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Extraction and characterization of hydroxyapatite (HA) from eggshell by precipitation method for bone implant coating: A Case Study

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Abstract: This research study was showed the extraction and characterization of hydroxyapatite (HA) from eggshell by precipitation method for bone implant coating. The eggshell were carried out using wet precipitation method in order to extract hydroxyapatite powder from the eggshell. Various parameters were used through the calcination process such as the first calcination process will be implemented at 900 °C for 1 hour while the second calcination process will be run at 1000 C within 2 hours. The different of pH value also used as parameters. The characterization of hydroxyapatite (HA) from eggshell powder have been conduct through scanning electron microscopy (SEM), X-Ray diffraction (XRD) and Fourier transform (FTIR). By SEM, the microstructure of hydroxyapatite (HA) based on different pH and calcination temperature could be analyse. The result were analyzed and discussed based on the results that gained from previous study by other researchers due to Movement Control Order (MCO). The purpose use of XRD is to categorize the crystalline material and define the unit cell. From XRD, SEM and FTIR findings, the effect of pH and effect of calcination temperature are determined. For FTIR, the elements and arrangement of molecules can be define. The result of analysis are retrieve from the previous research. According to the past research, pH value and calcination temperature are the strong influence on the SEM morphology. By comparing among the previous research, precipitation method the most method that applied and source of material from eggshell wastage. Thus, hydroxyapatite (HA) from eggshell could be prove by precipitation method for bone implant coating.

Keywords: Eggshell, Bone Implant Coating, Precipitation Method

1. Introduction

In the earlier decades the advancement in bone inserts has increased dramatically due to the changing human way of living. The problem associated with the body, such as bone fractures and bone infections, has caused a significant impact to the patients who require long time to recover from their

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common disease. At this time, the sharp rise in the amount of linked human population growth demands for quality production in the sense of impaired human protection and health (Kane et al., 2015). The biomaterial is also have been used as implanted in the body of a human being in order to provide the same biological and natural functions for bone implant applications, in particular for the removal or reconstruction of different tissues and bones (Giger et al., 2013).

For the last few years, researchers and scientists have been seeking to discover innovative biomaterials for skeleton tissue engineering. The most difficult problem is the synthesis of biocompatible and materials for bioactive bones. In this regard calcium Ceramic Hydroxyapatite (HAp) [Ca10 (PO4)6(OH) 2] dependent on phosphate is gaining significant popularity due to its excellent osteoconductant and bioactive characteristics (Lala et al., 2019). Commercial hydroxyapatite production typically requires synthetic chemicals such as calcium chloride (CaCl2), calcium hydroxide (Ca(OH)2), calcium nitrate tetra hydrate (Ca(NO3)2ή4H2O) and calcium acetate (Ca(CH3COOH)2) as sources of calcium (Khandelwal & Prakash, 2016). Hydroxyapatite (HA) is a compound that composes the body's tissues, such as bones and teeth. Hydroxyapatite (HA) is able to be synthesized from eggshells as they have high calcium content. Hydroxyapatite is commonly used as a medical application because of its properties, which demonstrate the strong biocompatibility, bioactivity and non-toxicity characteristics required as a biomaterial for medical use (Al-Sanabani et al., 2013).

1.1 Problem Statement

Stainless steel alloys were the first metals to be used in orthopedics as temporary bone implant. However, there are an issue of the materials when it is applied as permanent bone implant since it released toxic and harmful ion after longer time of implantation. There is still has a good material for bone implant such as titanium but it is very expensive. Thus, hydroxyapatite (HA) extracted from the wasted eggshells that suitable for application as a coating material on the metal implant to improve its biocompatibility will be synthesized and characterized.

1.2 Objective

This study consists of two main objectives which are to investigate the effect of pH during the precipitation process on the hydroxyapatite properties and to analyse the effects of calcination temperature on the hydroxyapatite characteristics.

