Research Article

Carbonate Hydroxyapatite and Silicon-Substituted Carbonate Hydroxyapatite: Synthesis, Mechanical Properties, and Solubility Evaluations

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The present study investigates the chemical composition, solubility, and physical and mechanical properties of carbonate hydroxyapatite (CO$_3$Ap) and silicon-substituted carbonate hydroxyapatite (Si-CO$_3$Ap) which have been prepared by a simple precipitation method. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray fluorescence (XRF) spectroscopy, and inductively coupled plasma (ICP) techniques were used to characterize the formation of CO$_3$Ap and Si-CO$_3$Ap. The results revealed that the silicate (SiO$_4^{4-}$) and carbonate (CO$_3^{2-}$) ions competed to occupy the phosphate (PO$_4^{3-}$) site and also entered simultaneously into the hydroxyapatite structure. The Si-substituted CO$_3$Ap reduced the powder crystallinity and promoted ion release which resulted in a better solubility compared to that of Si-free CO$_3$Ap. The mean particle size of Si-CO$_3$Ap was much finer than that of CO$_3$Ap. At 750°C heat-treatment temperature, the diametral tensile strengths (DTS) of Si-CO$_3$Ap and CO$_3$Ap were about 10.8±0.3 and 11.8±0.4 MPa, respectively.

1. Introduction

The use of hydroxyapatite (HA) as a bone substitute is well known for its bioactivity and osteoconductivity in vivo [1, 2]. However, the natural bone which differs from pure HA contains about 4–8 wt% carbonate along with several multi-substituted ions (Na$^+$, Mg$^{2+}$, K$^+$, F$^-$, Cl$^-$, etc.) in its structure [3–5]. Carbonate substituted into the HA structure (CO$_3$Ap) is of special interest because the CO$_3^{2-}$ ion has an impact on different pathologies of human tissues, such as dental caries [6]. CO$_3$Ap was also reported to be more soluble in vivo than HA and to increase the local concentration of calcium and phosphate ions that are necessary for new bone formation [7]. Moreover, CO$_3$Ap is resorbed faster by osteoclasts and replaced with the new bone at a higher rate compared to HA [8]. CO$_3^{2-}$ ion can replace OH$^-$ or PO$_4^{3-}$ ions giving A- or B-type CO$_3$Ap, respectively. If these substitutions take place simultaneously, an AB-type substitution occurs, as in the case of the bone mineral [7, 9].

It was reported that Si enhances and stimulates osteoblast-like cell activity [10] in vitro and induces a higher dissolution rate in vivo [11]. The solubility was observed to increase with a decrease in structural order due to the presence of the foreign ions (i.e., CO$_3^{2-}$, SiO$_4^{4-}$) in the HA structure [12]; nonetheless, only few papers have investigated ion release in synthetic fluids [11, 13]. Therefore, the development of synthetic HA powders with a fully completed ionic substitution in the HA lattice is of great importance in order to mimic that of the natural bone.

