

Phase transformation and structural characteristics of zinc-incorporated β -tricalcium phosphate

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Biological performance of bioceramics such as calcium phosphate has been proved to be improved by substitution of different ions like Mg, Sr and Si. In this study, different amounts of Zn ions in nitrate form were incorporated into β -tricalcium phosphate in which various molar ratios of Ca:Zn were achieved: 3:0, 2.8:0.2, 2.6:0.4, 2.4:0.6, and 2.2:0.8. The mixtures were heated at different temperatures ranging from 800 – 1100 °C. The phase composition, amount of each phase and lattice parameters of β -tricalcium phosphate were determined by means of X-ray diffractometry and coupled software. Also, solubility of the heated mixtures was investigated by determining the amount of Ca and Zn released into a simulated body fluid during 120 h. The results revealed that only limited amount of Zn ions could be incorporated into β -tricalcium phosphate lattice and ZnO phase was formed when high content of zinc nitrate was introduced in initial mixture. Both *a* and *c* lattice parameters of β -tricalcium phosphate were reduced by adding Zn. The release rate of calcium ions into the simulated body fluid was approximately constant during 120 h while for Zn minor release was observed.

Keywords: *bioceramics; tricalcium phosphate; zinc; bone filler*

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1. Introduction

Calcium phosphate (CaP) is of special significance for humans because it represents an the inorganic part of normal tissues in mammals, i.e. bones, teeth and deer antlers. Therefore, due to chemical similarity to biological calcified tissues the majority of artificially prepared calcium orthophosphates possess remarkable biocompatibility and bioactivity. Materials scientists use this property to construct artificial bone grafts that are either entirely made of or surface coated by biologically relevant CaP. Hydroxyapatite [HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] and β -tricalcium phosphate [β -TCP, $\text{Ca}_3(\text{PO}_4)_2$] are the most widely used CaP-based materials. Regardless of their favorable biological properties, both materials possess a number of drawbacks, i.e. low degradation rates (in the case of HA), poor mechanical properties, and low osseointegration ability [1–4].

To enhance the biological performance, modifying of chemical composition of bone substitute materials is effective. A bone consists of an organic phase (collagen and non-collagenous proteins) and an inorganic or mineral phase. The biological apatites (bones) are not pure HA but contain ions including zinc (Zn), strontium (Sr) and magnesium (Mg) that can substitute for the different cations present in the apatite structure [5, 6]. Zinc, an essential element and a cofactor for more than 200 enzymes, is present in nearly every type of cell in the body. Zn is important for cell proliferation, immunity, spermatogenesis, wound healing, and maintaining the senses of taste and smell. It also plays a very important role in the formation of bones. In fact, the concentration of Zn in a bone is higher than that in any other tissue. As an essential metal, Zn inhibits the differentiation of osteoblasts and/or promotes the activity of osteoblasts, affecting the formation of hard tissues, but too high Zn concentration can have serious side effects on cells. However, as Zn is an important essential metal with very strong bioactivity, and has serious side effects at high concentrations, control-

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ling of its release into blood is vital for therapeutic use. Bone-promoting effects of Zn-containing β -TCP have been reported [7–9].

The presence of zinc ions changes the structural, physical and chemical properties of calcium phosphates and induces formation of various calcium phosphate phases in a concentration-dependent manner. Numerous ion substituting evaluations have been done on hydroxyapatite [10]. In our previous study, the role of Mg and Sr on the structure and solubility of β -TCP was evaluated [11]. The evidence of the beneficial effect of Zn in bone growth motivated us to incorporate this element into β -TCP through solid state process at high temperatures, and evaluate the effect of concentration of Zn ions on lattice parameters and phase transformation.

2. Materials and methods

β -tricalcium phosphate was synthesized by solid state method as reported in our previous work [12], briefly: calcium carbonate (2069, Merck KGaA, Darmstadt, Germany) and dicalcium phosphate (2144, Merck KGaA, Darmstadt, Germany) were mixed at a molar ratio of 2:1 (for Ca/P = 1.5). Then, the mixture was ball-milled for 6 hours, followed by heating at 900 °C for 3 hours, and cooled down in a furnace to the ambient temperature. After that, the particle size and specific surface area of the synthesized β -TCP were measured by Laser-Particle Sizer (FRITSH analysette 22) and Brunauer–Emmett–Teller (BET) (Gemini 2375 Micrometrics), respectively. For incorporating Zn into β -TCP, zinc nitrate hexahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; Merck KGaA, Darmstadt, Germany) was employed at elevated temperatures. Therefore, thermal behavior of this salts was assessed using simultaneous thermal analysis (STA; PL-STA 1640) up to 1200 °C with a heating rate of 10 °C/min. β -TCP was poured into distilled water so that the concentration of 1.5 g/mL was achieved.

