Synthesis of Hydroxyapatite Crystal Nanowires by Using Clamshells

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Abstract

Hydroxyapatite (HA) is one of the most important calcium phosphate minerals due to its application in orthopaedics and dentistry. The applications of the synthesized HA powder depend upon the morphology of the HA. In this research, hydroxyapatite crystal nanowires are synthesized by simple hydrothermal method from clamshells. The results showed that the diameters of hydroxyapatite crystal nanowires distribute in range from 10 to 130 nm. The research also showed the influence of the pH value on the distributions of nanowire size. The purity of the synthesized phase was ascertained by X-ray diffractometry. The morphology and distribution of nanowire size were determined by scanning electron microscopy and ImageJ and Origin softwares while fourier-transform infrared spectroscopy was also used to confirm chemical bonds in HA powder.

Keywords: Hydroxyapatite, hydrothermal method, nanowires

1. Introduction

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a useful bio-ceramic. It is widely applied in the field of medicine, particularly in bone repairing and drug release due to its special biocompatibility, biodegradability and mechanical properties [1-3].

In the world, HA can be produced from biogenic, biowaste materials like coral, algae, fish, eggshell, bovine bone [4], shrimp shell [5], crab shell [6] and some synthetic methods: various techniques were developed for the synthesis of hydroxyapatites, based on solid state reactions [7-8], chemical precipitation reactions [9-10], thermal deposition, hydrothermal reactions and sol-gel methods [8,10] using different calcium and phosphorus containing starting materials. In Vietnam, one of such biowaste is clamshells. Approximately, 230,000 tons of clams are harvested annually in coastal delta provinces [11]. The clamshell represents about 89% of the total weight of clams and is contains mainly 95-97% of calcium carbonate ($CaCO_3$), small quantity of mineral and organic materials [12]. Being cheap and abundant in nature, conversion of these clamshells into HA can be highly advantageous. There are reported studies where bulk clamshells have been converted for synthesizing HA powder for different applications [13-14]. For instance, clamshell is used to synthesis HAp by hydrothermal method at 200^{o}C [15]. This technique is quite promising for synthesizing phase pure HA, however the HA structures required post machining to obtain desired shapes for implantation [16]. In another study reported, clamshells were also used to prepare HA powders at 800^{o}C. The problem with this technique is the lack of control over the particle size. It is quite known that HA nanostructures can significantly increase the biocompatibility and bioactivity of man-made biomaterials compare to their micron sized counterpart [17].

Morphologies and sizes of HA crystals also play a critical role in many applications. For example, in order to achieve self-hardening of bone cement, the addition of reinforcing agents such as fiber type HAp powders is required [18]. In addition, microspherical HA particles with higher number of surfaces delocalized electrons provide better osteoblast adhesion property are using for bone grafting [19]. In nanoscale, HAp nanoparticles can be used as carriers for drug, protein, and gene delivery [20]. Furthermore, plate-like HAp particles can be used as an appropriate adsorbent for protein chromatography and reinforcement materials [19,21].

For each application, the control of particle size and morphology of HAp is fairly important. In the present work we demonstrate synthesis of HA crystal nanowires using hydrothermal method. Influence of pH values on size distribution of hydroxyapatite nanowires is obtained by carefully controlling the pH.

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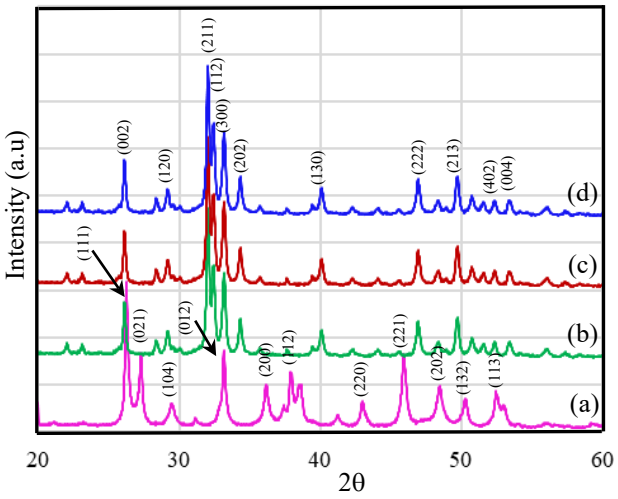


