CHAPTER 4

SYNTHESIS AND CHARACTERIZATION OF NANOCRYSTALLINE HYDROXYAPATITE FROM NATURAL BOVINE BONE

Overview - Nanocrystalline hydroxyapatite (HA) powder was synthesized from natural bovine bone by a vibro-milling method. The bovine bone had been deproteinized by hot water before it was calcined at 800°C for 3 h. The resulting product was crushed into small pieces and milled in a ball mill pot for 24 h. After that the powders were ground by the vibro-milling method with various milling times. Characteristics of the powders were then investigated by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDS). Nanoneedle-like shapes of HA powder with diameter less than 100 nm were revealed from the samples using vibro-milling time of 2, 4 and 8 h. Ca/P molar ratio in the powder was 1.66 which is close to the theoretical value that found in the pure hydroxyapatite.

4.1 Introduction

Hydroxyapatite (HA) is a class of calcium phosphate-based bioceramic, frequently used in the biomedical field because of their mineral component being similar to bone and teeth of the human body[23]. The stoichiometric HA has the chemical composition of $Ca_{10}(PO_4)_6(OH)_2$ with Ca/P molar ratio of 1.67. It is reported that HA is an excellent biocompatible material with hard tissues and exhibits

osteoconductive properties, non-toxic, non-immunogenic behaviour[2-5]. The HA derived either from natural sources or from synthetic sources can form a strong chemical bond with host bone tissue[2]. This makes it recognized as a good bone substitute material[3]. Since the early 1970s researchers have been investigating the use of HA in the treatment of bone fractures or defects[6]. After that many research investigations on HA were carried out and tested both in animals and in human. However, these previous studies concentrated on microscale particle of HA (>1 μm)[2]. Recently, nanoscale particle of HA (~10-100 nm) have received much attention because of their superior functional properties over their microscale counterpart. It is reported that sinterability, densification, and mechanical properties of HA ceramics can be improved by prepared from nanocrystalline HA, which is expected to have better bioactivity than the coarser crystals[3]. A number of techniques have been employed for producing HA nanostructures materials, such as sol–gel synthesis[20], co-precipitation[21], hydrothermal reaction[22], microemulsion syntheses[5] and mechanochemical synthesis[24], but most of them are limited to synthesis in small quantities. There has been a constant quest to scale up the process to bulk processing, thus the nanocrystalline HA powder produced from natural bone with conventional calcinations method could be another candidate.

In the present work, we have demonstrated a vibro-milling technique to produce HA nanopowders with high purity. The characteristic of the nanopowders were then characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy dispersive X-ray analysis.

4.2 Experimental procedures

The hydroxyapatite powder was derived from natural bovine bone by a sequence of thermal processes. The fresh bones in all parts of one cow were cut into smaller pieces and cleaned well to remove macroscopic adhering impurities. The bone samples were boiling in distilled water for 8 h for easy removal of the bone marrow and tendons. After that the bone has been deproteinized by continued boiling in water. The boiled bone samples were dried overnight at a temperature of 200 °C. The deproteinized bone was calcined at 800°C for 3 h, a temperature at which no prions or any disease-causing agents can survive. The resulting product was crushed into small pieces and milled in a ball mill pot for 24 h. Each 20 g of HA powders were reground by vibro-milling method (McCrone Micronizing Mill) using ethanol as a milling media and the milling time was varied from 0, 1, 2, 4 and 8 h, respectively. The phase identification and particle size of as-prepared powders have been examined via X-ray diffraction (XRD: Philip X'pert) techniques. For the microstructural analysis, the dried powder were mounted on stubs, gold-coated in vacuum and viewed under scanning electron microscope (SEM:JSM-6335F). Moreover, energy dispersive X-ray analysis (EDS) was employed for phase quantitative analysis. The nanopowder sample was ultrasonically dispersed in ethanol to form very dilute suspensions and then a few droplets were put on copper grids coated with carbon film for further examination by transmission electron microscopy (CM20 TEM/STEM). The crystallographic information could be examined by transmission electron diffraction (TED) pattern.

4.3 Results and Discussions

Fig. 4.1 shows the XRD patterns of the powders obtained after vibro-milling for various vibro-milling times comparing to the standard JCPDS File No.9-432 of pure HA[36]. The XRD patterns of all resulting hydroxyapatite powders correspond to that of pure hydroxyapatite phase. No impurity other than HA was detected by XRD, confirming the purity of this prepared HA within the limitation of the XRD method.