Then, the nitrate salt was added in different concentrations whilst the suspension was stirred. The amount of zinc nitrate hexahydrate was calculated so as to achieve different Ca (calcium ion in β -TCP) to Zn ratios: 3:0, 2.8:0.2, 2.6:0.4, 2.4:0.6,

and 2.2:0.8. Subsequently, the prepared suspensions were heated on a magnetic stirrer until evaporation of solvent and then dried in an oven at 100 °C for 24 h. In order to achieve better homogenization, the powder was fast-milled for 15 minutes, and finally, heated at different temperatures of 800 – 1100 °C. The same amount of each sample was used to evaluate the phase composition of heat-treated samples using an overnight automated X-ray diffractometer device (XRD, Philips PW3710). Cu- $\text{K}\alpha$ radiation was employed at operating condition of 40 kV and 30 mA and XRD data were collected over 2θ range of 10 – 50 degrees with a step size of 0.02 degree. The precise phase determination was done using X'Pert High Score Plus data processing software.

Changes in the lattice parameters of Zn^{2+} substituted β -TCP and the phase quantities were determined with Rietveld refinement analysis as described elsewhere [13, 14]. The structural model of ICDD card numbers 9-0169, 24-033 and 5-0664 was used for β -TCP, hydroxyapatite and ZnO, respectively and the software TOPAS 2, (Siemens, Germany) was employed for this purpose.

The Ca and Zn release behavior of powdered samples was carried out as described elsewhere [11]. Briefly, 1 mg of the powder was poured into 20 mL simulated body fluid (SBF) and kept at 37 °C for various times up to 120 h. The solution, at each period, was replaced by the fresh one and Ca and Zn concentrations of extracted solution were measured by inductively coupled plasma (ICP-AES).

It should be taken into account that SBF is a solution with pH of 7.25 that resembles inorganic composition of blood plasma and is prepared by dissolving chemicals (NaCl , KCl , $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, CaCl_2 , and Na_2SO_4) in deionized water in accordance to Kokubo's specification [15].

3. Results and discussion

3.1. Synthesized β -TCP

Fig. 1 illustrates the XRD pattern of synthesized β -TCP in comparison with its standard diffraction patterns based on ICDD (International Cen-

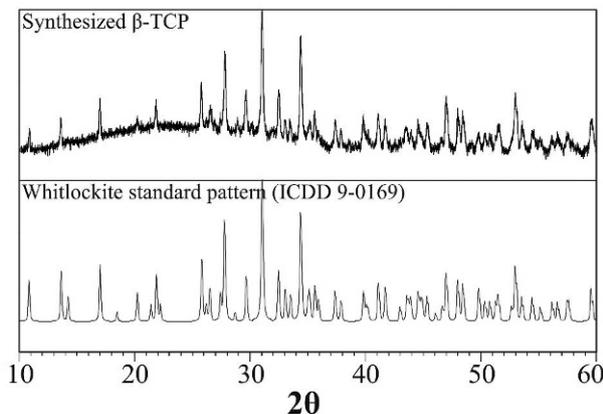


Figure 1. The XRD pattern of synthesized β -TCP (upper) and standard diffraction data according to ICDD 009-0169.

ter Diffraction Data) 009-0169. In the synthesized powder, all peaks belong to β -TCP corresponding to whitlockite mineral. Comparing the XRD pattern of the synthesized sample with the standard card, one can see that the β -TCP is pure and no impurity is observed. The highest peak of the whitlockite phase is produced at 31.04 degree, which corresponds to (0210) plane.

3.2. Thermal behavior of zinc nitrate

The STA (TG, DTA) of zinc nitrate hexahydrate is shown in Fig. 2. An endothermic peak at ~ 600 °C corresponds to decomposition of zinc nitrate into zinc oxide. In addition, the endothermic peaks at ~ 100 °C and ~ 250 °C are associated to removal of absorbed and crystalline water, respectively. Also, the 15 % weight loss at ~ 600 °C in TG is related to exhaustion of NO_2 gas. Decomposition of zinc nitrate salt occurs at about 600 °C. Thus, heat treatment of the mixture of zinc nitrate and β -TCP was done above this temperature.