2. Methodology

2.1 Eggshell preparation

In this research study, hydroxyapatite (HA) from eggshell was used as a biomaterial powder. To begin, the eggshell was washed and boiled in distilled water through preparation of eggshell. Next, the eggshell was experienced precipitation process where in this stage, the eggshell was testing with pH 8, 9 and 10 to get the ratio of pure HA which is 1.67. The next stage, the eggshells were placed in an oven to through the calcination process. The first stage consists of heating the eggshell to 900°C for 1 hour in tube furnace. At this temperature, any organic waste is expected to be burned. The second stage consisted of heating the samples to 1000°C for 2 hours. Through this stage, the eggshell is expected transform into calcium oxide by freeing carbon dioxide (CO2). The solution was filtered after the reaction was finished and the resulting material was dried. Then, the Fourier transform infrared spectrometry (FTIR) method that was used to identify the current powder group of functional or collecting elements. In addition, X-Ray diffraction analysis (XRD) was used to determine the ratio of HA materials and values. Scanning electron microscopes (SEM) have been used to determine the mixed powder microstructure.

2.2 Calcination process

Calcination is a heat treatment at low temperature. The process will enhance the crystallinity that is easily seen in X-ray diffraction patterns. Calcination is an important method to enhance a material's chemical and physical properties. In the study, calcium oxide powder (CaO) from the raw eggshell was placed in a sealed container before undergoing two stage of thermal treatment. The crushed eggshell in powder form was subjected to calcination process at 900˚C at 4˚C /min heating rate. In second calcination process, eggshells were heated to 1000˚C for 2 hours at a constant heating rate of 4˚C /min.

2.3 Drying process

Drying process took place due to remove excess water from the mixture by thermal drying. It has an importance in modern mineral processing. To carry out the drying process in the study is oven. The oven-dried method is effective and suitable in order to the material maintained during the drying process. The oven dried process was completed in a closed chamber with relatively low temperature, which is 90˚C for 15 hours.

2.4 Scanning Electron Microscope (SEM)

The morphological characterization of the synthesized powder is investigated using high resolution SEM (JEOL JSM-7600F). The samples are gold-plated by sputtering with a 230 V, 50 Hz direct drive 2-stage vacuum pump. The scale of magnification of the images is between 150-30000XX (Lala et al., 2019). The sample surface was studied by Energy Dispersion for X-ray analysis (EDX) (Damia et al., 2006). The morphology evaluation of the sample was determined using a 20 kV secondary electron image scanning electron microscope which is brand from Germany. Four runs at various locations on each sample were made to determine the chemical composition and to approximate the Ca/P ratio. Preparation of samples shall include: sectioning, assembling, grinding, polishing and gold coating (Agbabiaka et al., 2020).

2.5 X-Ray Diffraction Machine (XRD)

This X-ray diffraction (XRD) machine is used to classify the crystalline material, calculate the purity of the sample, determine the unit cell size, and also define the unknown material of the crystal. This machine is essentially a non-destructive analytical tool used to identify the crystalline forms that are called 'phase' and quantitatively analyse them. The machine will identify the form of arrangement of atoms and even the phase of crystals by diffracting the beam angle. The scanning electron will play the role of checking the pattern of the diffracted beam. All the pattern that collected will be compare with the computerized databases of Joint Committee of Powder Diffraction Standards (JCPDS file).

2.5 Fourier Transform Infrared Spectroscopy (FTIR)

The device used to observe the hydroxyapatite spectrum for the frequency graph formation of the hydroxyapatite powder during the hydrothermal reaction is Fourier transform infrared spectroscopy (FTIR). This FTIR equipment are also capable of analysing qualitative, quantitative, organic and inorganic scans. This machine aims to provide details on the chemical bonding of the molecular structure. The FTIR can be classified as non-destructive testing. The method to do the testing by get the sample of hydroxyapatite powder that calcination with different temperature of the eggshell part. Make sure the testing sample place cleaned with the distilled water before each testing start to avoid data error and get an accurate data of frequencies analysis graph. After the sample has been set, the machine needs to set the parameter to 32 scan with a wavelength of 600 to 4000. During the scanning process, allow some pressure to the sample by changing the graph frequency for improved graph frequency vibration. The scanning equipment can define the vibration of the chemical bonds and the characteristic graph pattern frequencies. The Frequency Graph can be used to check and classify the functional groups and compounds that suit the characteristics of the IR Band Positions Table.