Numerous research works have focused on the synthesis of HA biomaterial substituted with single- or multi-ion substitution of CO$_3^{2-}$ [14], Si$^{4+}$ [3, 15], and so forth, whereas the substitution of CO$_3^{2-}$ along with other cations in the apatite structure was restricted to the cosubstitution of HA with the ionic pair of Mg$^{2+}$/CO$_3^{2-}$ [4, 16], Sr$^{2+}$/CO$_3^{2-}$ [17], and Na$^+$/CO$_3^{2-}$ [18]. Although a few research works have been carried out on the synthesis of SiO$_4^{4-}$/CO$_3^{2-}$ cosubstitution in HA [13, 19], it is not clearly apparent whether SiO$_4^{4-}$...
present in the material substituted completely the \( \text{PO}_4^{3-} \) in
the HA structure or whether the replacement was partial. It was reported [12] that both \( \text{CO}_3^{2-} \) and \( \text{SiO}_4^{4-} \) reduced
HA crystallinity, and the structure could host only a limited
amount of the two ions before collapsing. Additionally, the
final product contained \( \text{CO}_3^{2-} \) and \( \text{SiO}_4^{4-} \), but there was a
lack of experimental evidence on the competitive substitution
of \( \text{CO}_3^{2-} \) and \( \text{SiO}_4^{4-} \) for \( \text{PO}_4^{3-} \. \) [12]. Recently, an
extensive study on the \( \text{SiO}_4^{4-} \) and \( \text{CO}_3^{2-} \) cosubstituted
HA was reported [18]. However, the preparation methods were
carried out under air atmosphere and used \( \text{CO}_2 \) from
the atmosphere as the \( \text{CO}_3^{2-} \) source, and as such, there was
no control of \( \text{SiO}_4^{4-} \) substitution level. Thus, the \( \text{CO}_3^{2-} \) ion
present could indeed be doped-HA, where the foreign ion is
just adsorbed on the surface of the crystals [12]. Moreover,
there were few research works that studied the mechanical
properties of the ion-substituted HA after heat-treatment.

Therefore, the purpose of the present work is to investi-
gate the simultaneous substitution of \( \text{SiO}_4^{4-} \) and \( \text{CO}_3^{2-} \) into
the HA structure in order to obtain a product which is closer
to the natural bone. The competition between \( \text{CO}_3^{2-} \) and
\( \text{SiO}_4^{4-} \) for substituting the \( \text{PO}_4^{3-} \) ions in the HA structure
was also investigated. The aim of the work was also to evaluate
the mechanical properties and the solubility of the silicon-
substituted carbonate HA as compared to that of carbonate
HA.

2. Experimental Procedure

A precipitation method was adopted to prepare \( \text{CO}_3\text{Ap} \) using
\( \text{Ca(OH)}_2 \) (96% purity, FLUKA, 21811) and \( \text{H}_3\text{PO}_4 \) (15M,
MERCK, 100573, Germany) with \( \text{CO}_2 \) gas as the carbonate
source [14]. The \( \text{Ca/P} \) molar ratio of the precursors was
designed to be similar to \( \text{Ca/P} \) molar ratio of biological bone,
which is 1.67 [2]. Initially, a solution of 300 mL of \( \text{H}_3\text{PO}_4 \)
1 M was gradually added to 500 mL of \( \text{Ca(OH)}_2 \) 1 M under
vigorous stirring at 400 rpm, whilst \( \text{CO}_2 \) gas was passed
through the reaction flask during the reaction. According to
Landi et al. [14], to obtain the highest carbonation degree and
favor B-type \( \text{CO}_3\text{Ap} \) precipitation with respect to A-type, the
\( \text{CO}_2 \) flow was set at 0.5 bubble/s as the outlet flux. Similar
to \( \text{CO}_3\text{Ap} \), the \( \text{Si}-\text{CO}_3\text{Ap} \) was prepared using silicon teta-
acetate \([\text{Si(COOCH}_3]_4 \) (98% purity, SIGMA-ALDRICH) as
the Si precursor. Based on the chemical formula proposed by
Gibson et al. [20] for silicon-substituted HA (Si-HA), the amount of reagents was calculated by assuming that one
\( \text{SiO}_4^{4-} \) ion would substitute for one \( \text{PO}_4^{3-} \) ion based on a sto-
ichiometric HA; \( \text{Ca/(P+Si)} \) molar ratio = 1.67. \( \text{Si(COOCH}_3]_4 \)
dissolved in the \( \text{Ca(OH)}_2 \) solution under continuous
stirring for 2 hours before adding the \( \text{H}_3\text{PO}_4 \) solution. In
this research work, the Si content was chosen to be 1.6 wt%
which had been shown to be the optimum amount for the
enhancement of the mechanical properties of Si-HA reported
in our previous study [21], where the \( \text{Ca/(P+Si)} \) ratio = 1.84.