3.3. XRD pattern of zinc-doped- β -TCP

As mentioned elsewhere, the highest peak in the whitlockite phase is formed at $2\theta = 31.04$ degree which corresponds to (0210) atomic plane. The XRD patterns of β -TCP with different amounts of incorporated zinc are illustrated in Fig. 3. Also, Fig. 4 shows a comparison of XRD patterns of the samples at a heating temperature of 1100 °C.

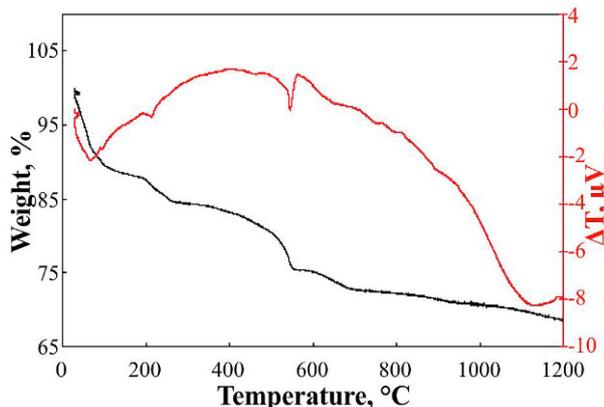


Figure 2. The STA curve of zinc nitrate hexaphosphate up to 1200 °C.

The absence of $(\text{Ca}, \text{Zn})_3(\text{PO}_4)_2$ phase was confirmed by checking ICDD standard cards 20-0248, 20-0249, 20-0250, 20-0251 and 01-084-1569. In zinc doped samples with Ca/Zn:2.8/0.2, HA and ZnO existed at low temperatures of 800 °C and 900 °C, and as the temperature was increased up to 1100 °C, HA disappeared and just slight amount of ZnO remained. β -TCP exists as a predominant phase at this temperature.

The same behavior can also be seen in other mixtures (Ca/Zn: 2.6/0.4, 2.4/0.6, and 2.2/0.8) but the concentration of ZnO increases as a result of increased amount of the added zinc nitrate salt. Furthermore, in 1100 °C, ZnO and HA phases can be observed. It seems that at temperatures lower than 1100 °C, the ions have not enough energy to migrate into the lattice of β -TCP.

Table 1 presents the quantities of whitlockite, ZnO and hydroxyapatite phases in the powders heated at 1100 °C. In Ca/Zn:2.8/0.2 (6.66 mol% Zn) slight amount of ZnO is observed and the concentration of HA is negligible. The quantity of ZnO and even HA increases by adding more nitrate salt. In Ca/Zn:2.2/0.8, whitlockite and ZnO are dominant phases and some amount of HA is also noticeable.

Fig. 5 reveals a shift in the XRD patterns of the Ca/Zn:2.8/0.2 sample (heated at 1100 °C) in comparison to pure β -TCP. Right-shift of the patterns indicates a decrease in the atomic plane distance caused by Ca substitution by smaller Zn ions.

