

# Influence of Si- substitution on the conversion of hydroxyapatite nano-particles into $\beta$ - tricalcium phosphate

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Calcium phosphate ceramics were considered as one of the most important biomaterial with wide biomedical applications.  $\beta$ - TriCalcium Phosphate ( $\beta$ -TCP) was proved to have the advantage among other calcium phosphate phases that it is resorbable in vivo with new bone growth replacing the implanted TCP. Conversion of HydroxyApatite (HA) into  $\beta$ -TCP was achieved at much lower temperature that occurs in case of calcination of HA and this conversion was enhanced with doping of HA with varying concentrations of Silica ( $\text{SiO}_2$ ) ranging from 2 to 10 weight % during simple wet chemistry method of preparation of hydroxy apatite. Analysis of microstructure was performed using Transmission Electron Microscope (TEM) as well as X-Ray (XRD) Diffraction studies revealed that  $\beta$ -TCP phase ratio in the calcium phosphate nano-powder increase with the increase of  $\text{SiO}_2$  wt% added. The produced calcium phosphates were characterized using TEM, ThermoGravimetric Analysis (TGA), XRD and FTIR. The presence of  $\beta$ -Tricalcium phosphate (as a result of transformation) was confirmed by the use of TEM, TGA, XRD and FTIR-techniques.

تعتبر أملاح فوسفات الكالسيوم من أهم المواد الحيوية والتي لها تطبيقات متعددة في المجالات الطبية وخاصة كبديل للعظام. من ضمن هذه الأملاح يتميز ملح بيتا فوسفات ثلاثي الكالسيوم بأن له خاصية إحلالية تساعد على استبداله بالنسيج العظمي المتكون حديثاً. عملية تحول الهيدروكسي أباتيت إلى بيتا فوسفات ثلاثي الكالسيوم يتم في هذه الدراسة عند درجات حرارة أقل كثيراً من تلك التي يتم عندها التحول في الظروف العادية وذلك بإضافة أوزان صغيرة من السيليكا تتراوح نسبتها ٢ إلى ١٠% خلال عملية التحضير للهيدروكسي أباتيت مما يحفز تحول الهيدروكسي أباتيت عند درجة حرارة ٦٠٠ درجة مئوية وجد أن زيادة نسبة السيليكا تزيد نسبة طور بيتا فوسفات ثلاثي الكالسيوم في الملح الناتج. أملاح فوسفات الكالسيوم المحضرة والأطوار المختلفة منها تم التعرف عليها ودراسة خواصها باستخدام الأجهزة التالية (TEM, TGA, XRD and FTIR).

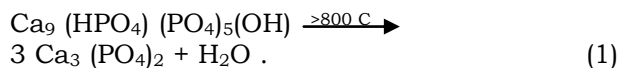
**Keywords:** Chemical synthesis, Biomaterials, Nano-structure, TriCalcium Phosphate (TCP)

## 1. Introduction

During the past few decades health care industries and the development of human life quality attracted much more attention. Calcium phosphate ceramics are considered as one of the most important biomaterials which have many biomedical applications such as Orthopedics, dentistry and drug delivery because they exhibit more biological affinity and activity than the other biomaterials currently synthesized [1]. Particular attention is placed on hydroxyapatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  and  $\beta$ -Tricalcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$  and this attention is due to their marvelous biological response to physiological environments [2].

These two calcium phosphate phases are widely used as bone substitutes or scaffold materials for bone regeneration [3]. Calcium phosphates are prepared by means of many methods including mechanochemical synthesis [4-8], sol gel technique [9] and various wet chemistry techniques. Commonly used technique is the precipitation technique [10-13]. Hydroxyapatite phase showed an advantage due to its chemical similarity with mineralized bone of human. Synthetic HA exhibits strong affinity to host hard tissue [1, 14]. On the other hand  $\beta$ -TCP is proved to be resorbable in vivo with new bone growth replacing the implanted  $\beta$ -TCP [15, 16].  $\beta$ -TCP exist in four crystalline structures [17].  $\beta$ -TCP phase with a theoretical density of  $3.01 \text{ g/cm}^3$