The reactions took place in a reaction flask which was
placed in a heating mantle to control the reaction temperature
at 40°C ± 1. The pH of the solution was monitored using
a pH meter. \( \text{NH}_4\text{OH} \) 29% (J.T.Baker, USA) was added to
maintain the \( \text{pH} \) of the solution at 9.4 ± 0.1. After the reaction
was completed, the slurry was continuously stirred for 2 h
without \( \text{CO}_2 \) gas. It was then allowed to mature at room
temperature for 24 h. Subsequently, it was filtered and washed
with deionized water to remove any residue before being
dried in an oven at 70°C for 24 h. The dried \( \text{CO}_3\text{Ap} \) and Si-
\( \text{CO}_3\text{Ap} \) powders were then ground with an agate pestle
and mortar. For the DTS test, the \( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) powders
were compacted by uniaxial hydraulic pressing equipment
using a die with 8 mm diameter at a pressure of 10 MPa. The
thickness of samples was about 2.91–3.25 cm. Alcohol 70%
was used to clean the mold. The compacted samples were then
heat-treated at different temperatures of 650, 700, and 750°C
with a heating rate of 3°C/min and soaked for 2 h in \( \text{CO}_2 \)
3 atmosphere (80 mL/min) which was passed through 150 mL
distilled water. The syntheses of \( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) were
repeated three times to confirm the reproducibility of the
materials.

The as-synthesized and heat-treated powders were charac-
terized using an X-ray diffractometer (XRD; D5000 Siemens)
for phase identifications. Peak (002) was chosen for
determining the crystallite size since it is one of the strongest
peaks without any overlapping in the \( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) patterns. The lattice parameters (\( a \) and \( c \)) of the as-prepared
\( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) samples were determined through
the \( (hkl) \) peaks position of the apatite from XRD patterns
according to (1) as follows [22, 23]:

\[
\frac{1}{d^2} = \frac{4}{3} \left( \frac{l^2 + k^2 + l^2}{a^2} \right) + \frac{l^2}{c^2}.
\]

Fourier transform infrared spectroscopy (FTIR; Perkin-
Elmer FT-IR 2000, FTIR spectrometer) was used to study the
silicon and carbonate substitutions of the different functional
groups, such as \( \text{OH}^- \), \( \text{PO}_4^{3-} \), \( \text{CO}_3^{2-} \), and \( \text{SiO}_4^{4-} \) in
the \( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) samples. The carbonate content of
powders was analyzed using an elemental analyzer (CHN test;
Perkin Elmer series 2, 2400 CHN/O). The chemical
composition (Si and Ca) was determined by inductive cou-
pled plasma (ICP) spectrometer (ICP/AES, ARL-3410). X-ray
fluorescence spectrometer (XRF; Rigaku RIX-300 wavelength
dispersive) was used to study the \( \text{Ca/P} \) ratio of the as-
prepared powders. The particle size of the powder (with ultra-
sonic dispersion) was measured using a Malvern Mastersizer
X (Malvern Instruments, Malvern, UK). The powder before
being characterized had been passed through a 75 μm sieve.

The densities of the heat-treated \( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) compacts were measured using Archimedes’ principle. The
diametral tensile strengths (DTS) of the heat-treated \( \text{CO}_3\text{Ap} \) and Si-\( \text{CO}_3\text{Ap} \) compacts were tested at a strain rate of
0.5 mm/min. The DTS test involves compressing a sample
diametrically, inducing a stress that causes the sample to yield
in tension. In this test, a disk sample was placed between
two platens and then vertically compressed until it broke
[24]. During loading, the applied force was recorded and the
tensile stress was calculated using (2)

\[
F_t = \frac{2P_{\text{max}}}{\pi dh},
\]

where \( P_{\text{max}} \) is maximum load at failure (N) and \( h \) and \( d \) are
the thickness and diameter of the compacts (mm), respectively.
The solubility evaluation was performed in triplicate on the as-synthesized CO$_3$Ap and Si-CO$_3$Ap compacts (8 mm diameter, 10 MPa) by immersing the compacts in a simulated body fluid (SBF) solution at 36.5°C. The SBF solution was prepared according to the procedure described by Kokubo and Takadama [25]. The tests were carried out within 1 and 7 days. After the predetermined soaking time, the samples were removed and the liquid mediums were analyzed by ICP. The released ion was estimated by subtracting the initial ion concentration of the SBF solution from the ion concentration of the SBF solution after immersion.