In order to study the effect of vibro-milling time on the crystallite size of the HA powders, the Scherrer equation in the equation (4.1) was used: [35]

$$B_{crystalline} = \frac{k\lambda}{L\cos\theta} \qquad (4.1)$$

where $B_{crystalline}$ = the broadening due to small crystallite sizes, λ = the wavelength of the X-rays, θ = the Bragg angle, L = the average crystallite size, k = a constant (at the best assumption, k = 1.0). Assuming that the XRD peaks have a Gaussian profile and the instrumental broadening and the broadening due to lattice strains of all powder samples are similar, it can be approximated that $B_{crystalline}$ varies linearly to the full width at half maximum (FWHM) of the observed X-ray peak. By using equation (4.1), it may be assumed that λ, $cos\theta$ and k values are constant for the same reflection, as in this case, the (202) reflection of all powder samples was used for determining the FWHM data. Moreover, the same instrument was employed for collecting X-ray data. Thus, it may be roughly estimated that the FWHM is inversely proportional to the average crystallite size as shown in equation (4.2).

$$FWHM \propto \frac{1}{L} \qquad (4.2)$$

By using this assumption, the FWHM of the (202) reflection and the estimated crystallite size from all powder samples were plotted against vibro-milling time and

the relationship between the vibro-milling time and crystallite size was subsequently revealed as illustrated in Fig. 4.2. The result indicates that there is negligible change in FWHM with increasing vibro-milling time, which in turn implies that the crystallite size of the HA powder is not responding to the extent with vibro-milling time by the aid of equation (4.2).

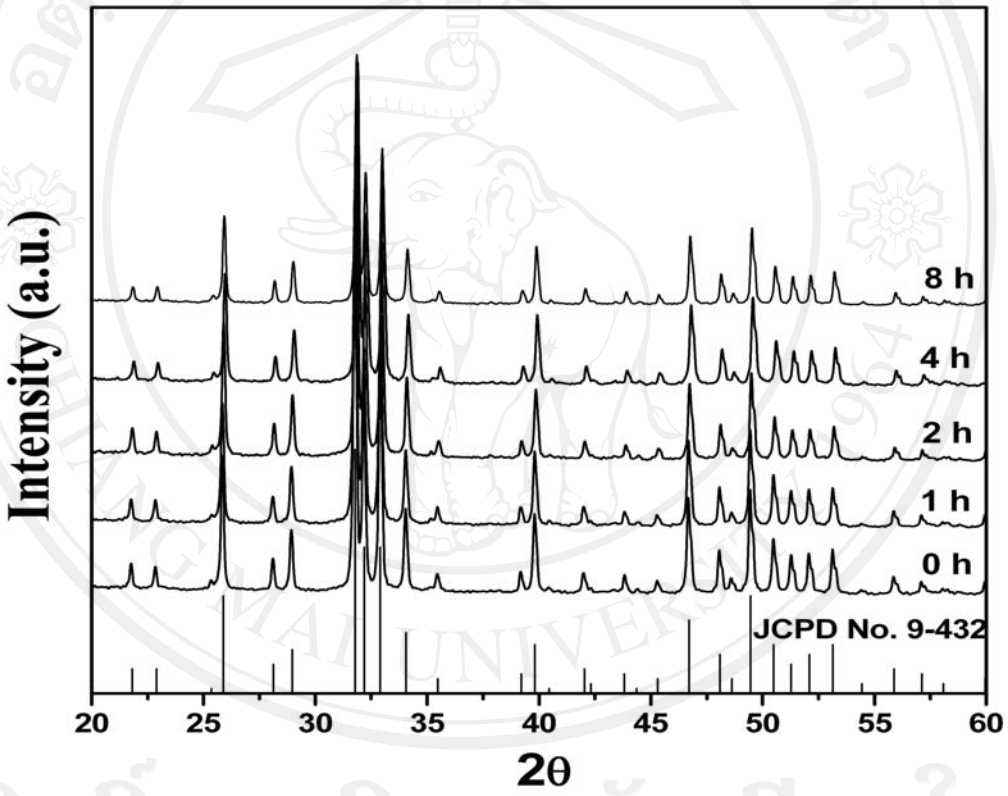


Fig. 4.1 X-ray diffraction patterns of HA powders at various vibro-millng times compared with JCPDS file 9-432 of pure HA.