is stable up to 1125 °C. Heating above this temperature,  $\beta$ -TCP transforms an  $\alpha$ -TCP with 7.24% volume expansion with lowering the theoretical density down to 2.86 g/cm<sup>3</sup>,  $\alpha$ -TCP, is stable up to 1430 °C. The other two crystalline structures of TCP are  $\delta$ -TCP which is stable above 1430°C, and  $\gamma$ -TCP which is a high pressure form difficult to be obtained because it is metastable phase. The high reactivity and consequently the high rate of dissolution of  $\alpha$ -TCP make it difficult to be applied in the field of biomedical applications. Meanwhile, the chemical stability, the lower dissolution and the mechanical strength of  $\beta$ -TCP make it more suitable for biomedical application [18, 19]. The  $\beta$ -TCP phase is synthesized by solid state methods [4-8], meanwhile wet chemical methods are commonly used for preparation of calcium deficient hydroxyapatite (CDHA) with chemical formula  $\text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5(\text{OH})$  and hydroxyapatite with chemical formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  [10-13].  $\beta$ -TCP is usually obtained by calcination of CDHA or HA above 800°C according to the following eq. (1).



The preparation of  $\beta$ -TCP at milder condition is considered a valuable issue to be studied as it is much more energy saving to achieve the material properties needed at lower temperature. The mineral of natural bone has a similar chemical composition of hydroxyapatite, but in addition it contain several ionic substitution such as  $\text{Si}^{++}$ ,  $\text{Fe}$ ,  $\text{Mg}^{++}$ ,  $\text{F}^-$  and  $\text{Na}^+$  [20]. Nowadays, there is a tendency in the research to study the physicochemical effect of addition of some ions to the calcium phosphate phase. Machi et al. and Kannan et al. [21,22] studied the effect of Mg-ion substitution. Cuneyt Tas, et al. [13] studied doping of  $\beta$ -TCP with  $\text{Zn}^{++}$  ions. Kannan, et al. [23] studied the addition of potassium ions to HA and HA-  $\beta$ -TCP mixtures.  $\text{SiO}_2$  is one of the substituents that attract some researchers to reveal the effect of doping calcium phosphate with it [24-27]. Introduction of  $\text{SiO}_2$  into the bone tissue was found to improve the mechanical properties of hydroxyapatite [28]. Also, the bioactivity of

calcium phosphate was improved [29]  $\text{SiO}_2$  played an important role in biochemical interaction in implantation [30], that  $\text{SiO}_2$  enhanced the occurrence of transition gel that exist on the surface of silicate- phosphate as a unique substrate for adhesion of cells as well as this gel adsorb protein and elements helping in the formation of bone tissue. Literature cited that addition of  $\text{SiO}_2$  produce what is called silicon stabilized tricalcium phosphate (Si-TCP). This phase has the same unit phase symmetry as  $\alpha$ -TCP but differs in lattice parameter because of the substitution of silicon into tetrahedral sites of phosphorous. The goal of this study focus on the attempt to develop an aqueous coprecipitation process followed by a calcinations step at 600 °C to obtain silicon stabilized  $\beta$ -TCP.

## 2. Materials and method

Hydroxyapatite powder was prepared by wet chemistry using the analytical reagent grade calcium nitrate tetrahydrate;  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  (99%, Aldrich Chemical company, Inc., USA) as the Ca sources, and ammonium dihydrogen phosphate;  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$  (Vaz Pereira, Portugal) in Ca: P ratio 1:6. The pH of the reaction mixture was adjusted to be 10 by adding ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) solution. The reaction mixture was stirred for an hour at 90°C and then the temperature was kept at 120°C for 6 hours, with continuous stirring. The reaction mixture was aged for 72 hours at room temperature. After completion of the reaction the hydroxyapatite precipitate filtered and washed with distilled water followed by n-butanol to decrease the aggregation of the powder. The product was dried at 120°C overnight and calcined at 600°C for 3 hours. Various percentages (2, 4, 6, 8, and 10%) of silica prepared from sodium silicate and acetic acid were added to calcium nitrate tetrahydrate and ammonium dihydrogen phosphate mixture and the reaction was carried out as mentioned previously. All produced calcium phosphates were characterized by means of ThermoGravimetric Analysis (TGA), XRD, FTIR and TEM.

