THE EFFECT OF FLUORIDES AND MILLING ON TEMPERATURE REDUCTION OF NANO B-TRICALCIUM PHOSPHATE PARTICLES FORMATION

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Homogeneous β-TCP particles have been synthesized by introducing fluoride in the TCP precursor. The effect of ZnF_2 additive and milling on the phase transformation and the micrograph of the prepared β-tricalcium phosphate (β-TCP) particles are investigated. The samples were characterized by different techniques such as, X-ray diffraction, Fourier transform infrared spectra and Transmission electron microscopy. Implementing Zinc fluoride (ZnF_2) additive and milling led to retardation of the transformation temperature and modification of the β-TCP particle shape. The non-agglomerated nano β-TCP particles were proved to be at their finest size (30-40 nm) at 750°C.

INTRODUCTION

Bioactive synthetic substrates capable of incorporation into the natural process of bone remodeling are of interest in applications which include in vitro bone cell assays [1] , in vivo resorbable bone cements [2, 3] implantable coatings which enhance the bonding of natural bone to the implant[1], various forms of implantable prostheses and bone repair agents [4]. The prime objective for such materials in vivo is to combine stimulation of osteogenic activity in associated bone tissues for optimum healing, with the capability to be progressively resorbed by specialized bone cells (osteoclasts) during normal continuous remodeling [5]. In vitro, related functions are to provide standardized laboratory test substrates on which bone cell resorptive function (osteoclast activity) or production of mineralized bone matrix (oteoblast activity) can be assessed and quantized [6]. Such substrates must be stable and insoluble in the biological environment until acted upon by osteoclasts, the special "bone mineral resorbing cells. Bone substitutes need to be biocompatible and have been integrated by the surrounding bone tissue. It is generally acknowledged that calcium phosphate ceramics like hydroxyapatite (HAp) and tricalcium phosphate (TCP) are a good candidate for bone substitutes due to their chemical similarity to bone mineral [1,7].

β-tricalcium phosphate [β-$Ca_3(PO_4)_2$] (β-TCP) and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (HA) powders are widely applied in the biomedical fields because of their biocompatibility and osteoconductivity [8, 9]. HA is thermodynamically the most stable phase in physiological conditions and has the ability for direct chemical bonding to the bone, while β-TCP is found to be resorbable in vivo with new bone growth replacing the implanted β-TCP [10]. Tricalcium phosphate is one of the most important biomaterials based on phosphates, currently recognized as ceramic material that significantly simulates the mineralogical structure of bone [8]. It has three polymorphic forms: β, α and α′. β-TCP below 1180°C, α-TCP between 1180°C and 1430°C, and α'-TCP above 1430°C [11]. The latter is of no interest because it transforms into the α-form during cooling. β-TCP is stable at room temperature and reconstructively transforms at 1125°C into α-TCP, which is metastable retained until room temperature during the cooling [9, 10]. It is the low-temperature phase in the $CaOP_2O_5$ phase diagram. As it was mentioned, β-TCP transforms to α-TCP at 1125°C and above this, up to 1430°C and α-TCP is a stable phase. Above 1430°C, the super α-TCP form becomes stable until the melting point of 1756°C. The ideal Ca/P ratio of β-TCP is 1.5 and the theoretical density is 3.17 g cm^{-3} as reported by Levitt et al., [11]. According to Li et al. [2], tribasic calcium

phosphate is nonstoichiometric compound often bearing the formula of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. TCP is a resorbable temporary bone space filler material. When implanted, TCP will interact with body fluids and form HA in accordance with the following equation:

$$4Ca_3(PO_4)_2 + 2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 \text{ (surface)} + 2Ca^{2+} + 2HPO_4^{2-} \quad (1)$$

