

# In Vitro Bioactivity Study of Pure Wollastonite Prepared from Local Raw Materials

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CaSiO<sub>3</sub> ceramics have been proposed as a medical material for artificial bone and dental root, since they proved to be bioactive and biocompatible. In this study, CaSiO<sub>3</sub> powders were synthesized by mixing of Calcium oxide (CaO) and (SiO<sub>2</sub>) for 3 h, and subsequent calcination of the mixture at 710 °C for 2 h. The compacts were conventionally sintered at 1150 °C for 2 h. Moreover, the in vitro bioactivity of wollastonite was investigated by soaking the powders in simulated body fluid (SBF) for various time periods to analyze the nucleation and growth of hydroxyapatite (HA) on the surface of the powders. The obtained results showed that hydroxy-carbonate-apatite (HCA) can be formed on the surface of  $\alpha$ -CaSiO<sub>3</sub> particles soaked in SBF solution for 3 days, and a continuous layer of dense deposits of HCA covered the surface of  $\alpha$ -CaSiO<sub>3</sub> powders after 15 days of soaking in SBF solution. Besides this,  $\alpha$ -CaSiO<sub>3</sub> showed strong hydration when soaked in SBF solution and the hydration was favorable for formation of HCA. Finally, obtained results indicate that  $\alpha$ -CaSiO<sub>3</sub> showed to be highly bioactive through the formation of homogeneous apatite layer.

DOI: [10.12693/APhysPolA.127.925](https://doi.org/10.12693/APhysPolA.127.925)

PACS: 81.05.Je, 81.05.Mh, 87.85.jj

## 1. Introduction

Wollastonite (CaSiO<sub>3</sub>) is a raw material mainly used for traditional ceramics [1]. Since the discovery of Bioglass by Hench and co-workers in 1970 [2], various types of biomaterials containing CaO-SiO<sub>2</sub>, such as wollastonite, have been investigated as bioactive biomaterials for tissue repair and replacement [3–5]. Moreover, some investigators have reported that wollastonite and pseudowollastonite ceramics are bioactive and observed that the formation of apatite on CaSiO<sub>3</sub> ceramics is faster than that on other bioglasses and glass-ceramics in Simulated Body Fluid (SBF) [6–10]. Furthermore, there are many wollastonite preparation routes: from its constituent oxides SiO<sub>2</sub> and CaO, sol-gel process and solid state reaction method. Because of their potential costs and relatively complicated sample preparation, the first two routes are discarded. For example, when powders were synthesized by sol-gel process many expensive and toxic chemicals (such as acids) are generally used. Consequently, an alternative process is proposed.

This process consists of replacing these more expensive starting materials by other raw materials, less expensive (and non toxic) and which are abundantly available in our country (Algeria). The main of these raw materials are: calcite (CaCO<sub>3</sub>), dolomite (CaCO<sub>3</sub>·MgCO<sub>3</sub>), bones (natural derived hydroxyapatite: HA: Ca<sub>10</sub>(PO<sub>4</sub>)<sub>6</sub>(OH)<sub>2</sub>), kaolin, feldspar and quartz. Many works have already been published in

three main interesting research topics for the valorization of these native raw materials. These topics are advanced ceramics [11–15], ceramic membranes [16–23] and bioceramics [24–30]. Indeed, replacing the more expensive starting materials, by other low cost raw materials is significantly important. So, what does 'low cost raw materials' mean? The price of calcite is incomparable with the generally used starting chemicals. These are at least about 100 times more expensive than the calcite. Another important advantage is the substantial saving of energy by decreasing the sintering temperature from about 1300 °C to about 1050 °C, when the proposed process is applied.

Consequently, the main goal of this study is to evaluate the CaSiO<sub>3</sub> powders bioactivity by examining hydroxyapatite (HA) formation on their surface in SBF.