Fig. 1. XRD pattern of the raw clamshells (a) and HA powders autoclaved at different pH values: (b) pH = 7÷8; (c) pH = 8÷9; (d) pH = 9÷10

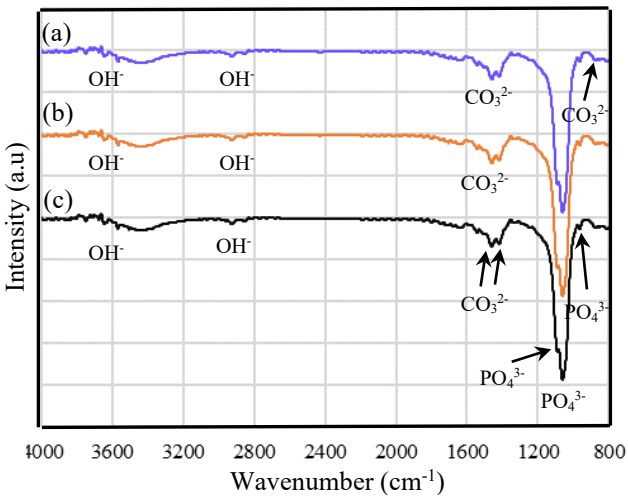
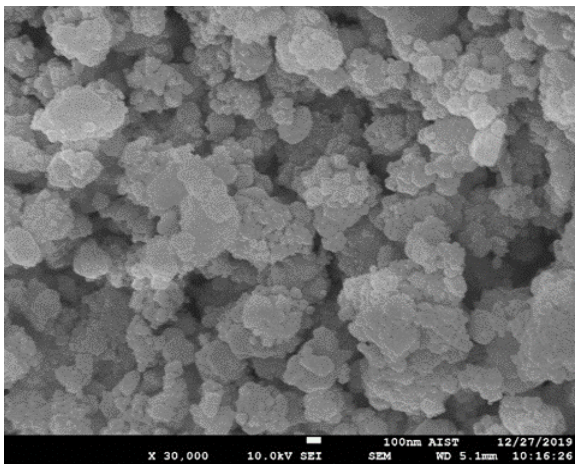
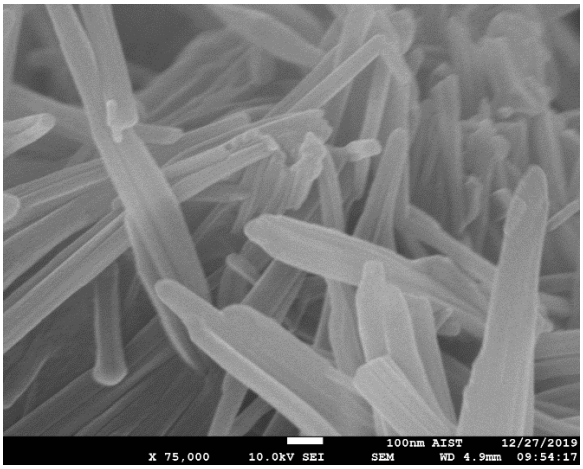


Fig. 2. FTIR pattern autoclaved HA samples at pH = 7÷8 (a); pH = 8÷9 (b) and pH = 9÷10 (c)



(a)



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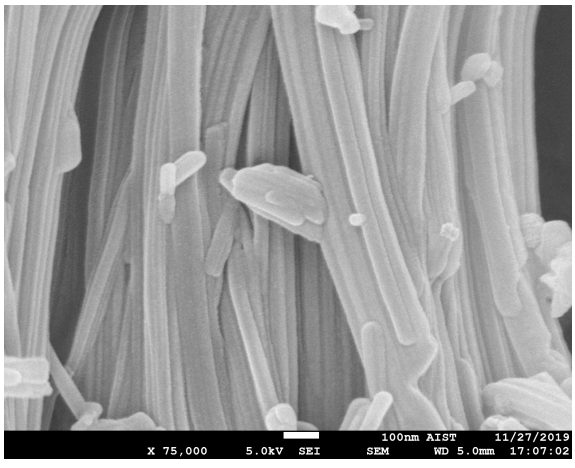