The reaction rate will decrease with increasing pH of the local solution and further increase the solubility of TCP. Theoretically, resorbable TCP is an ideal implant material. After implantation, TCP will degrade with time and be replaced with natural tissues. It leads to the regeneration of tissues instead of their replacement and so solves the problem of interfacial stability [12]. Synthesis of calcium phosphate nanopowder is relevant in biological fields, because the dimensions of large biomolecules such as proteins and DNA as well as those of many important subcellular structures fall in the size range between 1 and 1000 nm [13]. Fluorine ion, which exists in human bone and enamel, can be incorporated into HA crystal structure by substitution of fluorine ions for OH-groups to form Fluoro- Hydroxyapatite (FHA, $Ca_{10}(PO_4)_6(OH)_{2-x}F_x$, where $0<x<2$ is the degree of fluoridation and x = 0, pure HA; x = 2, pure FA). Incorporation of fluorine into HA, or fluoridation, reduces its solubility, while maintaining a comparable biocompatibility to that of HA [12]. Many methods, namely, solid-state reaction, wet precipitation and sol-gel processing have been developed for the preparation of FHA powders and ceramics [14,15]. β-TCP powders are reportedly prepared by liquid-solution methods, such as sol-gel, hydrothermal, micro emulsion and precipitation, as well as gas phase reactions [16]. Precipitation method is a wet chemical method that does not need high pH value or high sintering temperature. This method offers a molecular mixing of calcium and phosphorus, which improves the chemical homogeneity of the resulting material. Furthermore, the high reactivity of the sol-gel powders would result in the decrease of calcining and sintering temperature [17,18]. This examination will be conducted through the applying ZnF_2 additive and milling to reduce the transformation temperature for β-TCP.

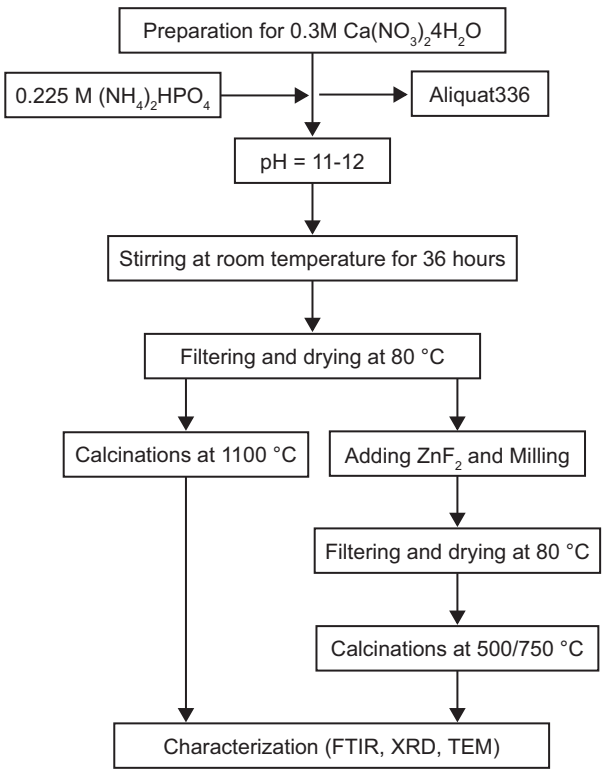


Figure 1. A flow chart of fabricating β-TCP bioceramics.

EXPERIMENTAL

Detail procedure and experimental conditions for preparation of β-TCP powder by wet chemical process is shown in Fig. 1. β-TCP nanopowders were synthesized by the reaction of calcium nitrate tetra-hydrate ($Ca(NO_3)_2 \times 4H_2O$, 98%, Merck) with diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, 99%, Merck) with the molar ratio of Ca/P = 1.33. Briefly, 100 mL of 0.3 mol $(NH_4)_2HPO_4$ solution was vigorously stirred at room temperature, and 100 mL of 0.225 M mol $Ca(NO_3)_2$ was added drop wise and then 25.807 wt.% Aliquat336 as a surfactant was gradually added to it over 150-200 min to produce a white precipitate. Throughout the mixing process the pH of the system was maintained at pH = 10-11 by adding of 0.1 M sodium hydroxide (NaOH, 99%, Merck). The obtained white suspension was then stirred for 36 h. The synthesized precipitate was washing with distilled water to improve the dispersion characteristics. The suspension was filtered and dried at 80°C for 24 h. Then, the as-dried powders were crushed by using mortar and pestle and calcined in alumina crucible at 1100°C for 2 h. The next step for temperature reduction of nano β-TCP formation, is to prepare the solution through implementing calcium nitrate tetra-hydrate and diammonium hydrogen phosphate as well as Aliquat336as a surfactant. The solution was stirred for 36 hour continuously and filtered to obtain the powders. Then, alcohol was applied to be milled with the powders for 24 hours after Zinc fluoride (ZnF_2) additive (2 wt %) was added to the solution.

