

Synthesis of Hydroxyapatite from Cockle Shell Wastes

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Abstract—Cockle shell wastes are abundant and easily available. It rich in calcium carbonate (CaCO_3) that can be converted into hydroxyapatite. HAP has numerous benefits especially in biomedical applications. The overall objective of this study is to determine the effect of reaction time on producing HAP derived from cockle (*Anadara granosa*) shells waste. There are two reaction times used in this study which are 3 h and 5 h. The processes involved in synthesizing the HAP were pre-treatment of the cockle shells, formation of CaCO_3 in aragonite form, calcination of the CaCO_3 to form CaO , production of $\text{Ca}(\text{OH})_2$ and finally reaction of the $\text{Ca}(\text{OH})_2$ solution with the phosphate source to obtain HAP. All samples have undergone physicochemical analyses to determine their crystallinity, purity, functional group, surface morphology and elemental compounds using X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM) and Electron Dispersive X-ray Analysis (EDX). This study found that 5 h reaction time produced powder is better HAP compared to the 3 h powder. Overall, this study proved that reaction time plays a vital role in the quality of producing HAP. Longer the reaction time has enhanced the quality of the HAP formed.

Keywords—Hydroxyapatite; cockle shell; biomaterial; wet slurry precipitation; reaction time

1. INTRODUCTION

Cockles which known scientifically as *Anadara granosa*, live predominantly on intertidal mudflats and are an important protein source in the South East Asian region [1, 2]. It is one type of sea molluscan that is widely consumed in South East Asian countries in various delicacies. This, however could contribute to the generation of waste shells after the cockle were consumed and it became abundant. The shell wastes usually being dumped into landfill or in the sea since the proper disposal procedure is relatively expensive [3, 4]. The landfills often cause unpleasant view to surroundings and give out nauseating smell.

To minimise the negative impacts on the environment, researchers around the world are studying on transformation of shell wastes into valuable products. The cockle shells could be used as replacement material in concrete structure [5, 6], catalyst for biodiesel production [7-10], adsorbent for treating industrial effluent and polluted river water [11-13], drug delivery applications [14-18] and used in biomedical applications especially for bone and teeth [1, 19, 20].

In fact, this cockle shell consists of high composition of calcium carbonate (CaCO_3) of more than 95% by weight [21-23]. The CaCO_3 could be a promising source to produce hydroxyapatite (HAP). With the chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, HAP is

widely used in biomedical applications mainly as bone replacement, regeneration, and dental restoration materials because of its biocompatibility and bioactivity [24-27] as well as due to its chemical resemblance to the mineral phase of bone and teeth [24, 27-29].

Besides cockle shell wastes, there are plenty of wastes that have been used by other researchers around the world to produce HAP. For examples, egg shell wastes [30, 31], dead snail shell wastes [32, 33], green mussel shell wastes [9, 21], scallop shell wastes [34], abalone shell wastes [35], oyster shell wastes [36], fish scale wastes [37], bovine bone wastes [31, 37] and chicken bone wastes [31, 38]. Cockle shell waste is used in this study due to the excessive and easily available of cockle shell wastes in Malaysia.

The formation of HAP using cockle shell waste could reduce the production cost as well as the HAP price in the market. Thus, this study is aiming to synthesize HAP from cockle shell wastes by varying the reaction time during the process. The samples will be characterized using TGA, XRD, FTIR and SEM with EDX analyses.

2. MATERIALS AND METHODS

A. Materials

Cockle shells were purchased from local market at Kuantan, Pahang. Potassium Dihydrogen Phosphate (KH_2PO_4) was purchased from Sigma Aldrich to be used as phosphate source for HAP production. Deionized water in this experiment was obtained directly from the lab.

B. Production of HAP

The cockle shells were boiled and cleaned to remove organic materials and impurities that were attached to it. The cleaned shells were dried in the oven for 1 h, at temperature of 110 °C. The cleaned shells were crushed using the mortar into small particles size and grinded into fine powder particles. Then, it was sieved using sieve shaker to get powders size ranging between 71 to 125 μm . The cockle shell powder was calcined at 800 °C for 3 h to get CaO. Afterwards, wet slurry precipitation technique was used to produce HAP based on Shariffuddin et al. [9]. The CaO was then dissolved in water to obtain $\text{Ca}(\text{OH})_2$. Afterwards, KH_2PO_4 solution was dropped into $\text{Ca}(\text{OH})_2$ solution to form hydroxyapatite. The mol ratio of CaO to KH_2PO_4 was fixed at 1.67. The reaction time was adjusted between 3 h to 5 h. The mixture was then centrifuged at 3000 rpm for 10 min to get the milky white precipitate that is referred as hydroxyapatite.

