

# The impact of pH on the properties of $\beta$ -Tricalcium phosphate synthesized from hen's eggshells\*

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## ABSTRACT

Everyday millions of tons of eggshells are produced as biowaste around the world. Most of this waste is disposed of in landfills without any pretreatment. Eggshells in landfills produce odors and promote microbial growth as they biodegrade. The present study provides a simple wet chemical method to obtain high-purity  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) using eggshells as the calcium source (Ca) in the form of calcium nitrate and ammonium phosphate as the phosphate source (P) and controlling the pH of mixture solution in a range from 7 to 9. The observed phases of the heated mixtures were dependent on the mixing pH values. The synthesized TCP was characterized by X-Ray Diffraction (XRD), Fourier Transformed Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM) and EDX analysis.

**Keywords:**  $\beta$ -tricalcium phosphate; eggshells; wet chemical method; pH control.

## 1 INTRODUCTION

Tricalcium phosphate (TCP) has the chemical formula,  $\text{Ca}_3(\text{PO}_4)_2$ , with the Ca/P ratio being 1.5. It is a compound of great interests mostly because its chemical composition is close to natural bone, as well as its good bioactivity and biodegradability [1–3]. Moreover, its high dissolution rate in the human biological environment advances bone growth during the progressive degradation. This property imparts significant advantage to  $\beta$ -TCP compared to other biomedical materials which are not easily resorbed and replaced by natural bone [4, 5]. Therefore, TCP is frequently used as bone repairing materials. Besides, there are other applications of this compound, which involve drug carrier, luminescence materials and catalyst [6–9]. TCP have been synthesized by wet chemical methods [10–12]. However, the methods resulted in the formation of nonstoichiometric powders [3–6]. TCP has little nonstoichiometry [10]. Thus, in the Ca/P range out of stoichiometry, TCP and a little  $\text{Ca}_2\text{P}_2\text{O}_7$  can exist according to the phase diagram  $\text{CaO}-\text{P}_2\text{O}_5$ .

TCP can exist under three polymorphs, such as:  $\beta$ -TCP stable below  $1120^\circ\text{C}$ ,  $\alpha$ -TCP stable between 1120 and  $1470^\circ\text{C}$  and  $\alpha'$ -TCP above  $1470^\circ\text{C}$ . The latter is of no interest because it transforms into the  $\alpha$ -form during cooling.  $\beta$ -TCP is stable at room temperature and reconstructively transforms at  $1125^\circ\text{C}$  into  $\alpha$ -TCP, which is metastably retained until room temperature during the cooling [13]. The ideal Ca/P ratio of  $\beta$ -TCP is 1.5. As reported by Dickens and al. [14, 15], the  $\beta$ -TCP crystallizes in the rhombohedral space group  $R\bar{3}c$  with unit-cell parameters  $a = 10.4121(3)$ ;  $c = 37.3517(5)$  Å [16].

Many methods are used to synthesize the  $\beta$ -TCP [17–22]. The most conventional is the precipitation in aqueous medium starting from  $\text{Ca}(\text{NO}_3)_2$  and  $(\text{NH}_4)_2\text{HPO}_4$  as raw materials. However, the synthesis of a pure  $\beta$ -TCP by this method requires a close control of many parameters such as, reaction pH, ripening time, temperature, stoichiometry of the raw materials. A light variation of these experimental parameters can generate drastic variations in composition of the final product and reveal the pyrophosphate calcium phase ( $\text{Ca}_2\text{P}_2\text{O}_7$  or CPP) or the hydroxyapatite phase ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  or HA). Thus, a 1wt.% excess of calcium ni-

trate causes the formation of about 10wt.% hydroxyapatite in the synthesized powder. The fact that raw materials are never perfectly pure or chemically homogeneous and that it can undergo various reactions as hydration or sublimation show the difficulty to obtain a perfectly stoichiometric product.

The purpose of this work is to find the optimal pH value of  $\beta$ -TCP synthesis obtained by wet chemical method with the use of eggshells as the calcium source, as well as to study of the properties and characteristics of the synthesized samples.