After the powders were dried at 80°C, they were calcined at 500°C and 750°C for 2 hour. As the final stage, mortar and pestle were used in order to crush the calcined powders.

Phase identifications were performed by X-ray diffraction (XRD) PW1800, of Philips Company, using nickel filtered Cu Ka radiation in the range of $2\theta = 10°$-60° with a scanning speed of 5° per minute. A Fourier transform infrared spectrometer (FTIR) by Perkin

Elmer Spectrum 100 series was used with the universal attenuated total reflection (UATR) method. An ultrasonic bath was applied to suspend the calcined powder in ethanol, and subsequently, a few droplets of it were used for microstructural evaluation by Philips (Zeiss, Germany) transmission electron microscope (TEM).

RESULTS AND DISCUSSION

The synthesis of nano β-TCP was achieved with the concurrent addition of Aliquat336as a surfactant and increasing the stirring time, to prevent excessive grain growth and aggregation of nanoparticles. By increasing the stirring time the particle size was decreased and the finest particle size (40-50nm) was obtained after 36 hours stirring time. The reactions involved in the formation of β-TCP during the chemical precipitation can be expressed as follows:

$$(NH_4)_2(HPO_4) + 3Ca\,(NO_3)_2.4H_2O \rightarrow \rightarrow Ca_3\,(PO_4)_2 + 6NH_4NO_3 + 4H_2O \quad (2)$$

The XRD analysis was performed using the X-ray diffractometer. The straight base line and sharp peaks of the diffractogram in Fig. 2 confirmed that the products were well crystallized. The XRD pattern in Fig. 2 indicated that the samples contain mostly β-TCP phases with some HA phases.

Fig. 3 shows that the XRD pattern of β-TCP with the addition of ZnF_2 as an additive and milling on the phase transformation of TCP at different temperatures. As it was shown, the characteristic peaks of β-TCP emerged at 500 °C with a quite weak intensity and amorphous background. The crystal size of the β-TCP powders is inversely proportional to the peak width according to the Debye-Scherrer equation [12,19].

$$\Delta = 0.9\,\lambda / D\cos\theta \quad (3)$$

where Δ (2θ) represents the peak width at half-maximum intensity of the reflection; λ is the wavelength for CuKα (λ=0.15418 nm); and D is the crystal size in nanometers.

The particle size of the prepared β-TCP powders was estimated about 11 nm through Scherrer formula. With increasing the calcinations temperature up to 750°C, high intensities of β-TCP were observed, and the amount of β-TCP was considerably improved. It was shown that β-TCP crystallite rapidly grew up to 42 nm as transformation was completed.

Table 1. The phase structure and crystallite size of the prepared β-TCP powder.

	Calcinations temperature (°C)		
	500	750	
Crystallite size/nm	11	30-40	40-50
Main Phase structure	β-TCP	β-TCP	β-TCP

The phase structure and crystallite size of the prepared β-TCP powder which is calculated through Scherer formula are presented in Table 1.

FTIR analysis was carried out for nano β-TCP precursor in the wave number region of 4000-200 cm^{-1}. The related spectrum is presented in Fig. 4. Absorptions were recorded at about 3000 and 3500 cm^{-1}, which were because of the stretching vibration as well as the deformation vibration characteristics of hydroxylate (O–H) [20].

The absorption peaks presented at 900-1000 cm^{-1} belonged to HPO_4^{-2} and the bonds at 700-730 cm^{-1} were due to the phosphate band P_2O_7 as well as the band at 480-600 cm^{-1} was the characteristic of phosphate (PO_4^{-3}) [1]. Carbo-related peaks appearing in the range of 1400-1600 cm^{-1} show the substitution of the carbon in the samples that is in the result of synthesis operations and absorbing carbonate of CO_2 in the air. With regard to Fig. 5, by increasing the ZnF_2 additive and milling, there is no significant change and new absorption bonds in both samples in comparing with pure β-TCP.