Statistical analysis was performed to evaluate the statistical differences between the sample sets by employing one factor analysis of variance (ANOVA) when comparing more than two sample populations. Significant differences were considered at the 95% level ($P < 0.05$).

### 3. Results and Discussion

#### 3.1. Physical and Chemical Composition Analyses

Table 1 shows the physical and chemical properties of the as-synthesized CO$_3$Ap and Si-CO$_3$Ap samples. The mean particle size of the as-synthesized Si-CO$_3$Ap sample is significantly smaller than that of the as-synthesized CO$_3$Ap sample. This can be attributed to the substitution of Si in the HA structure, as reported in previous research works [21, 26].

In the same table, the Ca/P molar ratios of the as-synthesized CO$_3$Ap and Si-CO$_3$Ap samples show much higher values than those of the predetermined ratios. This indicated that the substitution of CO$_3^{2−}$ and SiO$_4^{4−}$ ions for the PO$_4^{3−}$ groups in the HA had taken place. These substitutions reduce the amount of PO$_4^{3−}$ group, thus leading to an increase in the Ca/P ratio [14, 20]. However, the Ca/P ratio in this study was in the range of the Ca/P molar ratio of CO$_3$Ap reported previously, which was of 1.7–2.6 [27].

The Si contents are also included in Table 1. Si measured in the as-synthesized Si-CO$_3$Ap sample is about 0.85 wt%, and this is much lower than the starting value (1.6 wt%). The rest of the Si unaccounted for will be explained in the FTIR analysis. It was suggested that an amount of only 1 wt% Si substituted into HA was sufficient to elicit important bioactive improvements [12], and, hence, the Si-substituted CO$_3$Ap in this research work could be considered to approach this enhancement.

After heat-treatment at a temperature range of 650–750°C, the carbonate amount slightly decreases compared to the as-prepared samples (Table 2). This is due to the fact that carbonate absorbed had desorbed upon heat-treatment. The amount of carbonate is close to the typical amount of carbonate in human bone [28].

#### 3.2. XRD Analysis

Figure 1 shows the XRD patterns of the as-synthesized CO$_3$Ap and Si-CO$_3$Ap powders. The broad peaks indicate the formation of HA phase with low crystallinity, and no secondary crystalline phases were observed. The poor crystallinity was due to the low synthesis temperature and the substitution of SiO$_4^{4−}$ and CO$_3^{2−}$ ions limited the crystallization of the HA phase [18, 21].

The crystallite size determined using Scherrer’s equation and the lattice parameters are given in Table 3. The CO$_3^{2−}$ and SiO$_4^{4−}$ substitutions in HA structure led to changes in the crystal lattice parameters [4, 18]. Previous studies had shown that the $a$-axis decreased and the $c$-axis increased with increasing CO$_3^{2−}$ or SiO$_4^{4−}$ in the HA structure [3, 6]. The values presented in Table 3 for the as-prepared powders in this present research work also show a similar trend with previous works. The SiO$_4^{4−}$ groups are larger and have a more negative charge than either PO$_4^{3−}$ or CO$_3^{2−}$ ions [15, 18]. Additionally, the substitution of SiO$_4^{4−}$ and CO$_3^{2−}$ for PO$_4^{3−}$ contributes to reducing the crystallite size, as has been observed previously in other studies [12, 18, 21].