3. Results/Analysis/Discussion

3.1 Microstructural analysis

The aim of Microstructural analysis is to observe and study the internal structure which is morphology of hydroxyapatite. (Ummartyotin & Tangnorawich, 2015) stated that typical illustration of eggshell morphology as in Figure 3.1.

3.2 Phase Analysis

Phase analysis by using X-ray powder diffraction (XRD) is to analyse the specimens of HAp in reflection mode with Cu Kα (λ=1.5405 Å) radiation.

3.3 FTIR Analysis

Fourier transform infrared spectroscopy is capable of detecting chemical bonding as well as the presence of functional groups in samples. The reason of FTIR in the study is to gain information on the vibrational origins of functional groupings for example carbonate (CaCO3), amide group (CO-NH) and phosphate (PO43).

3.4 Result review

From the reported by (Ummartyotin & Tangnorawich, 2015), calcium oxide was derived from eggshell waste through wet chemical precipitation. From the study, it noticeable that eggshell has a great thermal stability, having been calcined for 3 hours at the calcination temperature 700, 800, 900 and 1000 °C. The single phase of hydroxyapatite was verified by XRD. Through XRD pattern, the peak position was existed at 2θ of 28° and it was analyzed as the calcium carbonate residual. The CaO peak with the greatest intensity was identified at a 2θ of 37°, which corresponded to the lattice plane (200) and at different calcination temperatures, the temperature rises from 800 to 1000 °C. The irregular shape with size variation was provided by morphological characteristics. The crystal size was detected at peak (200) which is 70 nm.