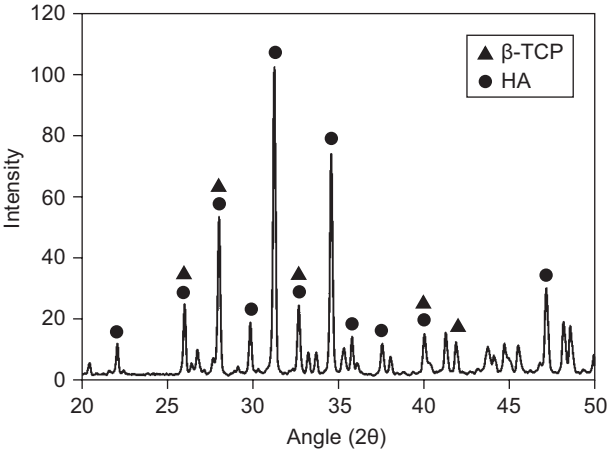


Figure 2. XRD pattern of β-TCP at 1200°C.

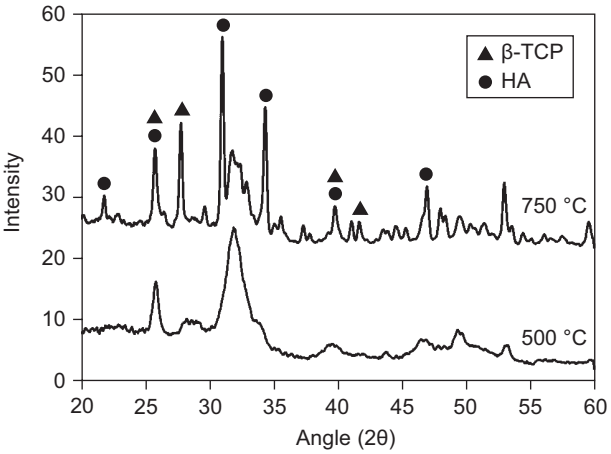


Figure 3. XRD patterns of the as-synthesized precursor calcined at different temperatures.

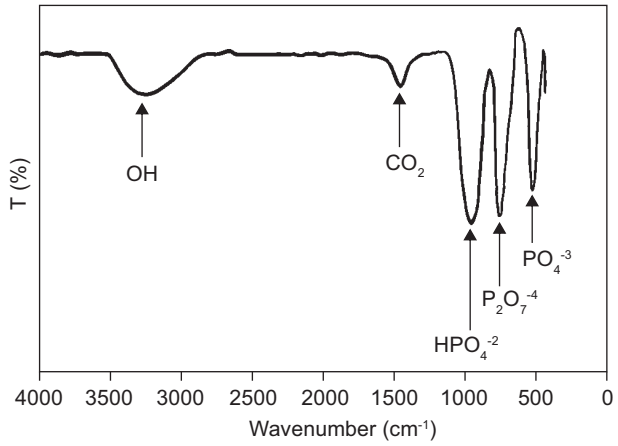


Figure 4. FTIR spectra of pure β-TCP at 1200°C.

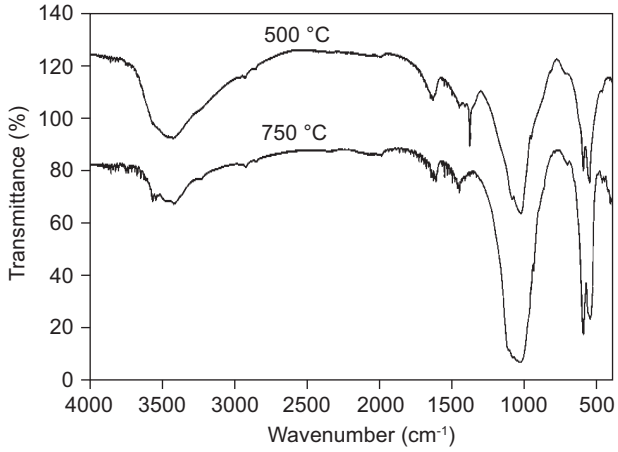


Figure 5. FTIR spectra of the as-synthesized precursor calcined at different temperatures.