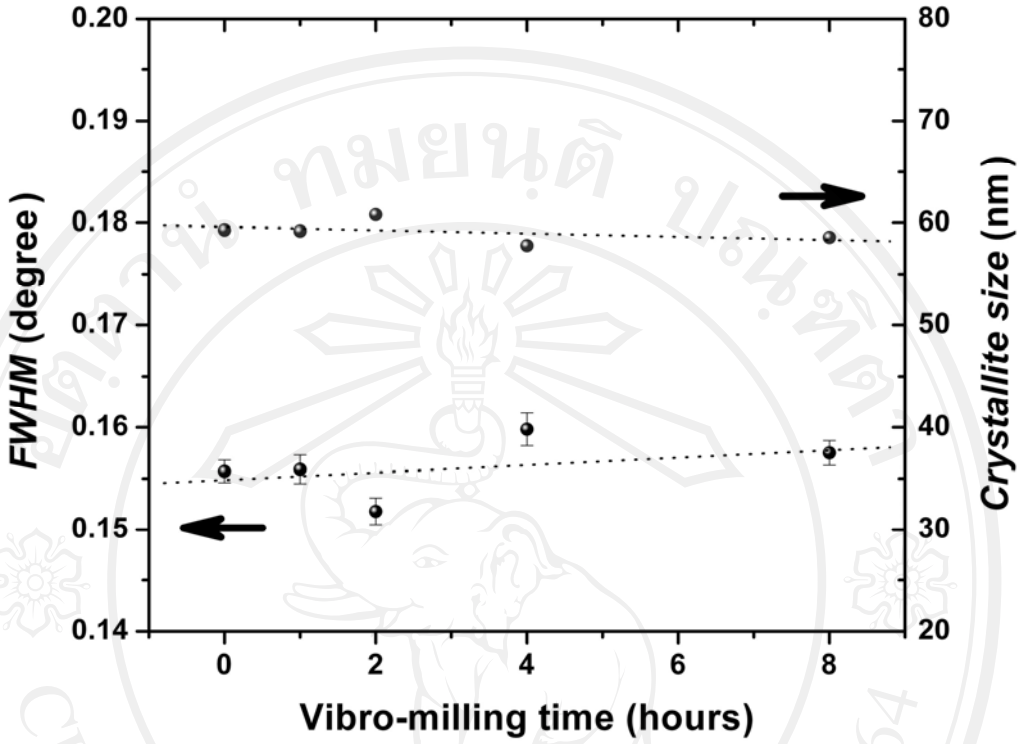
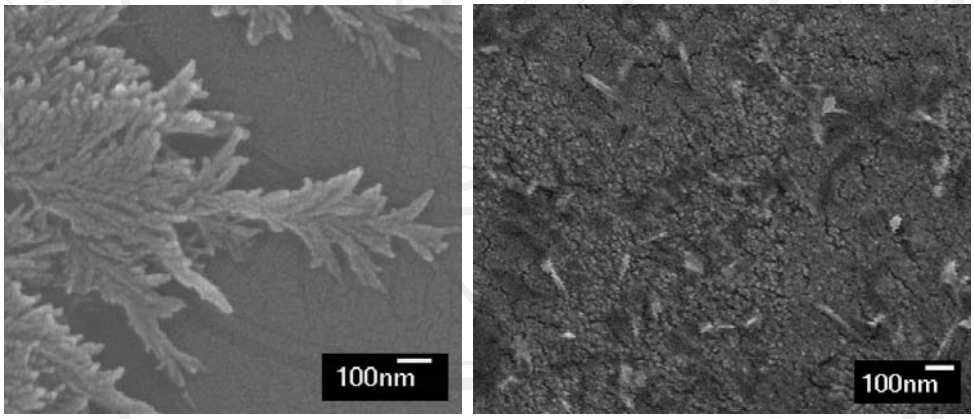


Fig. 4.2 The linear regression of $B_{crystalline}$ versus vibro-milling time using the same reflection of (202) plane from the XRD pattern of each HA powder.

The SEM images of all resulting HA powders for various vibro milling time are given in Fig. 4.3. It is clearly seen that the nanoneedle-like shape of hydroxyapatite powder with diameter less than 100 nm can be obtained from the powders using vibro-milling time of more than 2 h. This is closely related to the crystallite size of the HA powders with the values between 58-62 nm as estimated by Sherrer equation (Fig. 4.2). The more the vibro-milling time was employed, the better distribution of the nanoneedle HA crystals, was obtained. However, the long vibro-milling time of 8 h broke the HA powder into small pieces, giving rise to the inhomogeneous size and shape of the HA nanocrystals as seen in Fig. 4.3(e).