### 3. Results and discussion

#### 3.1. ThermoGravimetric Analysis TGA.

TGA was carried out on Calcium Phosphates with different weight percentage of Silica using TGA-50 Shimadzu – Japan analyzer under nitrogen atmosphere with 20 ml/min. flow rate and 10°C/min. temperature rate and is illustrated in fig. 1. TGA traces of the precursors showed in all samples, from room temperature to around

100°C, an initial weight loss between 5% and 6%, which corresponded to the loss of surface adsorbed water [31]. The shoulder observed from 130 – 310 °C could be represent the lattice water molecules and probably some residual ammonium and nitrate ions and appeared clearly in the undoped sample meanwhile it appeared in the other samples less clearly. It was difficult to find a direct correlation between the TGA weight loss behavior and the Si-dopant level.

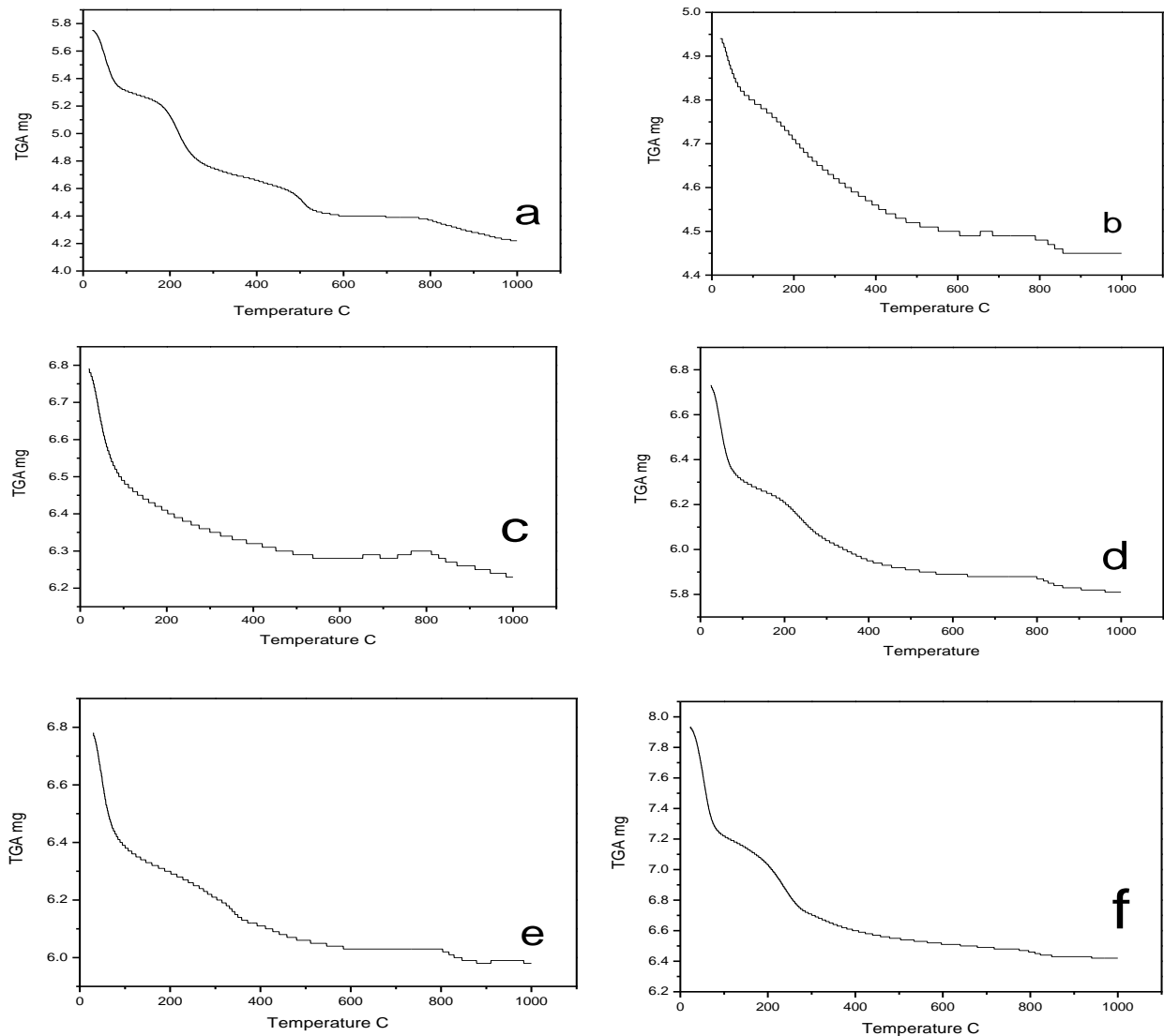


Fig. 1. TGA of calcium phosphates with different weight percentage of Silica added (a) zero % (b) 2% (c) 4% (d) 6% (e) 8% and (f) 10%.