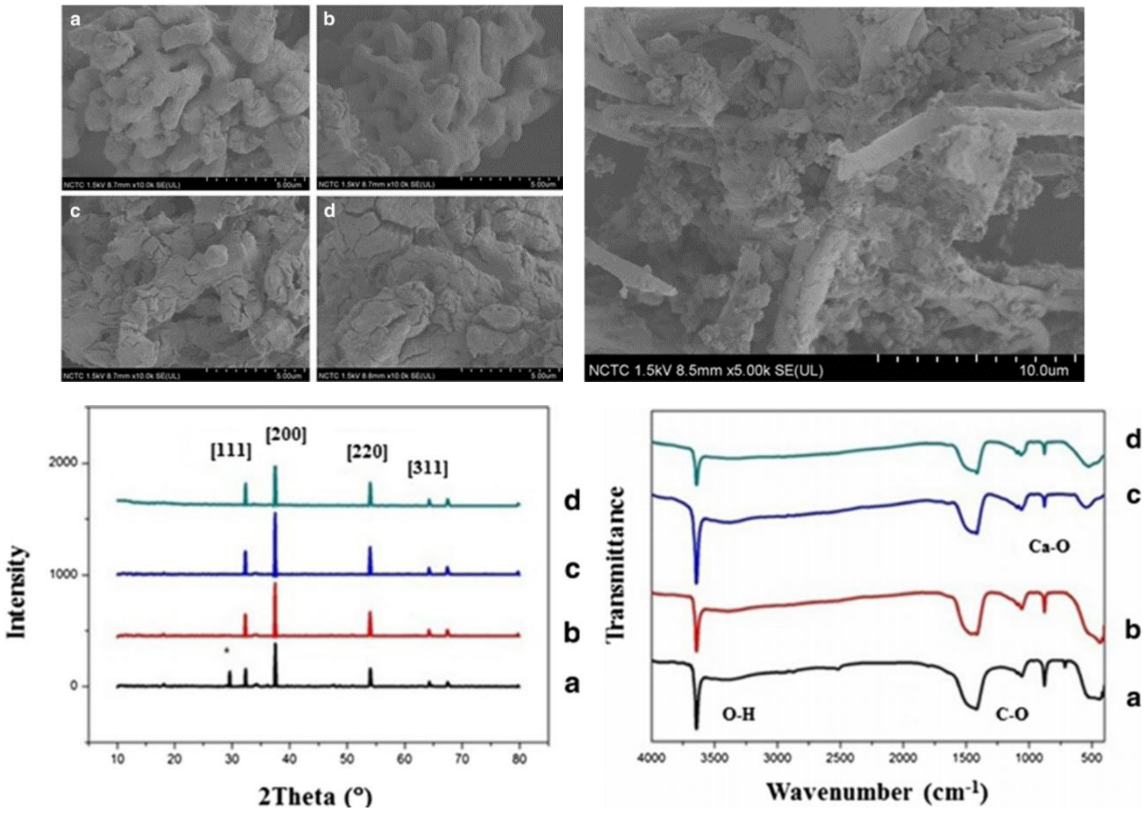


Figure 3.1: The result reported by (Ummartyotin & Tangnorawich, 2015)

While, the hydrothermal technique was used to produce pure HA phase using eggshell waste as a Ca precursor according to (Toibah* & , F. Misran, 2019). The pH of the beginning precursor influenced crystallite size, crystallinity, structure, and crystallisation temperature. At higher pH, the presence of HA and calcium hydrophosphate crystals increase while at low pH, only produce larger crystallite sizes. The pH used are 5 and 9. By SEM, the HA powders that were synthesized in acidic condition of pH 5 produced spherical and rod-like shaped particles whereas the powders synthesized in a pH 9 starting solution produced rod-shaped particles. Besides, XRD patterns showed that HA was the only main phase present in the as-synthesized powder after being calcined at 400°C. However, EDX analysis detected the presence of Mg as trace element which originated from the eggshell as starting materials.