Therefore, the optimum vibro-milling time for producing HA nanopowders should be in between 2-4 hours. Vibro-milling is considered to be a successful method for producing HA nanopowder from natural bone of in the short period of time. Generally, the natural bone consists of plate- or needle-like HA nanocyrstals with dimensions of about 50–100 nm in length and 1-10 nm in diameter [24], therefore the mechanical action of vibro-milling method may provide a sufficient amount of kinetic energy to separate the HA single crystals from the bone scaffold and the more time spent results in better distribution of the HA nanopowders (Fig. 4.3d). Additionally, the longer vibro milling time of about 8 h breaks the HA needles into finer particles of the same diameter as seen in Fig. 4.3e.



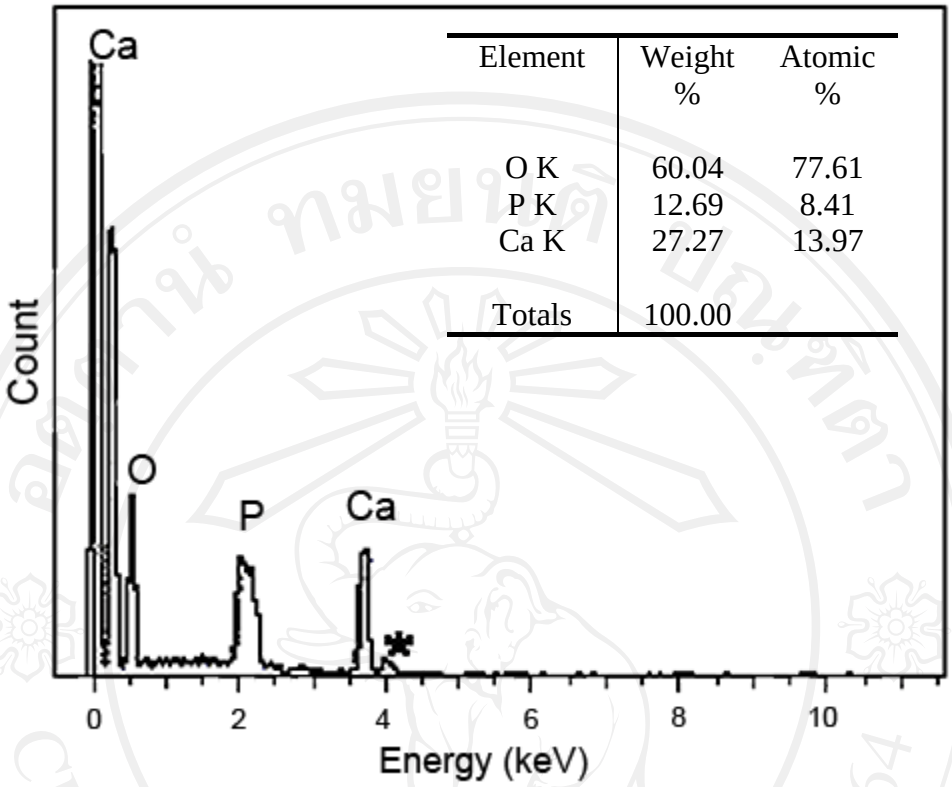
(a) (b)

Fig. 4.3 SEM micrographs of HA powders at various vibro-millng time, (a) 2h (b) 8h.

Fig. 4.4 shows a representative EDS spectrum of nanocrystalline HA powder, consisting only of calcium, phosphorous, oxygen and gold peaks. There are no significant elements detected apart from those elements, which confirms the high purity of nanocrystalline HA powder. The gold element was detected in the EDS

spectrum as a result of the gold coating on the sample surface. The Ca/P molar ratio in the nanocrystalline was determined as 1.66 which is closed to the theoretical value of the pure hydroxyapatite (1.67).