### 3.2. X-Ray diffraction study

X-Ray Diffraction (XRD) analysis was performed. To evaluate the phase composition, XRD spectra were obtained with a 30 kW Rigaku rotating anode diffractometer fitted with a copper target. XRD spectra were obtained between  $20^\circ$  and  $80^\circ$  (2Theta) in continuous scan with 4 degree/ min. using the standard  $\theta - 2\theta$  geometry. The spectra were compared to standard spectra from the Joint Committee on Powder Diffraction Standards (ICDD) database. The XRD patterns of the samples prepared and dried at  $120^\circ\text{C}$  and calcined at  $600^\circ\text{C}$  for 3 hours were carried out. The diffractograms were shown in fig. 2 the samples are denoted as zero for the one without addition of silica and 2%, 4%, 6%, 8% and 10% for that doped with different weight percentages of silica. The diffractogram of zero sample shows the diffraction peaks of well crystalline hydroxyapatite (HA) phase ICDD number 00-001-1008 [32] as a predominant phase [33]. The diffractogram of the 2% doped sample exhibit the appearance of the diffraction peaks corresponding  $\beta$ -TCP phase ICDD number 00-003-0691 [34]. As the percentage of silica increase the diffraction peaks of  $\beta$ -TCP phase increase and the diffractograms show both phases HA and  $\beta$ -TCP. The pattern of 10% weight percentage of silica doping showed that the diffraction peaks belongs to  $\beta$ -TCP phase was the predominant predicted phase [35].

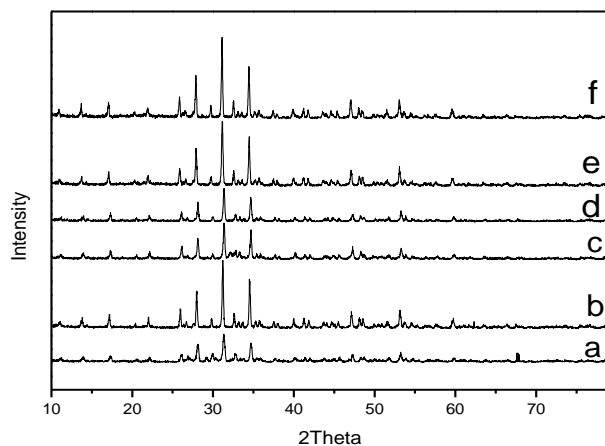


Fig. 2. XRD diffractogram of Calcium Phosphates with different weight percentage of Silica added (a) zero %, (b) 2%, (c) 4%, (d) 6%, (e) 8% and (f) 10%.

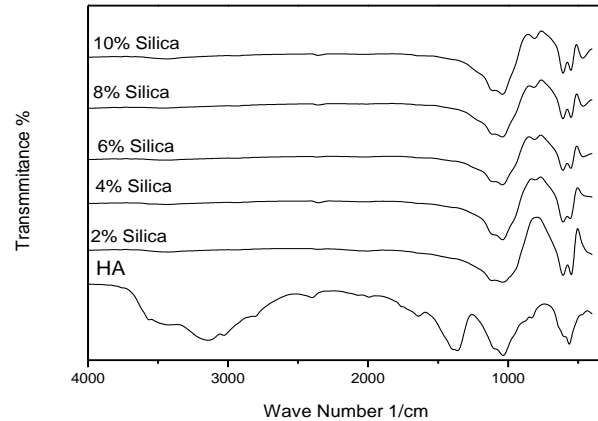


Fig. 3. FTIR spectrum of Calcium Phosphates with different weight percentage of Silica added (from zero to 10 %).