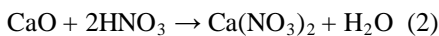
## 2. TECHNOLOGY OF SAMPLES SYNTHESIS

Wet chemical method is the basis of the elaborated TCP preparation technology. A special feature of our method is producing the initial component for a chemical reaction calcium oxide (CaO) by treating the hen's eggshell. Eggshell is chosen as source since it consists of 95% calcite  $\text{CaCO}_3$ , the rest of it comprises an organic component i.e. several layers of interlaced protein fibers, as well as various mineral salts (1%) arranged on the protein fibers just as calcite. Under heating  $\text{CaCO}_3$  is dissociated to yield CaO and  $\text{CO}_2$ :



During anneal the organic component of eggshell is burnt out and the residue contains CaO with low ( $\leq 1\%$ ) content of impurities. TCP synthesis involves step-by-step precipitate preparation. Preliminarily the eggshells of hen containing  $\text{CaCO}_3$  were collected. The membranes of eggs were removed and washed to remove adhesion. In order to eliminate as much organic matter as possible, the eggshells were first boiled in an aqueous solution. Uncrushed eggshell was calcined in an air atmosphere at  $900^\circ\text{C}$  for 1h, and then cooled in a furnace to ambient temperature. Then, these shells were ground into powder using an agate mortar. At the temperature of  $900^\circ\text{C}$ , the eggshells become fragile and very white in color. At this temperature the new phase of eggshell is calcium oxide (CaO). The eggshells transformed into calcium oxide by releasing carbon dioxide ( $\text{CO}_2$ ) according to equation (1).

The samples of  $\beta$ -TCP were synthesized by precipitation method using a Ca/P molar ratio equal to 1.66. Under rigorous stirring, the calcium oxide thus obtained was converted to calcium nitrate by dissolving in requisite amount of nitric acid  $\text{HNO}_3$  to obtain  $\text{Ca}(\text{NO}_3)_2$  solution with the following reaction:



When the CaO powders were completely dissolved in nitric acid, they were diluted in distilled water. The pH of the solution was adjusted by addition of ammonium hydroxide  $\text{NH}_4\text{OH}$ . Calcium solution, vigorously stirred for 4 hours, was added drop by drop into  $(\text{NH}_4)_2\text{HPO}_4$  solution. During synthesizing process, the pH value varies between 7 and 9. After completion of mixing, the solution was subjected to aging treatment for 24 h and then filtered. The filtered cake was further heated at  $80^\circ\text{C}$  for 24h and then crushed in a mortar. The powders were calcined at  $800^\circ\text{C}$  for 1 h.

### 3. CHARACTERIZATION OF THE PRODUCT

Fourier transform infrared (FTIR) spectroscopy analysis; VERTEX 70, Genesis Series; was carried out to identify the functional groups. The spectrum was recorded in the  $4000\text{-}400\text{ cm}^{-1}$  region with  $4\text{ cm}^{-1}$  resolution.

A Shimadzu 6100 X-ray diffractometer (XRD), using  $\text{CuK}\alpha$  radiation and operating at 40 kV and 30 mA, was used to identified crystalline phases of  $\beta$ -TCP samples. XRD patterns were collected over the  $2\theta$  range of  $10\text{-}60^\circ$  with a step size of  $0.02^\circ$  and a count time of  $0.5^\circ/\text{min}$ . Crystalline phases detected in the patterns were identified by comparison to the standard patterns from the ICDD-PDF (International Center for Diffraction Data-Powder Diffraction Files). The crystallite dimensions (D) were calculated using Debye-Scherrer Eq. (3) [23]:

$$D = 0,89\lambda / \text{FWHM} \cos\theta \quad (3)$$

Where D is the crystallite size (nm),  $\lambda$  the wavelength of X-ray beam ( $0.15406\text{ nm}$  for  $\text{Cu-K}\alpha$  radiation), FWHM the full width at half maximum for the diffraction peak under consideration (rad), and  $\theta$  is the diffraction angle ( $^\circ$ ). The crystallinity noted by  $X_c$  corresponds to the fraction of crystalline  $\beta$ -TCP phase in the investigated volume of powdered sample, evaluated by the Eq (4):

$$X_c = 1 - V_{300/0210} / I_{0210} \quad (4)$$

Where  $I_{0210}$  is the intensity of (0 2 10) reflection of  $\beta$ -TCP structure and  $V_{300/0210}$  is the difference between the intensity of the (3 0 0) and (0 2 10) reflections [12, 24]. The relative intensity ratio of the phase ( $R_{ir}$ ) corresponding to the major phases observed in the XRD spectra of powders calcined were computed using the relationship given in Eq. (5):

$$R_{ir} = \text{Intensity of major line of phase} / \sum \text{Intensity of major lines of all phases.} \quad (5)$$

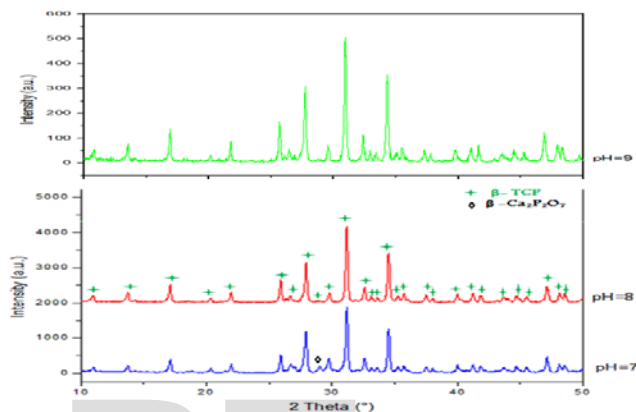
The size, morphology and the chemical constituents of fine powders were observed on a transmission electron microscope (Philips CM10, Eindhoven, The Netherlands) equipped with energy-dispersive X-ray microanalysis that operated at the accelera-

tion voltage of 100 kV.