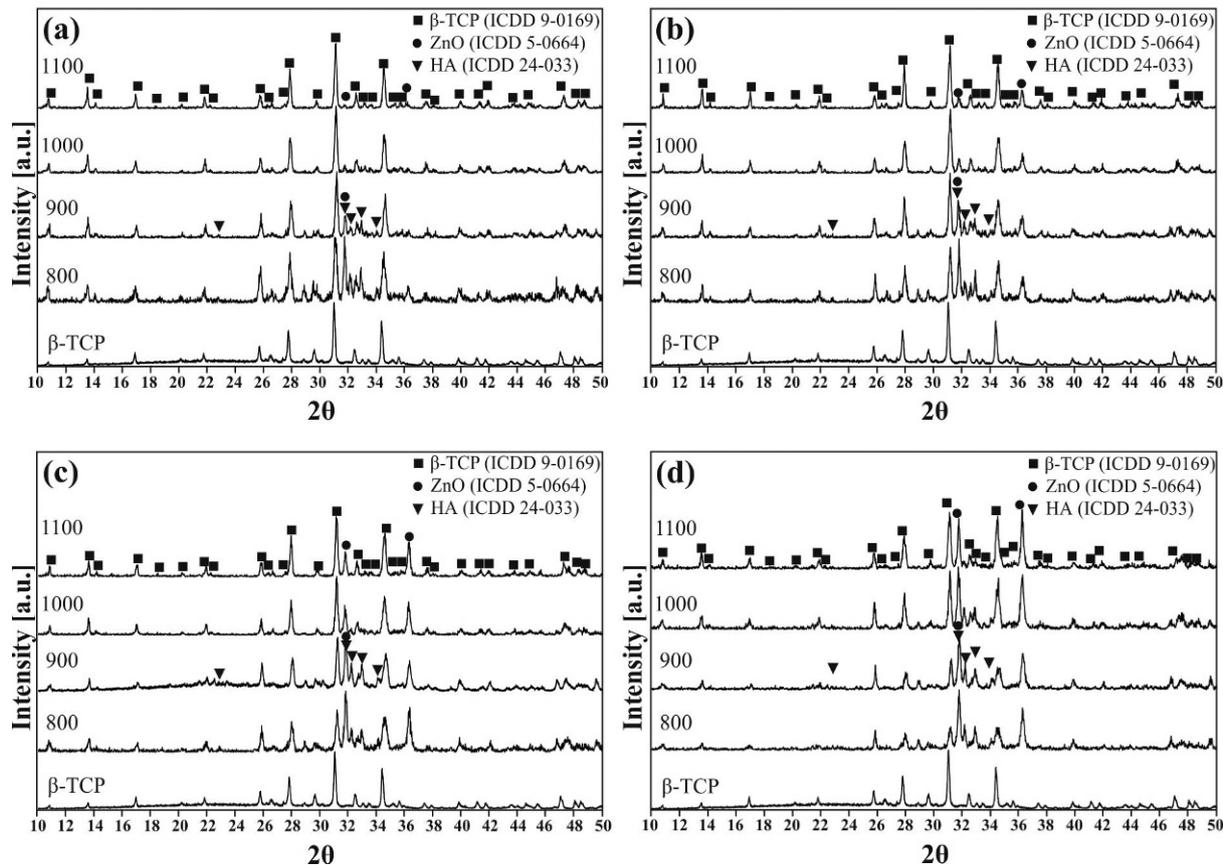


Figure 3. The XRD patterns of zinc doped samples heat-treated at different temperatures in the range of 800 – 1100 °C (a) Ca/Zn:2.8/0.2, (b) Ca/Zn:2.6/0.4, (c) Ca/Zn:2.4/0.6 and (d) Ca/Zn:2.2/0.8.

Table 1. The results of phase quantification of powders heated at 1100 °C.

Zn (mol%)	Ca/Zn	Whitlockite (mass%)	Hydroxyapatite (mass%)	Zinc oxide (mass%)
0	3/0	100	-	-
6.66	2.8/0.2	94.5 ± 0.2	0.3 ± 0.1	5.2 ± 0.2
13.33	2.6/0.4	61.9 ± 1.3	3.4 ± 0.2	34.7 ± 1.0
20.00	2.4/0.6	53.9 ± 1.5	7.4 ± 0.1	38.7 ± 1.1
26.66	2.2/0.8	46.1 ± 1.9	10.3 ± 1.6	43.6 ± 2.1

Besides, as it can be seen in Fig. 6, adding Zn ions at the amount higher than 6.6 mol% does not induce further peak shift. This confirms the limited substituting capacity of β -TCP by Zn ions.

The limited substituting capacity of other calcium phosphates for Zn ions has been reported in other studies. Zn-containing apatite has been obtained as a result of hydrolysis of Zn-containing α -TCP powders [5]. The starting material was ob-

tained as a single phase above 1300 °C. The higher the heating temperature, the more Zn was incorporated into α -TCP, with a maximum Zn content of 1.26 wt.%, obtained at 1450 °C. Soaking of Zn-containing α -TCP powders for 12 h in water resulted in α -TCP conversion to the apatite structure with almost complete transfer of Zn.