## 2. Materials and methods

### 2.1. Preparation of specimens

Wollastonite (CaSiO<sub>3</sub>) powders were synthesized by solid state reaction method according to our previously published study [26]. The method was based on mixing of high purity SiO<sub>2</sub> (99.9%) and CaO extracted from local CaCO<sub>3</sub>. The mixture was calcinated at 900 °C for 12 h, then hydrated in distilled water and calcinated for a second time at 800 °C for 2 h. A mixture of CaO and high purity SiO<sub>2</sub> (99.9%), with CaO/SiO<sub>2</sub> molar ratio equal to one, was prepared using wet ball-milling for 3 h. Then, the mixture was calcined at 710 °C for 2 h. Subsequently, the powder mixtures were compacted at 150 MPa, and then sintered at 1150 °C for 2 h.

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### 2.2. In vitro tests

The in vitro properties of the sintered specimens were investigated by their apatite forming ability in SBF. The wollastonite samples sintered at 1150 °C for 2 h were ground and sifted through a 40–100  $\mu\text{m}$  sieve, then soaked in SBF solution in which the ion concentrations were nearly equal to those of the human body blood plasma, as it is shown in Table I [31]. The SBF solution was buffered at  $pH = 7.4$  with trimethanol aminomethane-HCl. The samples were immersed in SBF solution at 37 °C for 4 h and 1, 3, 7, 15 days. After soaking for varying periods of time, the samples were gently removed from the fluids using deionized water. This was followed by drying at room temperature before further characterization.

Concentration of SBF in comparison with human blood plasma. TABLE I

Types	Ion concentrations (mM)							
	Na <sup>+</sup>	K <sup>+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Cl <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	HPO <sub>4</sub> <sup>2-</sup>	SO <sub>4</sub> <sup>2-</sup>
SBF	142.0	5.0	1.5	2.5	148.5	4.2	1.0	0.5
Blood plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

### 2.3. Characterizations

The surface structure and morphology of specimens before and after soaking in SBF solutions were characterized by: X-ray diffraction (XRD) (BRUKER, D8 ADVANCE) (Karlsruhe, Germany) with a CuK $\alpha$  radiation ( $\lambda = 0.154$  nm) and a Ni filter, working voltage 40 kV, and working current of 30 mA, Scanning Electron Microscopy (SEM) (HITACHI, JSM-6301 F) (Tokyo, Japan) working at accelerating voltage of 7 kV and Energy Dispersive Spectrometry (EDS) (JEOL JSM-6400).

## 3. Results and discussion

### 3.1. Formation of HAp on the surface of CaSiO<sub>3</sub> powders

The XRD patterns of both the pure CaSiO<sub>3</sub> powders sintered at 1150 °C before and after soaking in SBF solution for various time periods are shown in Fig. 1. Only  $\alpha$ -CaSiO<sub>3</sub> peaks appear for the powders before soaking in SBF solution.

It is obvious that the characteristic peak intensities of pure CaSiO<sub>3</sub> powders decrease with the increase of the soaking time and disappear after 7 days of soaking. New peaks for Ca<sub>4</sub>H(PO<sub>4</sub>)<sub>3</sub>·3H<sub>2</sub>O were apparent after 7 days, while the characteristic peaks of HA at  $2\theta = 32$  and  $26$  appeared after 7 days of soaking, indicating the formation of HA on the surface of  $\alpha$ -CaSiO<sub>3</sub> powders.

The surface morphology of pure CaSiO<sub>3</sub> powders before and after soaking in SBF solution for 3 and 15 days are depicted in Fig. 2. In comparison with the particles before soaking in SBF solution (Fig. 2a) of pure CaSiO<sub>3</sub>,