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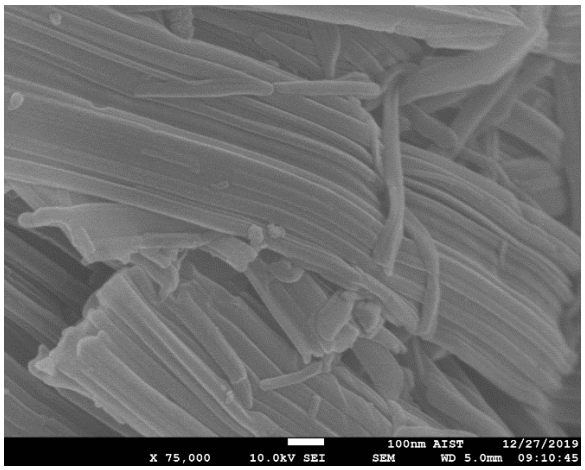

(d)

Fig. 3. FE-SEM images displaying the morphology of (a) ball-milled clamshell powder, (b) HA powders prepared at pH = 7÷8; (c) pH = 8÷9 (b) and (d) pH = 9÷10

2. Materials and methods

In this research, clamshells were collected from clam farm in Thai Thuy district, Thai Binh province. The triethyl phosphate was imported from Himedia, India (purity > 99.8%). The acetic acid solution was obtained from I.C.I.S, South Korea (purity > 99%) and the ammonia solution (28% NH_3 in water) was collected from Vietchem, Viet Nam. At the beginning step, biowaste clamshells were thoroughly cleaned using distilled water and dried in air at 90ºC for 6h. These shells were mechanically crushed, and ball milled in a 350 mL stainless steel jar for 5h at 250 rpm to obtain fine powders. Following step, 0.4g of these ball-milled powders was completely dissolved in 35 mL acetic acid under stirring condition. To this solution, triethyl phosphate was added such that the molar ratio of Ca:P was kept constant at 1.67. The pH of the solution was maintained at pH = 7÷8, 8÷9 and 9÷10 by addition of ammonia. This solution mixture was autoclaved in a teflon lined container at 140ºC for 12h and furnace cooled. The obtained precipitate was washed and filtered with distilled water followed by drying at 90ºC before subsequent characterization by using field emission scanning electron microscopy (model JEOL JSM-7600F) in Advanced Institute for Science and Technology, Hanoi University of Science and Technology. The X-ray diffraction equipment (model D8-Advance) and fourier-transform infrared spectroscopy (model FT/IR-6300 TypeA) were used to analyse phase compositions and chemical bonds of collected powder. Both of equipment are in Faculty of Chemistry, VNU University of Science Hanoi. The diameter size distribution of the powder was carried out using Image J and Origin software. The pH values were determined by using pH Pocket Tester (model ADWA AD110) in School of Materials Science and Engineering, Hanoi University of Science and Technology.

3. Results and discussion

Fig.1 shows the XRD pattern of the raw clamshell and HA powders autoclaved at different pH values at 140ºC. In Fig.1a, the strongest diffraction peaks appearing at planes (111), (021), (012), (112) and (221). The peaks were in agreement with the published data [22] and belonged to JCPDS 41-1475, these confirm that the ball milled shell powders showed the presence of pure calcium carbonate peaks.

In Fig.1b-d, XRD patterns of HA powders with strongest peaks at planes (002), (211), (112), (300), (202), (130), (222) and (123). The peaks were in agreement with the published data [23] and belonged to JCPDS 9-432, these results confirm that HA powders were successfully synthesized by using hydrothermal method. The powders were characterized with absence of secondary phases indicating that the synthesized HA were pure.

Fig.2 presents the FTIR patterns for autoclaved HA samples at different pH values. Presence of weak band at about 3573 cm^{-1} and 2930 cm^{-1} indicated the presence of OH^- group [24]. A strong band of PO_4^{3-} group [25] was observed at about 1087 cm^{-1}, 1032 cm^{-1} and 958 cm^{-1}. The band values obtained for respective phosphate and hydroxyl groups were in agreement with other published data [26] for pure HA. A weak band of CO_3^{2-} was detected in the region around 1459 cm^{-1}, 1420 cm^{-1} and 877 cm^{-1}. These bands indicate mode of CO_3^{2-} group in the HA structure.

Based on the above observations, the possible reaction involved in the formation of HA during the hydrothermal process can be expressed as follows:

$$10CaCO_3 + 6(C_2H_5)_3PO_4 + 10H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18C_2H_5OH + 10CO_2 \quad (1)$$

In Fig.3 shows FESEM images of clamshell and HA powders. Clamshell after milling in stainless steel jar for 5h at 250 rpm give fine powders with particles size is focusing in range of 0.3÷5 μm (in Fig.3a). The result in Fig.3b shows that the HA powders from reaction (1) are in nanowires shape with diameter several tens of nanometers. That confirmed that hydroxyapatite crystal nanowires can be synthesized by using clamshells with hydrothermal method.