### 3.3. Fourier transform Infrared spectroscopy FTIR

FTIR analysis was performed using Fourier transform infra red spectrophotometer FTIR-8400 S Shimadzu- Japan. FTIR spectra showed two bands corresponding to OH vibration in hydroxyapatite at  $3564\text{ cm}^{-1}$  and  $601\text{ cm}^{-1}$  in zero sample (with zero % of  $\text{SiO}_2$ ). These two bands are disappeared with adding  $\text{SiO}_2$  indicating the transformation of hydroxyapatite phase with chemical formula  $\text{Ca}_9(\text{HPO}_4)(\text{PO}_4)_5(\text{OH})$  to tri-calcium phosphate phase with chemical formula  $\text{Ca}_3(\text{PO}_4)_2$  [36]. IR also records two bands at  $1092\text{ cm}^{-1}$  and  $1097\text{ cm}^{-1}$  corresponding to the stretching vibration of phosphate group the bending vibration of P-O bond is detected at  $601, 565\text{ cm}^{-1}$  [37]. The  $\text{SiO}_2$  stretching and bending vibrations are recorded to be at  $1140\text{ cm}^{-1}$  and  $463\text{ cm}^{-1}$  respectively appears at  $\text{SiO}_2$  percentage from 6% added to the hydroxyapatite and a band appears in the same sample which corresponds to Si-OH vibration [38].

### 3.4. Transmission electron microscopy TEM

The morphology of the synthesized powder was studied by Transmission Electron Microscope (TEM). The samples were prepared by sonication for 30 minutes and denoted as zero, 2%, 4%, 6%, 8% and 10% respectively referring to the weight percentages of silica added. The TEM micrograph show in zero

sample the ultra fine structure of hydroxyapatite nano-rods is of homogenous microstructure [39]. The obtained nano-rods have a length of approximately 85-100 nm and diameter of approximately 15-17 nm. With the addition of SiO<sub>2</sub> the spherical morphology of  $\beta$ -TCP was observed [14]. It was obvious that the spherical  $\beta$ -TCP particles are around 8-29 nm in diameter with observed growth in

hydroxyapatite rods. As the percentage of SiO<sub>2</sub> added increased, the spherical morphology of  $\beta$ -TCP became the major Morphology in the sample. In the sample of 10% of SiO<sub>2</sub> the main crystalline phase was for  $\beta$ -TCP indicated in the enhancement of the transformation of hydroxyapatite phase into  $\beta$ -TCP phase by adding SiO<sub>2</sub>.

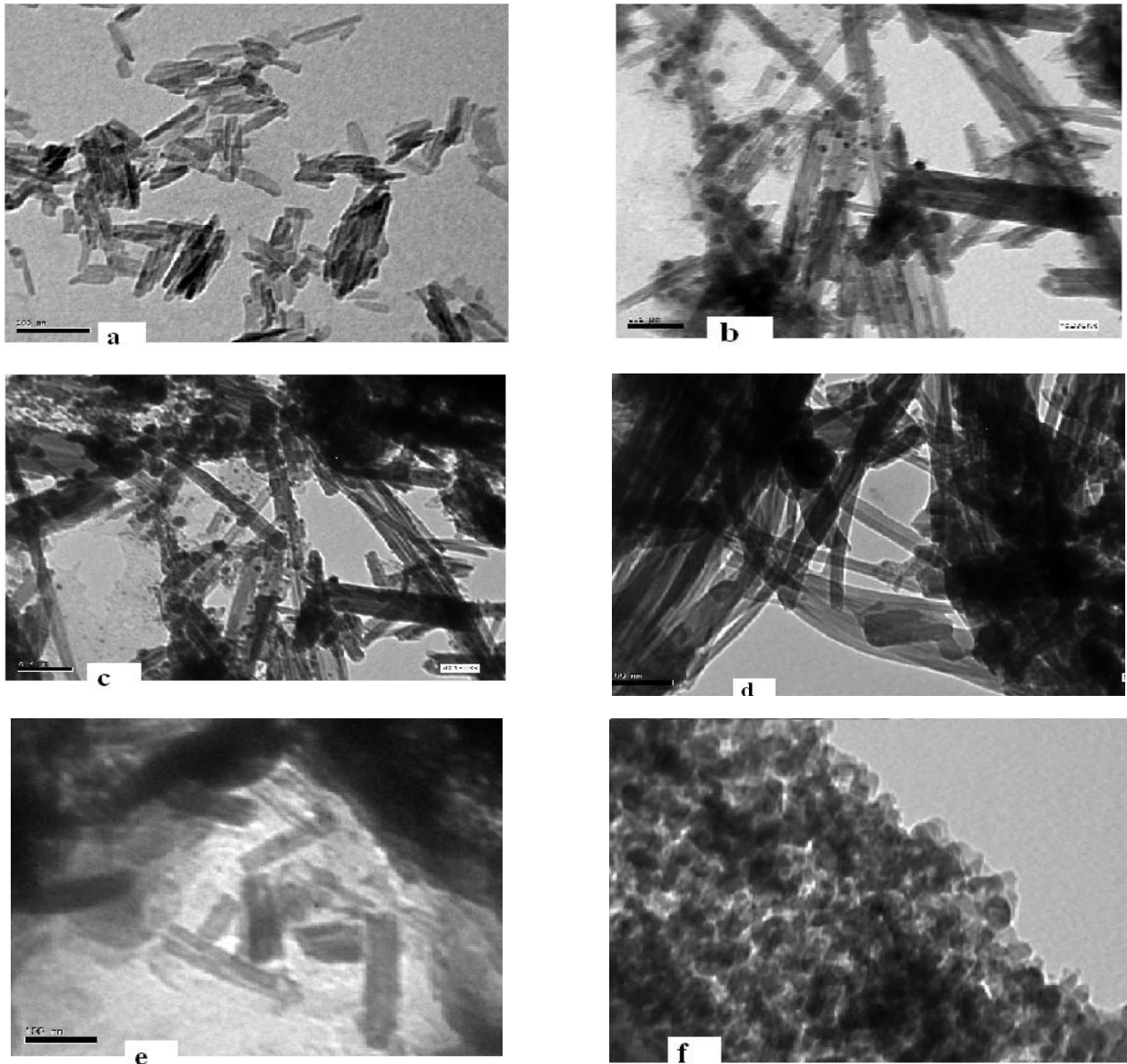
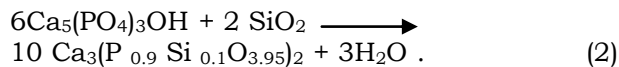