Zn ions could be quantitatively incorporated into HA lattice by direct synthesis under mild con-

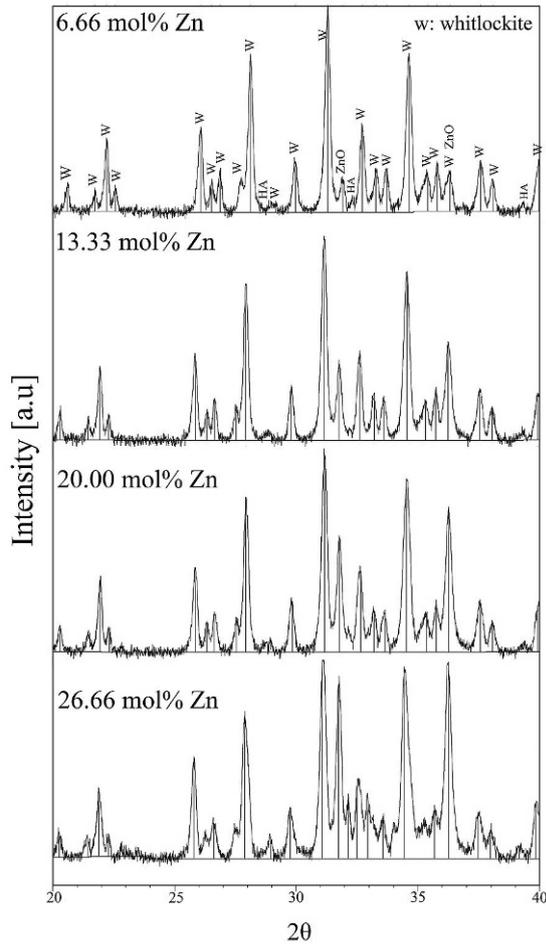


Figure 4. The XRD patterns of whitlockite containing different amounts of Zn after heating at 1100 °C.

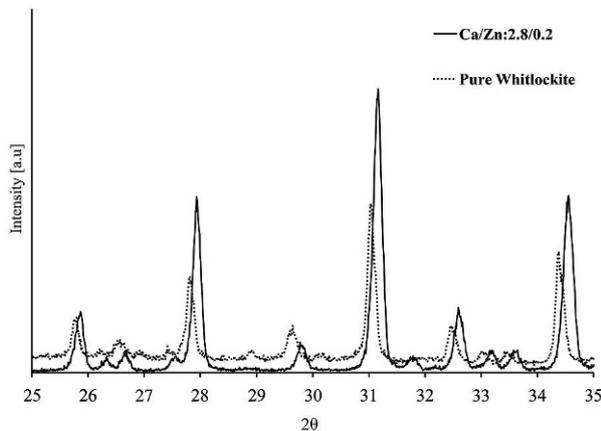


Figure 5. Comparison of XRD patterns of pure β -TCP and Zn-doped β -TCP.

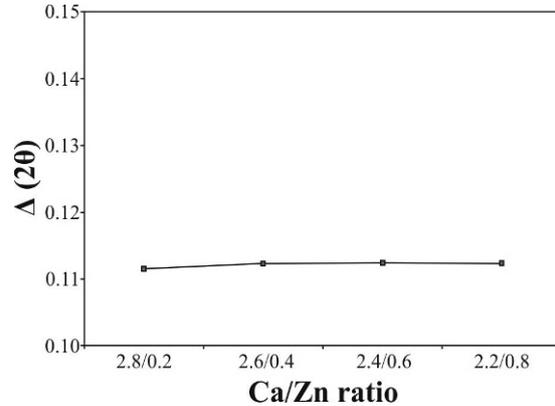


Figure 6. Shift in maximum diffraction peak of β -TCP added by different amounts of zinc.

ditions. The substitution for Ca ions occurs up to about 20 at.%, whereas the apatite structure does not sustain higher Zn incorporation [10].

In this study, incorporation of Zn ions into the lattice of β -TCP phase has been also evaluated by tracking the change in the lattice parameters.

Fig. 7 shows the a and c parameters of a whitlockite lattice as a function of Zn concentration for samples heat-treated at 1100 °C. The value of a parameter decreases when 6.6 mol% of Zn (corresponding to Ca/Zn:2.8/0.2) is added to β -TCP, whereas adding more Zn ions has no effect on this parameter. The c parameter also decreases with adding 6.6 mol% of Zn (Ca/Zn:2.8/0.2) followed by minute increment when adding 13.3 mol% of Zn (Ca/Zn:2.6/0.4). After that, the c parameter remains almost constant in spite of adding more Zn. The authors suggest that the lattice parameters do not change at higher amounts of Zn, because these ions do not enter into the lattice of β -TCP. These results agree with that of peak shifts and confirm that the substituting concentration of β -TCP with Zn ions is limited to about 6 mol%, and adding more Zn results in formation of ZnO. Therefore, it is obvious that the substitution of Zn^{2+} with Ca^{2+} results in a contraction of the network. As reported in literature [16], ions with bigger atomic radius, i.e. strontium and barium, could completely substitute with calcium while substitution of zinc is not simply possible because the reaction of zinc and TCP is limited due to the small atomic radius of Zn [17]. Low substitution of Zn into β -TCP net-