### 4. RESULTS AND DISCUSSION

The dried, raw eggshell showed  $\text{CaCO}_3$  phase, and CaO was observed in the calcined eggshell in the results of XRD analysis. The  $\text{CaCO}_3$  was completely decomposed at  $900^\circ\text{C}$  and turned to pure CaO.

The crystallization and phase composition of synthetic TCP powders with wet method were investigated by XRD synthesis. Fig. 1 presents the XRD spectra with different pH (7, 8 and 9) for calcined powders at  $800^\circ\text{C}$ . Powders exhibited sharp and intense diffraction peaks indicating a high crystallinity.



**Figure 1 : XRD spectra of  $\beta$ -TCP powders synthesized at different pH and calcined at  $800^\circ\text{C}$  .**

All XRD patterns shows diffraction peaks characteristics of  $\beta$ -Tricalcium phosphate presents in standards and in literature. The major phase, as expected, is  $\beta$ -Tricalcium phosphate, which is confirmed by comparing data obtained with the ICDD - PDF2 card: 00-009-0169. Conversely, in the  $\beta$ -tricalcium phosphate powders prepared at pH 7, beside the  $\beta$ -TCP, an additional peak is detected at  $2\theta = 28.95^\circ$  such peak corresponds to  $\beta$ -calcium pyrophosphate  $\beta\text{-Ca}_2\text{P}_2\text{O}_7$  (JCPDF 9-346) phase, the percentage of volume fraction  $R_{ir} = 9\%$ . In samples synthesized with pH 8 and 9, and as can be seen in Fig. 1, the most important peaks of  $\beta$ -TCP were observed in all the samples with no additional peaks correspond to other calcium phosphates. The obtained peaks were in concurrence with the ICDD card No. 09-0169. The determined amounts of crystallinity and crystallite size (determined by Scherrer equation) of calcined  $\beta$ -TCP are given in Table 1.

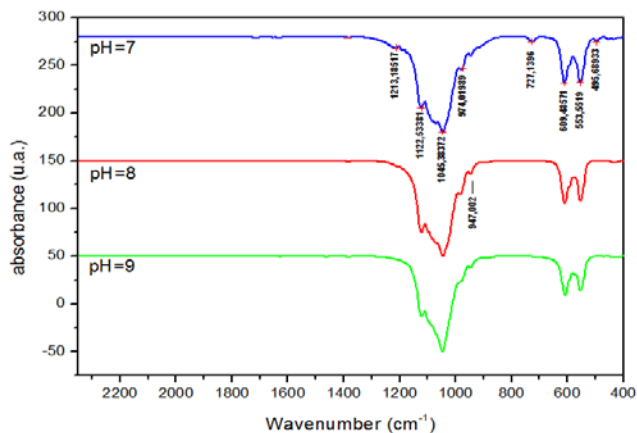
**Table 1: Characteristics of the synthetic powders at different pH values and calcined at  $800^\circ\text{C}$ .**

pH value	Crystallite size D (nm)	Fraction of crystalline phase ( $X_c$ )
pH = 7	39	77
pH = 8	41	86
pH = 9	43	86

The crystallite size and the degree of crystallinity increase slightly with pH. These results indicated that the crystallinity of  $\beta$ -TCP was slightly influenced by the pH. It has been found that

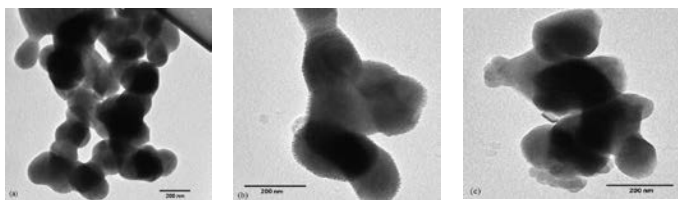
the control of the crystallinity of calcium phosphates is necessary for their biological applications [25]. Since calcium phosphates with low-level of crystallinity show high osteoconductivity, the synthesized powders can be used to promote osseointegration or as a coating to promote bone in growth in to prosthetic implants [26].

In order to identify the molecular arrangement of the precipitated powders, FT-IR analysis was performed. Fig. 2 illustrates the representative FT-IR spectra of the samples prepared at different pH and calcined at 800°C.