C. Chemical Characterization

The phases of the powders were analyzed using X-ray powder diffractometer (XRD Rigaku MiniFlex II, Japan). Samples were scanned at 2θ from 20°-80° with step size 0.02° at 1 s step time. FTIR (Thermo Fisher Nicolet iS5, USA) with diamond attenuated total reflectance (ATR) system was used to identify functional groups in the powders over a range of 400 to 4000 cm^{-1} . The morphology and elemental analysis of the powders were observed using SEM along with EDX (Hitachi TM3030 Plus, Japan). The samples were sputter-coated with platinum to prevent charging during analysis process.

3. RESULTS AND DISCUSSION

A. EFFECT OF REACTION TIME ON HAP PRODUCTION

Fig. 1 illustrates the XRD patterns of HAP produced at different reaction times. The HAP produced at 3 h reaction time shows broader peaks compared to 5 h reaction time. Broad peak indicated that the sample has less crystallinity. Besides that, there is calcium phosphate sulfide peaks appeared on XRD pattern of HAP with 3 h reaction time. This is probably due to impurity during pre-treatment of HAP powder. Meanwhile, HAP produced at 5 h reaction time has sharper and more apparent peaks. This indicated that HAP with 5 h reaction time has higher crystallinity compared to HAP with 3 h reaction time. Increase the reaction time has increased the crystallinity of the HAP. This finding was supported by [39] and [40].

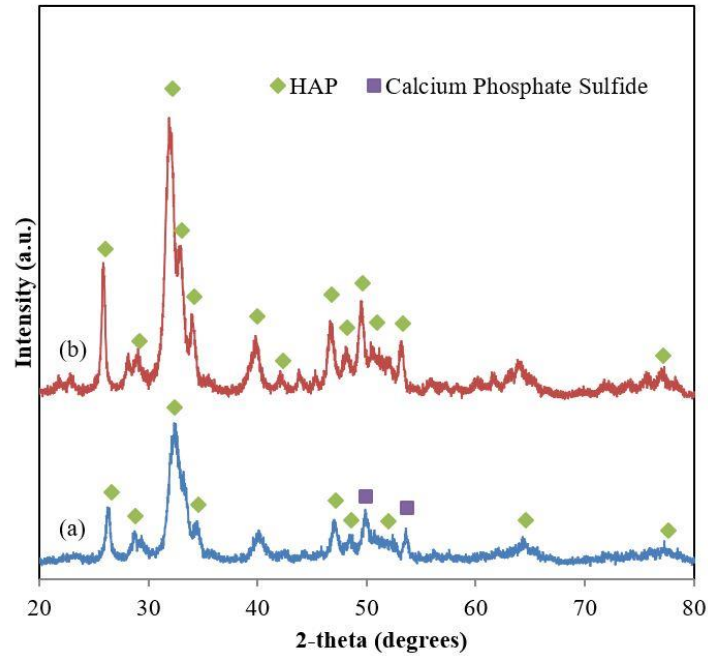


Figure 1 : XRD patterns of HAP at reaction time (a) 3 hours and (b) 5 hours.

Fig. 2 shows the FTIR spectra of HAP produced at 3 h and 5 h reaction time. HAP produced at reaction time of 3 h has C-O band presented at 1417 cm^{-1} and 877 cm^{-1} . Meanwhile, bands 1089 cm^{-1} , 1028 cm^{-1} , 962 cm^{-1} , 602 cm^{-1} and 561 cm^{-1} correspond with phosphate band. Other than that hydroxyl band can also be seen at 3347 cm^{-1} . For HAP produced at reaction time of 5 h, C-O band at 1413 cm^{-1} , 875 cm^{-1} . P-O band can be presented at 1089 cm^{-1} , 1029 cm^{-1} , 963 cm^{-1} , 603 cm^{-1} and 567 cm^{-1} indicated the presence of phosphate. Lastly, O-H band observed at 3343 cm^{-1} . Since FTIR analysis is meant to detect the functional group of compounds, these results proved that HAP has been produced even at shorter reaction time which is 3 h. Basically, both reaction time show that it has carbonated constituent since common bands of carbonated HAP was detected. Similar findings were also obtained by previous scholars [41, 42]. However, HAP produced at 3 h reaction time has intense C-O band around 1417 cm^{-1} and 877 cm^{-1} compared to 5 h reaction time. This indicate that HAP produced at 3 h reaction time contained higher carbonate ion concentration. According to McElderry et al. [43], increase in carbonate ion concentration has reduced the crystallinity of the produced HAP. This FTIR results are supported by XRD results in Fig. 1 where HAP produced at 3 h reaction time has lower crystallinity compared to 5 h reaction time.