Numerous studies showed that both $a$- and $c$-axis dimensions increased with the silicon content [18, 29, 30]. Considering the substitution of SiO$_4^{4−}$ in the CO$_3$Ap, it is possible that, $a$- and $c$-axis dimensions are higher than those of CO$_3$Ap (Table 3) because the ionic bond length of a Si–O bond (0.166 nm) is greater than that of a P–O bond (0.157 nm). The radius of the PO$_4^{3−}$ tetrahedron would be smaller than that of the SiO$_4^{4−}$ tetrahedron that results in the change of HA lattice parameters.

### Table 1: Physical and chemical properties of the as-synthesized CO$_3$Ap and Si-CO$_3$Ap samples.

| Sample  | Starting value | Si content (wt%) | Measured value (ICP/in powder) | Starting value | Ca/P | Measured value (XRF) | Mean particle size (μm) |
|---------|----------------|------------------|-------------------------------|----------------|------|----------------------|-------------------------|
| CO$_3$Ap | 0              | —                | —                             | 1.67           | 2.08 | 2.52                 | —                       |
| Si-CO$_3$Ap | 1.6          | 0.85             |                               | 1.84           | 2.16 | 0.98                 | —                       |

### Table 2: Carbonate contents in the CO$_3$Ap and Si-CO$_3$Ap samples before and after heat-treatment.

| Sample  | CO$_3$ (wt%) | As-prepared powders | CO$_3$ (wt%) | Heat-treated powders |
|---------|--------------|---------------------|--------------|----------------------|
| CO$_3$Ap | 10.75        | 10.1                | 10.05        | 10.05                |
| Si-CO$_3$Ap | 10.25       | 9.4                 | 9.4          | 8.4                  |

### Table 3: Lattice parameters and crystallite size of the as-synthesized CO$_3$Ap and Si-CO$_3$Ap powders.

| Sample  | Lattice parameters (Å$^2$) | Crystallite size (nm) |
|---------|---------------------------|------------------------|
| HA [26] | $a = 0.94366 \pm 0.003$   | $c = 6.8905$          |
| CO$_3$Ap | $9.3860 \pm 0.03$        | $6.8963$             |
| Si-CO$_3$Ap | $9.4061 \pm 0.03$       | $16.82 \pm 0.02$   |
Figure 1: XRD patterns of the as-prepared powders: (a) CO$_3$Ap and (b) Si-CO$_3$Ap.

Figure 2: XRD patterns of the samples after heat-treatment of Si-CO$_3$Ap at (a) 650°C, (b) 700°C, and (c) 750°C and of CO$_3$Ap at (d) 650°C, (e) 700°C, and (f) 750°C.

After heat-treatment at 650°C to 700°C, pure CO$_3$Ap and Si-CO$_3$Ap are still observed and no secondary phases are detected (Figure 2(a), (b), (d), and (e)). However, a new phase, CaCO$_3$, is clearly observed in Si-CO$_3$Ap samples heat-treated at 750°C due to the decomposition of the Si-CO$_3$Ap samples.

Sintering of CO$_3$Ap at high temperatures (>900°C) [15, 31] produces hydroxyapatite (HA) and CaO. In the CO$_3$-rich atmosphere, the CaCO$_3$ obtained was due to the reaction of CaO and CO$_2$. Therefore, a mixture of CO$_3$Ap and CaCO$_3$ is observed after heat-treatment in CO$_2$ atmosphere. The decomposition temperature decreased with an increase of the carbonate [31] and/or silicon content [15, 32]. Since the heat-treatment process was carried out at low temperatures, such decomposition did not occur in the CO$_3$Ap sample but did occur in Si-CO$_3$Ap sample at 750°C. The simultaneous substitution of SiO$_2$$^{4-}$ and CO$_3$$^{2-}$ ions for PO$_4$$^{3-}$ ions of the HA structure increased the defects in HA structure and produced more OH$^-$ vacancies [13] compared to CO$_3$Ap. The formation of OH vacancies has been proven to accelerate the decomposition process [23]. Thus, the formation of CaCO$_3$ in the Si-CO$_3$Ap could be explained by a similar mechanism as the decomposition of CO$_3$Ap.