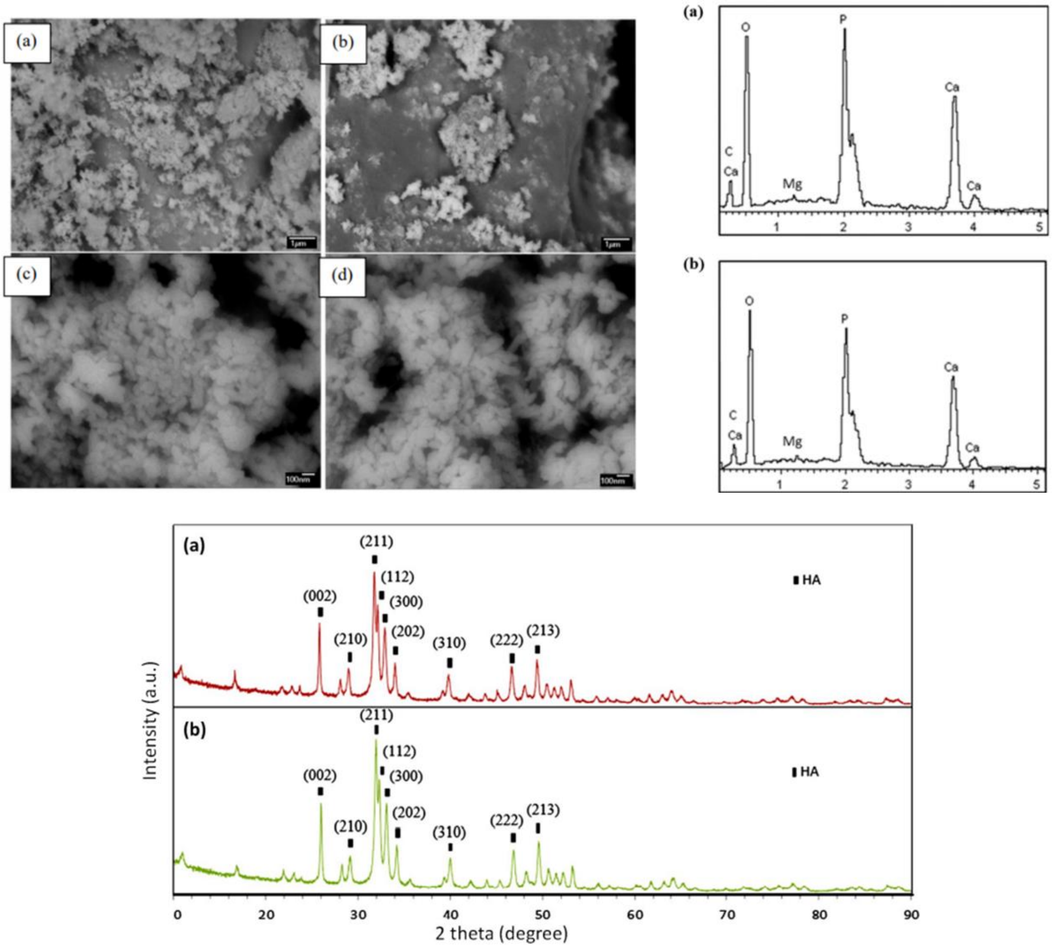


Figure 3.2: The result reported by (Toibah* & , F. Misran, 2019)

Next, based on report by (Le et al., 2012), the synthesized HAp from eggshell is from hydrothermal method. From SEM image, it found that large plates are present when it in acidic condition due to the pH is not controlled during precipitation. Although, when above pH 9, the plate is vanished. The existence of large plate correspond with the existence of calcium hydrophosphate in XRD pattern as shown in Figure 3.3. It conclude by the researcher that the big plates are calcium hydrophosphate, which is produced when the pH is acidic. The formation of hydroxyapatite nanoplates is depend on the desirable of pH and temperature condition. The plates are 1–2 µm wide and 80 nm thick.