The TEM was used to obtain better information regarding to the size and shape of the HA nanopowder. Results of TEM analyses are shown in Fig. 4.5. It is apparent from Fig. 4.5 that the powder particles have nano-sized needle-like morphology. However, the nanopowder was not well dispersed, suggesting that the aggregation of powders occurred due to van der Waals attraction[31]. The transmission electron micrograph in Fig. 4.5 confirmed that the HA powder consisted of mostly submicron aggregates of nano-sized particles. The TED pattern in the inset of Fig. 4.5 also confirms that HA has hexagonal crystalline structures with a plane spacing $d_{(hkl)}$ of 0.351 nm, 0.207 nm, and 0.128 nm determined from the three smallest R values. These observed $d_{(hkl)}$ values matched well with the d-spacing values from HA database (JCPDS File No.9-432) of the (201), (113), and (423) planes reflections, respectively. In addition, some of the TEM images revealed the lattice fringes, with interplanar spacing of 0.82 nm and 0.53 nm, corresponding to the (100) and (101) planes of hexagonal HA, respectively as shown in Fig. 4.6. The visible lattice fringes further verify that the as-obtained HA nanocrystals are single crystals.



Element	Weight %	Atomic %
O K	60.04	77.61
P K	12.69	8.41
Ca K	27.27	13.97
Totals	100.00	

Fig. 4.4 EDS spectrum of nanocrystalline HA powders and the result of the chemical analysis determined by EDS technique (inset).

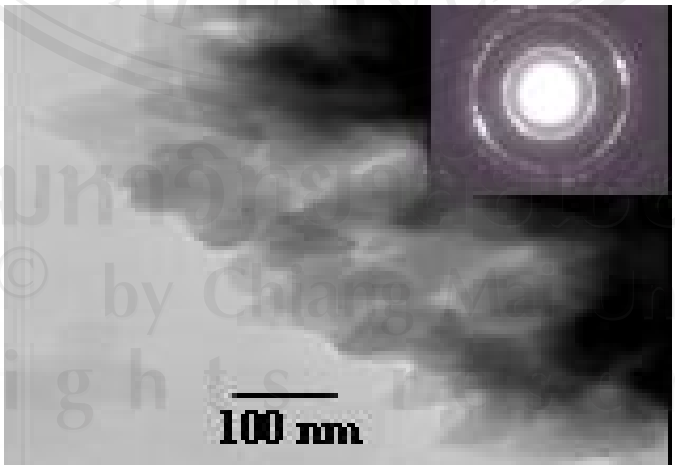


Fig. 4.5 TEM micrographs of nanocrystalline HA powders and corresponding diffraction ring pattern (inset).

4.4 Conclusions

The vibro-milling method successfully provided a simple route for synthesis of nanocrystalline HA powder from natural bovine bone. It was found that the vibro-milling time slightly affects the crystallite size of the HA nanopowders. However, the optimum period of vibro-milling time of about 2-4 hours can separate and disperse the HA nanocrystals from its parent bone structure. In addition, the as-prepared powders are confirmed to be pure nanocrystalline HA powder with their Ca/P ratio is close to that of stoichiometric HA.

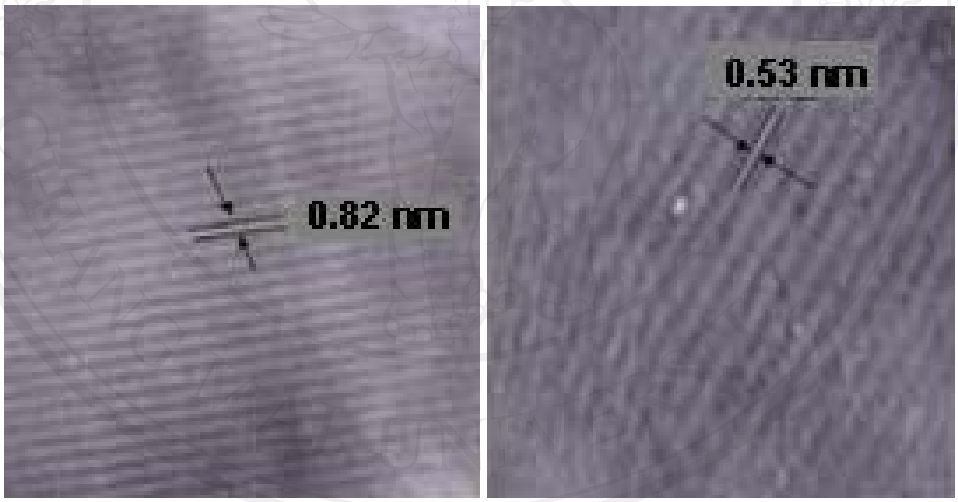


Fig. 4.6 TEM images of two lattice fringes of (a) (100) and (b) (101) planes.