3.3. FTIR Analysis. FTIR spectrum of each powder (Figure 3) shows the characteristic absorption bands of HA corresponding to stretching vibration of PO$_4$$^{3-}$ ions at 567, 604 cm$^{-1}$ (v4); 963 cm$^{-1}$ (v1); 1045 cm$^{-1}$ (v3); in all the as-synthesized powder bands. The broad band at about 1638 cm$^{-1}$ corresponds to in-plane water bending mode. The CO$_3$$^{2-}$ groups substituted in B-site were confirmed with typical bands around 874 cm$^{-1}$ (v2), 1470 cm$^{-1}$ [4, 18, 33], whereas the bands located at 1505 cm$^{-1}$ could be attributed to A-type CO$_3$Ap [28].

The characteristic OH$^-$ bands of HA at 630 cm$^{-1}$ are not clearly visible in all FTIR spectra. In fact, a similar decrease in the intensity of OH$^-$ signals was also observed due to the substitution of CO$_3$$^{2-}$ at the OH$^-$ lattice of HA [33]. In this case, the substitution of CO$_3$$^{2-}$ and SiO$_2$$^{4-}$ ions for PO$_4$$^{3-}$ would create an OH$^-$ loss needed to compensate the charge balance, thus resulting in the weak of OH$^-$ signal.

Additional bands are also observed in the Si-CO$_3$Ap sample at about 800 cm$^{-1}$ and 480 cm$^{-1}$ which do not appear in CO$_3$Ap sample. The band at 480 cm$^{-1}$ is assigned to the SiO$_2$$^{4-}$ in the apatite structure [15]. However, the band at about 800 cm$^{-1}$ might be assigned to either the silicate group [30] or to the O–Si–O bending in the SiO$_4$ amorphous phase [22, 34]. As detected by ICP, the amount of Si in Si-CO$_3$Ap sample is much lower than the starting value (Table 1); the silicate species which could not totally be incorporated in the apatite structure exist on the surface of the materials as an amorphous phase [22, 35] and/or remain in mother liquors after precipitation [36]. The remaining Si suggests that the competition arising between the SiO$_2$$^{4-}$ and CO$_3$$^{2-}$ ions occupies the PO$_4$$^{3-}$ sites. The polymerization of the silicate species at the surface was reported elsewhere [37]. In another research work [38], the amorphous SiO$_2$ phase in β-TCP containing Si-substitution showed a significantly higher MC3T3-E1 osteoblast-like cell number compared to pure β-TCP. Therefore, the presence of SiO$_2$ would not cause toxicity to the cells and would not affect cell differentiation.
The substitutions of CO$_3^{2-}$ and SiO$_4^{4-}$ groups for PO$_4^{3-}$ change the symmetry and stability of an apatite structure [39]. As a result of these substitutions, shifts and splitting of the PO$_4$ vibration bands at about 500–700 cm$^{-1}$ occur in the apatite IR spectra (Figure 3).

It has already been reported that the calcium phosphate apatite constituent of bone mineral consists of a mixed AB-type substitution [40]. The results from the present study confirm the formation of AB-type carbonated apatite along with the presence of Si in the structure. Thus, this complex substitution type is also of utmost importance when the development of a synthetic bone-substitute material is sought.

3.4. Evaluation of Mechanical Properties and Microstructure. The mechanical and physical properties were evaluated in terms of diametral tensile strength (DTS) and bulk density. In Figure 4, the density of CO$_3$Ap sample is higher than that of Si-CO$_3$Ap sample at any heat-treatment temperatures. This can be explained by the higher lattice parameters of both CO$_3^{2-}$ and SiO$_4^{4-}$ cosubstitution compared to the single CO$_3^{2-}$ substitution (Table 3).

