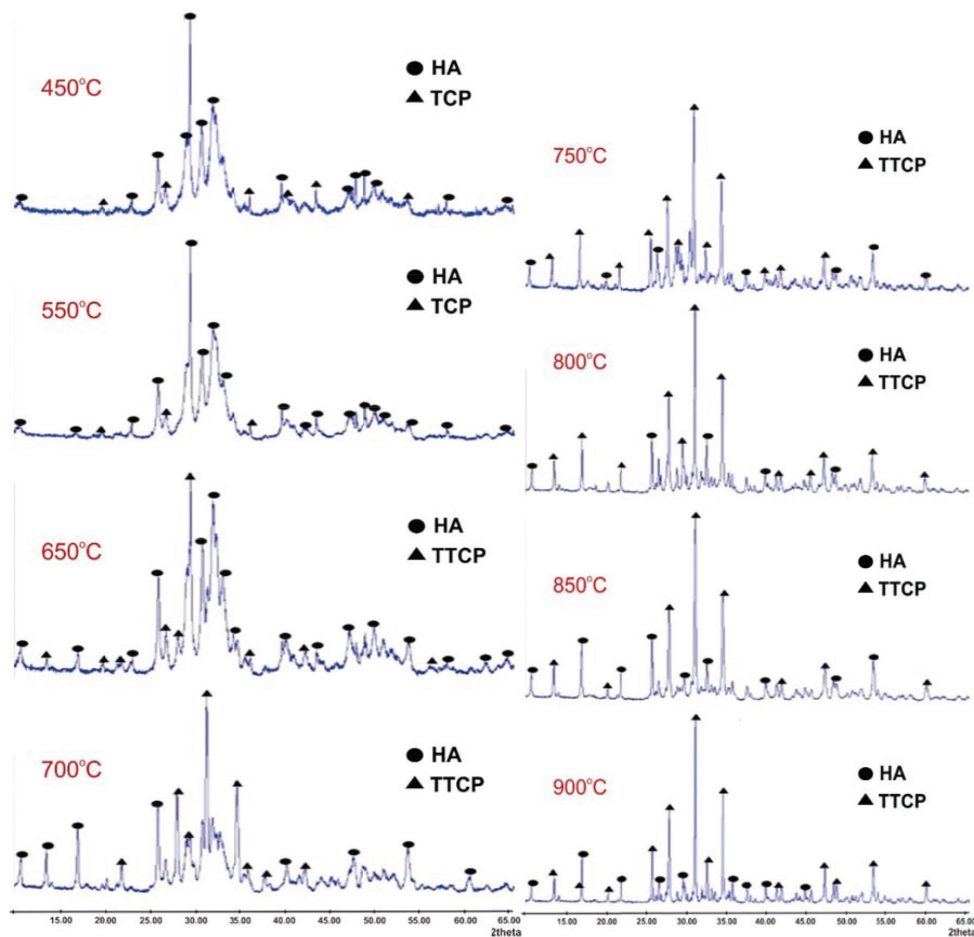


Fig. 2. XRD samples use the sol-gel method for temperature variations (450 °C - 900 °C)

the biomaterial (Kamal *et al.*, 2013).

Research conducted by Haruda *et al.* (2016) using the method of precipitation made from bovine bones obtained HA phase of 99.8%. While the research conducted by Henggu *et al.* (2019) made from cuttlefish using the hydrothermal method obtained calcium phosphate compounds with a Ca/P ratio of 1.66. Different results carried out by Aziz *et al.* (2018) using the method of sol gel made from eggshells obtained a Ca/P ratio of 1.77. From these data shows that the synthesis of calcium phosphate made from corals using the precipitation method produces HA biomaterials that have less impurity than synthesis by the sol gel method. However, the percentage of HA is lower than the percentage made from bovine bones.

### Conclusion

Calcium phosphate synthesis using the sol-gel method requires a lower sintering temperature compared to the precipitation method. The optimal temperature that produces stable calcium phosphate HA using the sol-gel method and precipitation respectively 550 °C and 900 °C. However the percentage of HA volume fraction using the precipitation method is higher than the sol-gel method which is 96.6% and 90.8%. For bone graft application candidates, the use of the precipitation method is recommended because it produces a Ca/P ratio that is close to ideal.

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