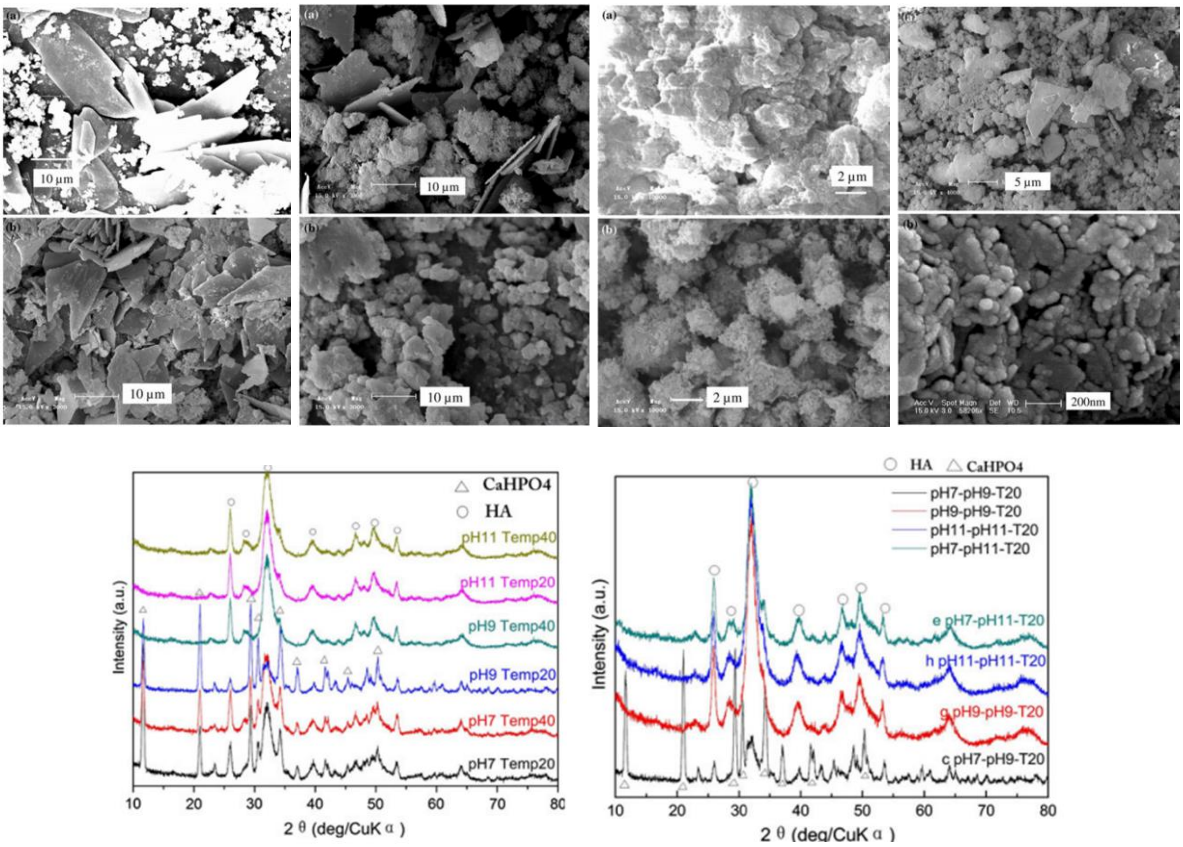


Figure 3.3: The result reported by (Le et al., 2012)

Furthermore, the standard precipitation method was used to make hydroxyapatite powders with various pH reaction temperatures, and Ca/P molar ratios according to (Lee et al., 2020). The formation of HA is detected at Ca/P=2.2 and pH >11 while α-TCP and β-TCP were obtained at pH 10 and 11, regardless of the Ca/P ratio. Ca was lost throughout the process, and the PO43– ions in HA were replaced by HPO42– ions, resulting in CdHA and degraded into to α-TCP or β-TCP after through heating process. When the pH was raised, however, the reverse reaction occurred, resulting in the production of stoichiometric HA. The impact of increasing the reaction temperature was reduced HA solubility and as a result, Ca loss in the production of HA. The form of the produced HA particles changed from rod to bamboo leaf-like as the reaction temperature increases, and their size reduced. Regardless of Ca/P, the majority of the spectra showed bands associated with PO_4^{3-} (470, 567, 603, 963, 1034, and 1092 cm^{-1}), OH^- (633 and 3570 cm−1), CO_3^{2-} (874, 1425, and 1452 cm^{-1}), H_2O (1641 and 3300 - 3500 cm^{-1}), and HPO_4^{2-} (874 cm^{-1}), represent to characteristic of HA and other organic compounds.