It can also be seen that the density of the CO$_3$Ap samples significantly increases with increasing heat-treatment temperatures, whilst there is only a slight change in the density of the Si-CO$_3$Ap samples. The substitution of Si reduced the density of the materials compared to HA as reported previously [15, 21] due to the change of unit cell parameters in the silicon-substituted materials. Therefore, the effect of silicon became significant which slowed down the densification process upon heat-treatment. In the present research work, the densities of CO$_3$Ap and Si-CO$_3$Ap are significantly lower compared to that of a fully dense HA (3.16 g/cm$^3$) due to the low heat-treatment temperatures.

Figure 5 shows that the DTS of both CO$_3$Ap and Si-CO$_3$Ap samples significantly increase with increasing temperatures. The increase in DTS value of CO$_3$Ap with the increasing heat-treatment temperatures can be explained by the increase in density as shown in Figure 4. However, although a slightly higher density was obtained for the Si-CO$_3$Ap, the DTS of Si-CO$_3$Ap increases significantly with increasing heat-treatment temperatures. This is due to the cosubstitution of CO$_3^{2-}$ and SiO$_4^{4-}$. This cosubstitution induced the smaller particle size (Table 1). In addition, Si substitution was reported to impede grain growth during heat-treatment [41] and so increased the DTS value.
By comparison, the DTS of CO$_3$Ap samples appear to be slightly higher compared to those of Si-CO$_3$Ap samples. This difference in strength was evaluated to be $\rho > 0.05$, and as such, this difference in DTS value of Si-CO$_3$Ap is insignificant compared to CO$_3$Ap. However, its density is significantly lower ($\rho < 0.05$) indicating the positive effect of SiO$_4^{4-}$ and CO$_3^{2-}$ cosubstitutions on this matter. As reported, the effect of silicon on the increase of mechanical strength was evidenced at higher heat-treatment temperatures, that is, 1200°C and above, as compared to Si-free samples [21]. Conversely, at lower temperatures, this effect was not so apparent where the strengths of Si-samples and Si-free samples were comparable based on previous studies [21, 41] and even lower [19] due to the lower density. Hence, due to the low heat-treatment temperatures employed in this research work, the difference in strength between CO$_3$Ap and Si-CO$_3$Ap samples is not that significant.

Figure 6 presents a comparison of the DTS values of the present materials at 750°C with those of samples in previous research works [19, 21]. Interestingly, at the same Si content (about 0.8 wt%), the DTS values of CO$_3$Ap and Si-CO$_3$Ap at 750°C are about 10.8 ± 0.3–11.8 ± 0.4 MPa, and these are higher than those of Si-substituted HA samples at 1250°C [21] and much higher than that of Si-HA sample at 1300°C [19]. This demonstrates that, at these low heat-treatment temperatures, the cosubstitution of carbonate and Si in the HA structure would increase the strength of the final product. In Figure 6, the DTS of Si-substituted HA sample [21] is higher than that of pure HA because the SiO$_4^{4-}$ substitution impeded grain growth at high temperatures and, therefore,
increased the strength of the materials [41]. The DTS of CO₃Ap and Si-CO₃Ap in the present work are also higher than that of pure HA. It was explained [42] that the CO₃²⁻ and SiO₄⁴⁻ substitutions also reduced the grain size of the final product and resulted in an increase of the strength of the samples.

3.5. Solubility Evaluation. In the case of crystalline HA, the degree of micro- and macroporosities, defect structure, and amount and type of other phases present have a significant influence on the dissolution rate [43]. In this study, the immersion of the CO₃Ap and Si-CO₃Ap compacts (surface area = 150.8 mm²) into SBF solution produced noticeable changes in the ion concentrations of the solution. Figures 7(a), 7(b), and 7(c) show the ion concentration of Ca, Si and changes in pH value of the medium after a certain period of immersion time, respectively. According to Boanini et al. [12], crystallinity and crystal dimensions significantly affected the solubility and, as a consequence, ion release. Thus, a decrease in structural order due to the presence of foreign ions might be responsible for the observed increase in solubility.