**Figure 2: FT-IR spectrum of the samples prepared at different pH and calcined at 800°C.**

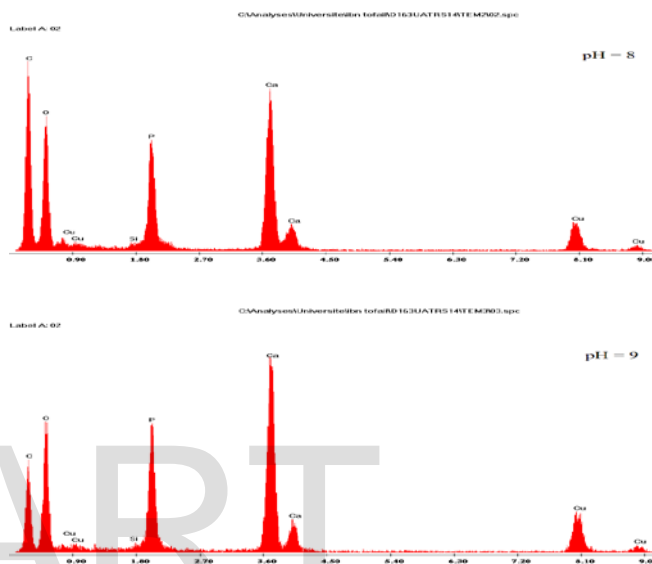
The bands at 1122 and 1045  $\text{cm}^{-1}$  are assigned to the components of the triply degenerate  $\nu_3$  antisymmetric P–O stretching mode. The 974  $\text{cm}^{-1}$  band is assigned to  $\nu_1$ ; the non-degenerate P–O symmetric stretching mode. The bands at 609 and 553  $\text{cm}^{-1}$  are assigned to components of the triply degenerate  $\nu_4$  O–P–O bending mode, and the bands in the range of 434–462  $\text{cm}^{-1}$  are assigned to the components of the doubly degenerate  $\nu_2$  O–P–O bending mode. In the case of sample prepared at pH = 7 besides the mentioned bands, additional vibration at 727 and 1213  $\text{cm}^{-1}$ . These vibrations belonged to pyrophosphate group ( $\text{P}_2\text{O}_7$ ) [27]. The presence of these weak bands confirms the existence of a small amount of calcium pyrophosphate in the product. Therefore, the obtained FTIR curves are consistent with the previous XRD results. Further the highly sensitive FTIR results indicate that there is no  $\text{CO}_3^{2-}$  stretching vibrational peaks.



**Figure 3: TEM images of the  $\beta$ -TCP samples synthesized at (a) pH = 7, (b) pH = 8 and (c) pH = 9.**

Fig. 3 shows the TEM images of the  $\beta$ -TCP samples. From the TEM images it is clearly seen that the particles grow obviously

compared with  $\beta$ -TCP precursors and the morphology is spherical shape. The size of the spherical shape is from 150 to 180 nm and the particle size is uniform. Also the particles exhibit high tendency to agglomerate. They clearly reveal the formation of well-crystallized single  $\beta$ -TCP crystals. This particle size was much smaller than that of the  $\beta$ -tricalcium phosphate powders synthesized through a solid-state reaction method [28]. It has been reported [29] that the  $\beta$ -tricalcium phosphate powders with a reduced particle size have a beneficial effect on the mechanical characterizations of the porous  $\beta$ -tricalcium phosphate scaffolds for an application of the synthetic bone graft substitute.



**Figure 4: EDX analysis data of the  $\beta$ -TCP samples at pH=8 and pH=9**

EDX data (fig 4) showed that the main elements of the calcium phosphate-based nanopowders were calcium, phosphorus, oxygen, carbon, and copper. The origin of the copper and the carbon is due respectively to measuring equipment and ambient air. On the other hand, the Ca/P ratio for  $\beta$ -TCP synthesized at a pH=8 and pH=9 was 1.44 and 1.48 respectively, which is already close to 1.5.

## 5. CONCLUSION

The present study suggests the eggshell as a possible material-recycling technology for future waste management and ecology. By using recycled eggshells hens, the  $\beta$ -tricalcium phosphate powders with high purity could be obtained through mixing  $(\text{NH}_4)_2\text{HPO}_4$  with  $\text{Ca}(\text{NO}_3)_2$  and controlling the pH of mixture solution in a range from 7 to 9. From the FT-IR and the XRD analysis result, we confirmed that the peaks of calcium pyrophosphate were observed at a pH = 7 but was not observed at a pH = 8 and pH = 9 and the  $\beta$ -tricalcium phosphate powder had a high phase purity. The particle size of  $\beta$ -tricalcium phosphate powders was in a range from 150 nm to 180 nm and these particles were uniformly distributed and were easy to aggregate. The particle shape of  $\beta$ -tricalcium phosphate powders was spherical.

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