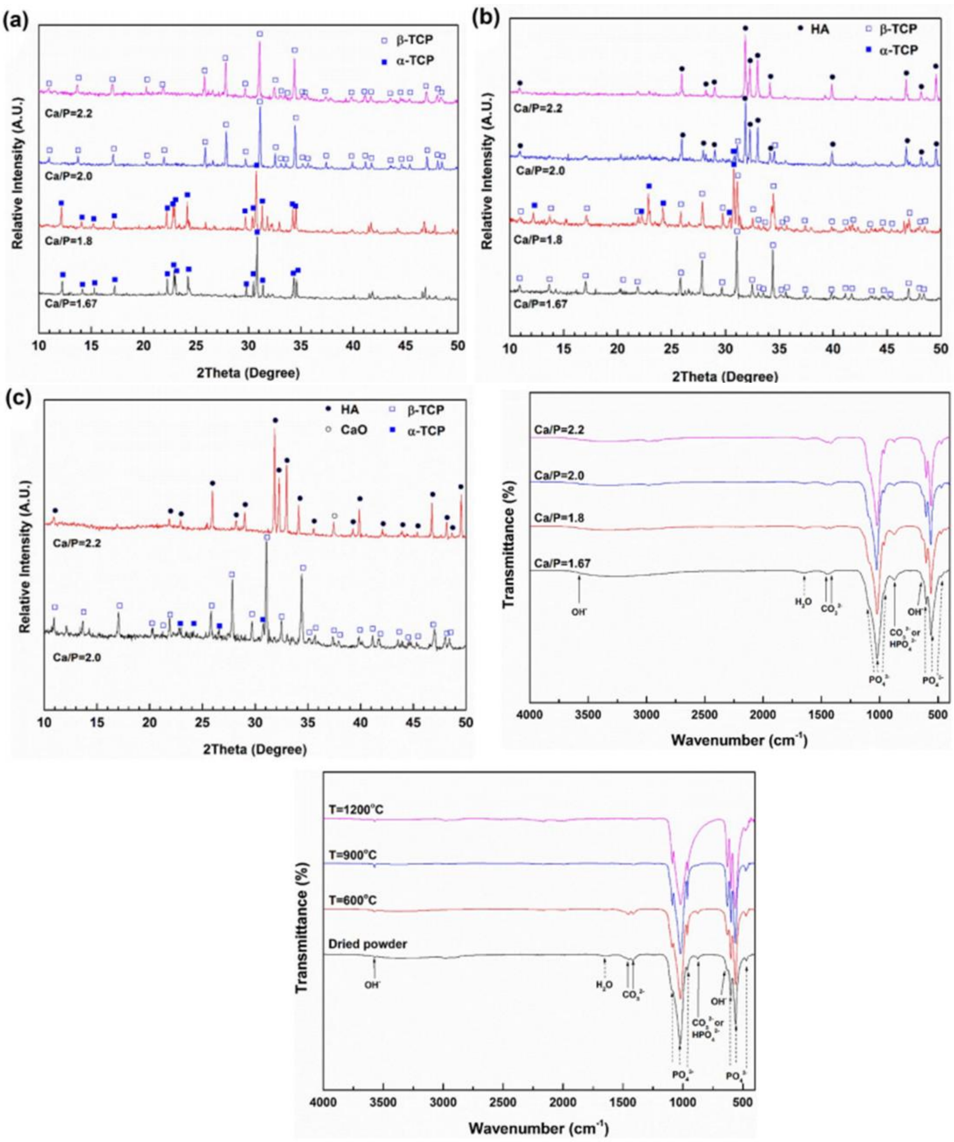


Figure 3.4: The result reported by (Lee et al., 2020)

Moreover, (Wu et al., 2015) said that the development of the HA phase is certainly beginning when the milled sample is sintered at 900 °C for 1 hour, based on XRD pattern. After that, sintering at 1200 °C for 1 hour produces extremely crystalline phase-pure HA (97.4%) and the β-TCP faded away which is progressively replaced by HA. This is due by through high temperature diffusion, the unreacted precursors are being integrated into the HA and β -TCP lattice. With an increment in heating temperature from 900 to 1200 °C for 1 hour, the average particle sizes progressively rise from 1.62 to 2.54 mm and their average crystallite size increased from 31.35 to 59.97 nm. The carbonate peaks in the specimen's FTIR spectra are almost as similar to A- and B-type carbonate HA.