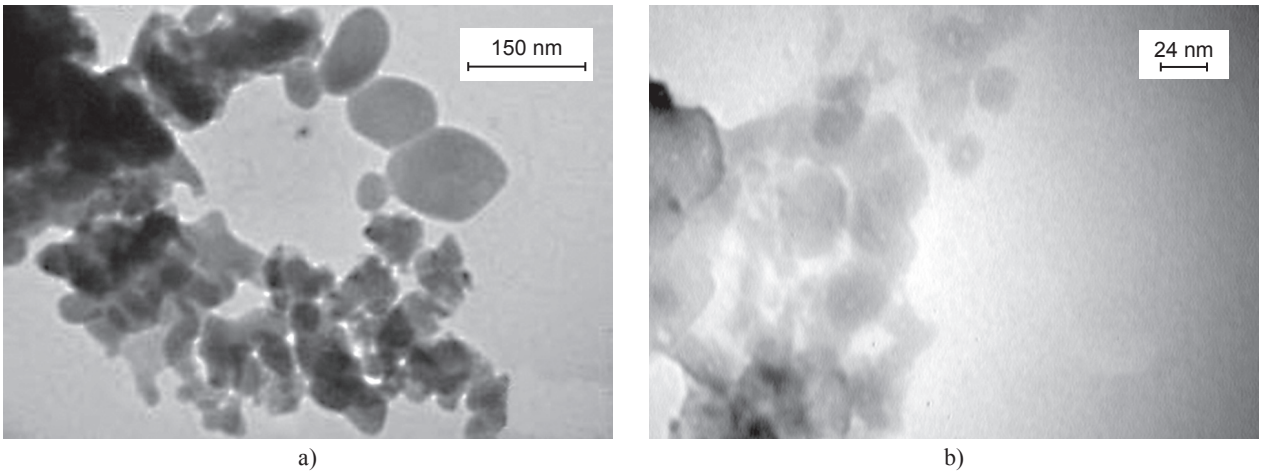
Fig. 6 shows TEM micrographs of β-TCP in presence of presence of Aliquat336 at 1200°C. As it could be seen, the range of particle sizes were about 40-50 nm with regular shape and good dispersion and less agglomeration.

Fig. 7 shows TEM micrographs of the synthesized precursors with addition of ZnF_2 at 500 °C and 750 °C. It can be seen that β-TCP crystallite couldn't nucleate very well at 500 due to the low temperature and there were a lot of agglomeration (Fig. 7a,b).Moreover, the size of particles calcined at 500°C is in the range of 10-11 nm with some agglomeration .In those conditions, β-TCP cannot be crystallized very well, hence, the crystallite obtained was very fine with a nearly spherical shape that is due to low transformation temperature (Fig. 7a,b).

At the temperatures higher than 750°C crystallite growth of grains were observed. As it is shown in Fig. 7, the particle size of β-TCP crystallite were in the range of 30-40 with the spherical shape and had a good quality of dispersion. As is clear, by increasing milling time, a decrease in annealing temperature was achieved. This is obvious because as the milling time was increased, the particle size of the powders would be decreased which led to lower annealing temperature [3,10,15]. Liu et al. [9] investigated the effect of mechanical milling on phase transformation of nanoscale particles. They had particle size distribution ranging from 40 to 200 nm with a lot of agglomeration after 50 hour milling.

Wang et al. [20] synthesized nanoscale particles by introducing fluorides in the precursor. It was also indicated that the fluoride ions enhanced the directional growth of the nanostructures. In summary, liquid-forming additives increased the transformation of TCP. Additive effects on sintering/shrinkage were similar except for the added effect of reaction sintering and a faster transformation kinetics due to the better contact between powders in a compact [20].

In the proposed method, nanoscale β TCP powder was synthesized at about 750°C when milling and ZnF_2 additive were used together. Consequently, in this research, the size of the particles formed was in the range of 30-40 nm when Aliquat336 surfactant and ZnF_2 additive were applied in 36 hour milling. Nano β-TCP



a) b)

Figure 6. TEM micrograph of β-TCP in the presence of Aliquat336 at 1200°C.