In Figure 7, the Ca²⁺ and Si⁴⁺ ion concentrations as well as the pH of the SBF solution increase with soaking duration which indicates the dissolution of Ca³⁺ and Si⁴⁺ ions. It had been reported that the initial dissolution of implant materials plays an important role in enhancing their bonding to the bone [32]. With an increase in the soaking duration, Ca²⁺ concentrations and pH value continuously increase due to the ionic exchange between H⁺ within the SBF solution and Ca²⁺ in the CO₃Ap and Si-CO₃Ap compacts [44, 45]. The increase of solution pH generally facilitates the nucleation of apatite [46].

The release of Si⁴⁺ ions was also observed continuously over the whole investigation period. It was reasoned out that the amorphous layer surrounding the apatite grains dissolved within the first period of immersion in SBF leaving a more stable and less soluble core [13]. As solubility is highly sensitive to the structural and chemical compositions of the apatite samples, the crystallite size is a key factor for in vitro behavior of synthetic apatite [47]. In this manner, the resorbability of CO₃Ap and Si-CO₃Ap could be promoted by a smaller crystallite size when CO₃²⁻ and SiO₄⁴⁻ were cosubstituted; the amorphous shell can be thicker and yield a more intense and prolonged ion release [13]. In addition, the Ca²⁺ release in Si-CO₃Ap compacts is slightly higher compared to CO₃Ap, which suggests a better solubility (Figure 7(a)) that leads to a faster super-saturation with respect to HA, a faster nucleation, and growth of apatite on the surface of the compacts [36].

By comparison, the Ca²⁺ release for CO₃Ap and Si-CO₃Ap samples in this study is much higher than that of Mg-substituted fluorapatite [48] and HA [44, 49] under the same conditions. It was reported that the solubility of materials increases with increasing ionic substitutions into the HA lattice and decreasing crystallinity which is represented by the higher ion release in the SBF solution [13, 16, 49]. Therefore, the CO₃Ap and Si-CO₃Ap obtained in this work are of higher solubility compared to the above-mentioned materials.

Based on the solubility evaluations using SBF, the solubility of CO₃Ap and Si-CO₃Ap is such that it is predicted that ions would continuously exist in actual physiological conditions. This is further reinforced by a previous work [13]. These materials could supply elements which are essential for osteoblast activity and new bone tissue formation [13]. The simultaneous presence of such elements can further enhance the cell response.

4. Conclusions

Carbonate hydroxyapatite and silicon-substituted carbonate hydroxyapatite powders were successfully synthesized by a simple and high-yield process. The crystalline and mean particle size of Si-CO₃Ap sample was significantly smaller than that of CO₃Ap sample due to the cosubstitution of SiO₄⁴⁻ and CO₃²⁻ in the HA structure. No secondary phases were detected in CO₃Ap and Si-CO₃Ap samples after heat-treatment in the temperature range of 650°C to 700°C. CaCO₃ was observed in Si-CO₃Ap sample after heat-treatment at 750°C, whilst the purity of CO₃Ap was retained. The SiO₄⁴⁻ and CO₃²⁻ cosubstituted HA structure led to a significant decrease in density compared to a single CO₃²⁻ substituted HA structure, whilst the DTS of both samples showed insignificant differences.

The competition between SiO₄⁴⁻ and CO₃²⁻ ions had taken place to occupy the PO₄³⁻ site. Si-CO₃Ap existed in the form of AB-type carbonated apatite, and the presence of SiO₄⁴⁻ in the structure is of utmost interest in developing a synthetic bone-substitute material. The total amount of carbonate and silicon and the crystal size of the powder obtained mimic those of biological apatites. The silicon substitution improved the solubility of Si-CO₃Ap which prolongs the ion release compared to that of Si-free CO₃Ap. The present materials possess low crystallinity and the CO₃²⁻ content is close to that found in natural bone, and, in combination with the high strength, these materials could be ideal for bone substitutes.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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