The pH value was known as a factor influence on size distribution of HA nanowires. In this research, the pH values were also varied to observe the size distribution of HA nanowire diameters. By using ImageJ software to measure diameter of HA nanowires and Origin software to determine size distribution with the normal function as shown in Fig.4. Results showed that pH values have influence on size distribution of nanowires. Although the diameters of nanowires on all samples are in the range of 10 to 130 nm, there has been a shift in the peak position of the nanowires diameter distribution curve from right to left as the pH value in the solution increases. At pH = 7÷8 and pH = 8÷9, the peak positions are about 59.5 and 53.5 nm, respectively (Fig.4a-b). The smallest peak position is about 51 nm with pH = 9÷10 (Fig.4c). This proves that when the pH value increases, it will allow to receive more amount of nanowires with smaller diameter. This might be because of the higher concentration of OH- ions present in the solution. OH- ions provide the template to the nucleation process resulting in the formation of lower diameter nanowires.

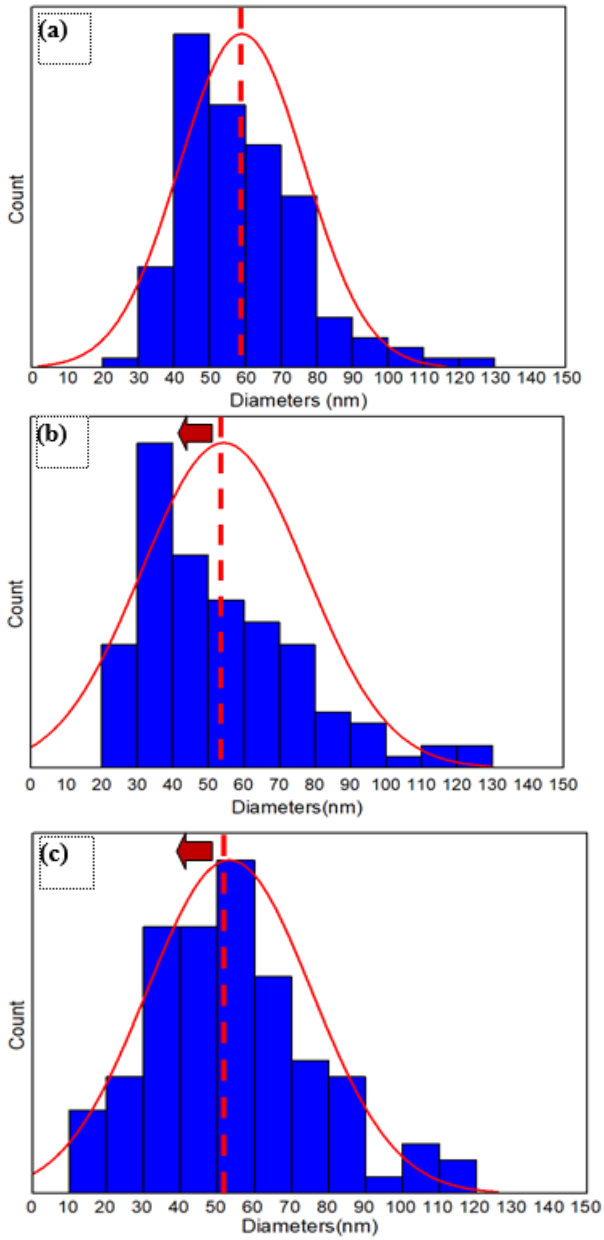

Fig. 4. Distributions of hydroxyapatite nanowire size with different pH values: (a) pH = 7÷8; (b) pH = 8÷9; (c) pH = 9÷10

4. Conclusions

In this study, clamshells were used as a rich source of calcium to synthesize hydroxyapatite crystal nanowires via hydrothermal method. These powders were autoclaved at 140ºC for 12h giving average diameter evaluated from FESEM observations showed proximate values between 10 and 130 nm. The pH value of the starting reaction solution is significant influence on altering the size of HA nanowires. At higher pH value, the size distribution of HA nanowires focuses in the smaller diameter range.

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