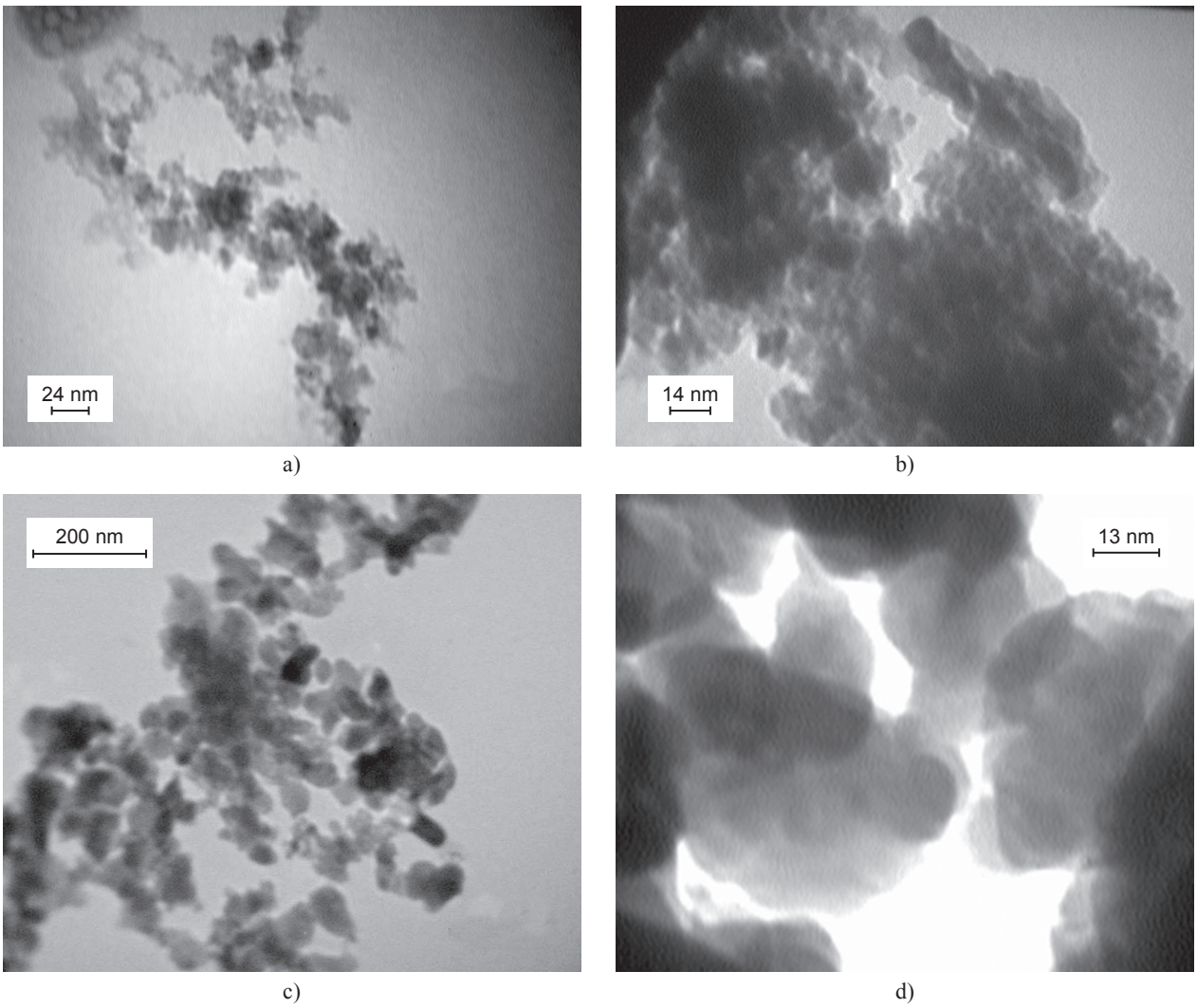


Figure 7. TEM micrograph of β-TCP in the presence of ZnF2 additive and milling at 500°C (a, b) and 750°C (c, d).

particles were plate-like in shape with a good quality of dispersion. Therefore, the presence of surfactant and ZnF_2 controls the size of the particles, their degree of aggregation, and their shape.

CONCLUSIONS

The precipitation method was used for the synthesis of nano β-TCP .The micro-structural observation showed that with 36 hour stirring time, nanoscale β-TCP powder was produced in the range of 40-50 nm. Moreover**,** the nanoplate β-TCP powders with average size of 35 nm were fabricated, using the process of milling and introduction of ZnF_2 that could reduce the transformation temperature. ZnF_2 could also modify the β-TCP particle shape and caused the morphology of the β-TCP particles to be plate-like.

Its calcination temperature was about 750 °C, which was 350 °C lower than general calcination temperature for preparing β-TCP particles. This process was available for a great number of compounds, with its great simplicity which provide ultra-fine powders at relatively low temperature.

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