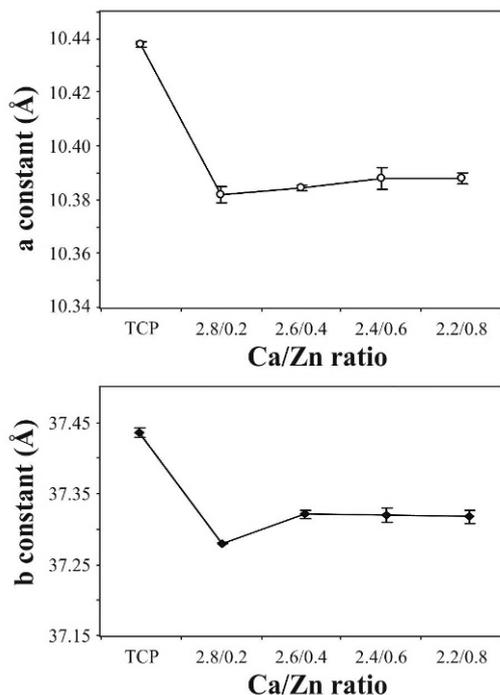


Figure 7. Lattice parameters of β -TCP as a function of Zn concentration for samples heated at 1100 °C: (upper) a parameter, (lower) b parameter.

work is due to the low solubility of this ion in this network.

3.4. Ion release into SBF solution

The concentration of Ca and Zn ions released from one of the samples (with Ca/Zn at a ratio of 2.8/0.2) into the SBF solution was studied to estimate its ion releasing capacity. Fig. 8 shows calcium concentration of SBF versus time in the above mentioned sample. Calcium release decreases up to 7 hours, and after that remains approximately constant up to 120 h. The release of zinc ions was limited and the values for all the samples were lower than 0.5 ppm during the evaluation period. Since Zn-doped β -TCP is only slightly soluble, the release of Zn from β -TCP is very slow and can be easily controlled. These limited concentrations of Zn ions may have an advantageous therapeutic effect on bone formation as high doses could produce cytotoxic effect. Song et al. [18] incorporated Zn into β -TCP. They reported higher miner-

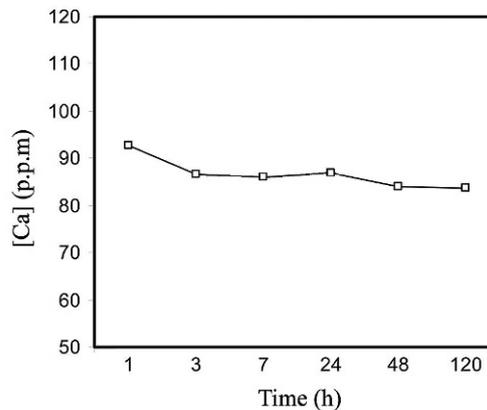


Figure 8. Calcium concentration of SBF solution as a function of time in the sample doped with 6.66 mol% Zn.

alization and degradability in Zn-doped β -TCP in comparison with TCP.

3.5. Suitability as bone substitutes

Zreiqat et al. [8] incorporated Zn into TCP, and found that the incorporation of Zn stimulated in vitro proliferation and differentiation of human bone derived cells and induced in vivo osteoconductivity after 3 and 6 weeks following implantation in tibia bone defects in rats. On the whole, the authors suggest that β -TCP sample doped with Zn (at Ca/Zn:2.8/0.2) is a proper choice to achieve optimum biological properties. It may repair bone defects even better than β -TCP though in vivo clinical experiments are required to confirm this suggestion.

4. Conclusions

From the results of this study the following conclusions can be pointed out:

1. Heating a mixture of zinc nitrate and β -TCP results in formation of Zn-doped β -TCP phase at a temperature of 1100 °C.
2. Incorporating of Zn ions into β -TCP lattice results in decreasing a and c lattice parameters, however, the amount of Zn ions doped into β -TCP is limited to the values lower than ~ 6.6 mol.%.

3. Along with β -TCP, HA and ZnO phases are produced at low heating temperatures (800 and 900 °C) but the quantity of these phases, especially HA, decreases with increasing the temperature.
4. The concentration of zinc oxide phase increases with an increase in the amount of zinc nitrate in the primary mixture.

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