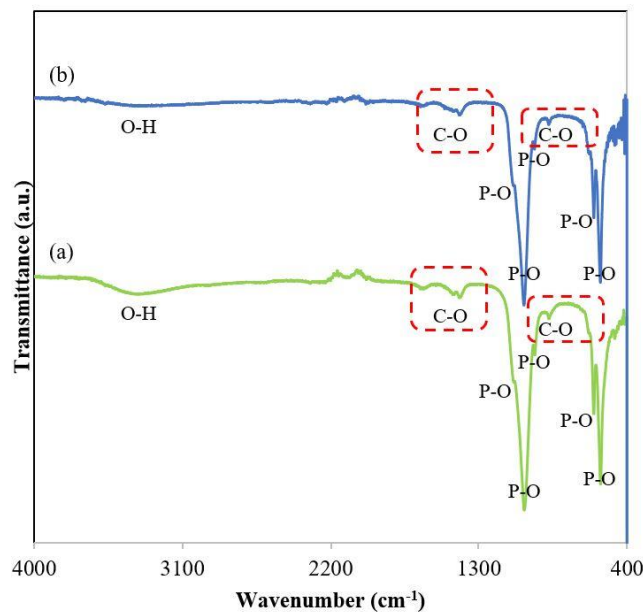


Figure 2 : FTIR spectra of HAP at reaction time (a) 3 hours and (b) 5 hours.

The surface morphologies and EDX analyses of HAP are shown in Fig. 3. From the SEM micrographs, HAP crystals from both conditions were irregular in shape and varied in size. These HAP morphologies were in agreement with the study done by Dey et al. [44] and Bogdanoviciene et al. [45] where the produced HAP have irregular shapes. HAP produced at 3 h reaction time has smaller particle compared to 5 h. Increase in reaction time has increased the particle size of HAP. This findings are parallel with previous research done by Yusoff et al. [46] where longer reaction time has produced larger HAP particle due to agglomeration process.

The EDX analysis of HAP powder had confirmed that HAP powder contains calcium, oxygen and phosphorus. These elements are in agreement to the FTIR results in Fig. 2 where phosphate groups are present in the powder. Other than these three main elements, potassium bands were found as well. The potassium was originated from the potassium dihydrogen phosphate solution that was used as the phosphate source [9]. However, the potassium content could be eliminated by sufficient rinsing with sufficient amount of deionized water. Carbon is present due to absorption of carbon dioxide during reaction. The platinum peaks were detected because of the coating used for running the SEM analysis.

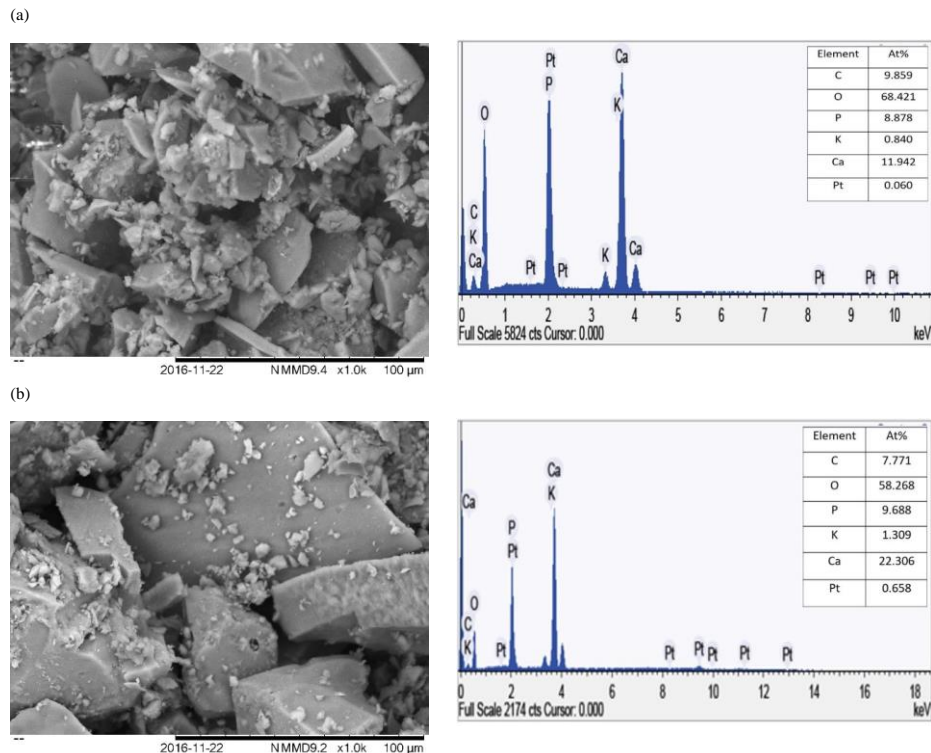


Figure 3 : SEM micrographs and EDX analyses of HAP at reaction time (a) 3 h and (b) 5 h at 1000× magnification.

4. CONCLUSION

In conclusion, the results show in this study have proven that HAP powders have been produced for both samples (HAP 3 h and 5 h) using cockle shell wastes as shown by XRD, FTIR and SEM/EDX. Based on XRD and FTIR results, it is confirmed that 5 h reaction time is better compared to 3 h as it has longer reaction time and produce higher crystalline HAP. It can be concluded that reaction time is important parameter in HAP production.

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