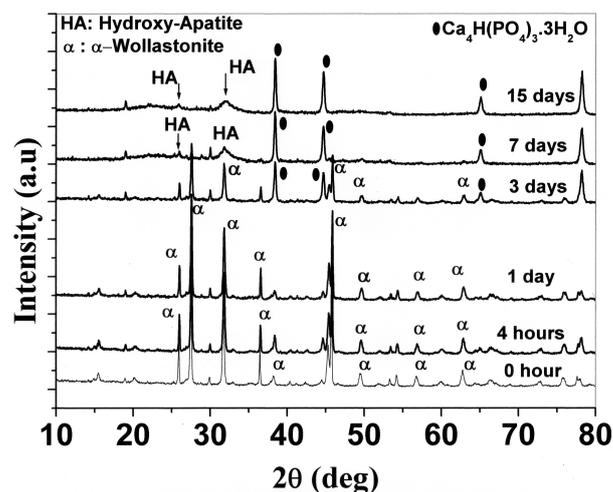


Fig. 1. XRD patterns of  $\alpha$ -CaSiO<sub>3</sub> powders sintered at 1150 °C for 2 h before and after soaking in SBF solution for various periods of time.

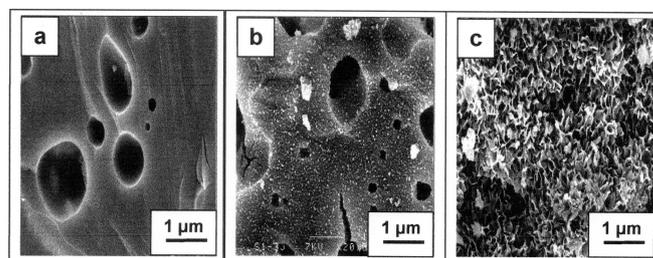


Fig. 2. SEM micrographs of  $\alpha$ -CaSiO<sub>3</sub> before and after soaking in SBF solution for (a) 0 h, (b) 3 d, (c) 15 d.

small ball-like particles appeared inside the pores and on the surface of pure CaSiO<sub>3</sub> powders, soaked in SBF for 3 days (Fig. 2b). After a prolonged soaking for up to 15 days, the surface morphology of powders has changed and a continuous layer of dense deposits formed on the surface of pure CaSiO<sub>3</sub> powders (Fig. 2c).

The formation mechanism of apatite was proposed by Hench *et al.* [32]. The exchange of calcium ions in pseudowollastonite with H<sup>+</sup> in SBF solution gives rise to the formation of silanol (Si-OH) in the surface layer, an increase in  $pH$  value at the pseudowollastonite-SBF, and eventually, to the production of a negatively charged surface with the functional group (Si-O<sup>-</sup>). The later has been proposed to be a catalyzing agent and could provide specific favorable sites for the apatite nucleation.

### 3.2. Concentration changes of Ca, P and Si in SBF solution

The changes of concentrations of Ca, P and Si in SBF solutions measured by ICP after soaking for various time periods was studied. It is obvious that the ion concentrations of SBF solutions changed markedly after soaking in SBF solution. The Ca and Si concentrations in SBF

increased with an increase of the soaking time to 7 days. The concentration of Ca in SBF decreased after 15 days of soaking. In contrast to the increase of Ca and Si, the P concentration of SBF solutions decreased during the soaking periods.

The increases in the Ca and Si concentrations were attributed to the dissolution of these ions from the  $\text{CaSiO}_3$  powders. Moreover, the decrease in the Ca after 15 days is due to a faster consumption of Ca ions during the subsequent formation of HAp on the surface of the powders. The decrease in the P concentration was attributed to the formation of the crystalline apatite on the surface of powders by consuming the P ions from the SBF solutions.

#### 4. Conclusions

Pure  $\text{CaSiO}_3$  powders were synthesized using solid state reaction. The obtained results indicate that pseudowollastonite ( $\alpha\text{-CaSiO}_3$ ) presents high reactivity in SBF solution by showing strong hydration when soaked in it, and that the hydration is favourable for formation of HCA on the surface of the powders.

The obtained results suggest that pure  $\text{CaSiO}_3$  powders have excellent in vitro bioactivity as shown by the formation of HAp in SBF solutions and are potential candidates as tissue repair biomaterials.

#### Acknowledgments

The authors gratefully acknowledge the partial financial support received from the DGRSDT, (Grant No 7/u250/823), and wish to thank Prof. H. Ourag DGRSDT, general director for equipment facilities (Hg-Porosimeter, BET, FTIR, . . . ), Algeria.

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