Fig. 4. TEM micrograph of Calcium Phosphates with different weight percentage of Silica added (a) zero % (b) 2% (c) 4% (d) 6% (e) 8% and (f) 10%.

It was cited in previous literature that the combination of SiO<sub>2</sub> and phosphates in the bioceramic materials had a considerable role in implantation with bone tissue, where hydrolyzed gel formed at first on the surface SiO<sub>2</sub>/phosphate particles help in adhesion of cells and tissue [40]. There are studies indicating that silicon substituted calcium phosphate have an enhanced biological more than the pure calcium phosphates [40]. It is previously hypothesized in literature [35, 40], that silicon stabilizes the TCP phase by substitution in tetrahedral phosphorous sites. That produces oxygen vacancies associated with OH decrease in order to make charge compensation. It was suggested that the transformation for hydroxyapatite into tricalcium phosphate in the presence of silica take place according to eq. (2) [35].



Assuming that one of every ten phosphorus atoms is replaced with silicon and it was predicted that the full conversion to saturated Si-TCP with formula suggested Ca(P<sub>1-x</sub>Si<sub>x</sub>O<sub>4</sub>)<sub>2</sub> will be in ratio 0.33 mol SiO<sub>2</sub> : 1 mole calcium phosphate in accordance with previous studies [41] it was assumed that the SiO<sub>4</sub> substitution for PO<sub>4</sub> is charge compensated by loss of OH from the structure. To achieve mass balance of the partially dehydrated apatite with formula Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3-x</sub>(SiO<sub>4</sub>)<sub>x</sub>OH<sub>1-x</sub>. The charge compensation by losing OH is limited by the number of OH ions that can be extracted from HA phase. If SiO<sub>2</sub> is added in excess the undoped TCP phase formed and many incorporate SiO<sub>2</sub> with lattice to form Si-TCP.

## 5. Conclusions

The synthesis of Si- substituted calcium phosphates has been achieved by simple aqueous precipitation technique during the preparation of hydroxyapatite by adding SiO<sub>2</sub> from 2 to 10 weight % in situ. The substitution of silicon into the lattice of the calcium phosphates enhance the transformation of CDHA or HA into TCP phase, which may be referred to as silicon

stabilized TCP with formula Ca(P<sub>1-x</sub>Si<sub>x</sub>O<sub>4</sub>)<sub>2</sub>. This transformation occurred at 600°C which is a lower temperature of transformation of CDHA or HA into TCP.

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