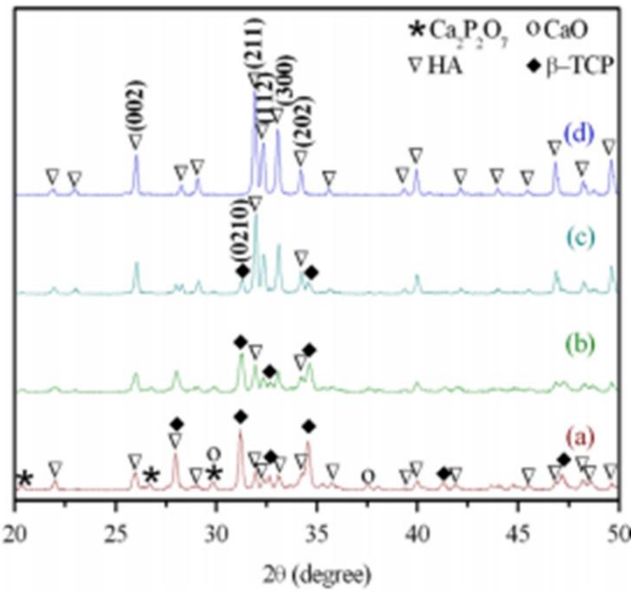
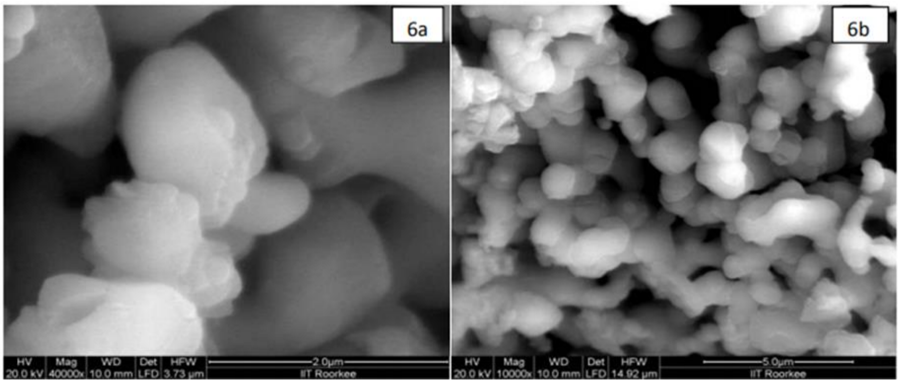
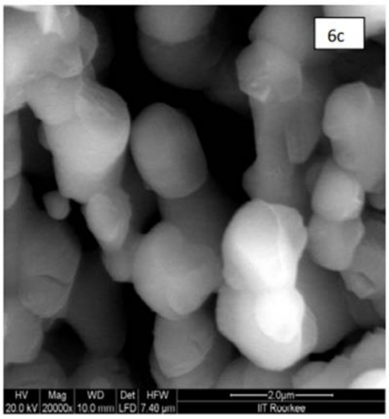


Figure 3.5: The result reported by (Wu et al., 2015)

(Hui et al., 2010), this researcher mention that thermal method only need a low temperature treatment which is 900 °C in comparison to the conventional technique, which uses a 1050 °C treatment temperature. From SEM view, the fiber diameters ranged from 10 nm–1 μm with a flexible orientation. The eggshell regarded as a high porosity due to the dissimilarity on size of fiber and its hierarchical orientation. (Hui et al., 2010) reported that the agglomerates can be found in the synthesized of HAp as in Figure 3.6. Fine particles formed up in size of around 400 nm, the forms were nearly same and the size was usually between 3µm and 5µm. The agglomerates were random in size, such as oval and spherical shapes. The main sizes were 3 to 5µm but the agglomerates forms as big as 10µm. It also can prove by the FTIR spectrum of synthesized HA. The nano-sized particles are typically spherical as revealed by FT-IR and XRD analysis. According to the study reported by (Hui et al., 2010), the record of diffraction pattern in the 2θ range of 10-80 with a 2° per min of scanning step. From the thermal processing of calcium oxide (CaO) in the phosphate solution (H_3PO_4) during high temperature, it produce the white solid material with high mechanical strength and irregular diameter porous structure. Around 1047.9, 604.1 and 566.7 cm^{-1}, it found that the asymmetric stretching (v3) and bending modes (v4) of PO_4^{-3} ion. While the symmetrical stretching for PO_4^{-3} ion is detected at approximately 961.4 and 470.4 cm^{-1}. For the OH^-, around 3571 and 1637cm, the liberation and stretching modes were found. Due to CO_3^{2-}, the stretching vibrations were also identified at about 1425.1 and 876 cm^{-1}. Adsorbed H2O is shown by bands at 3413.3 and 1637.5 cm^{-1}.





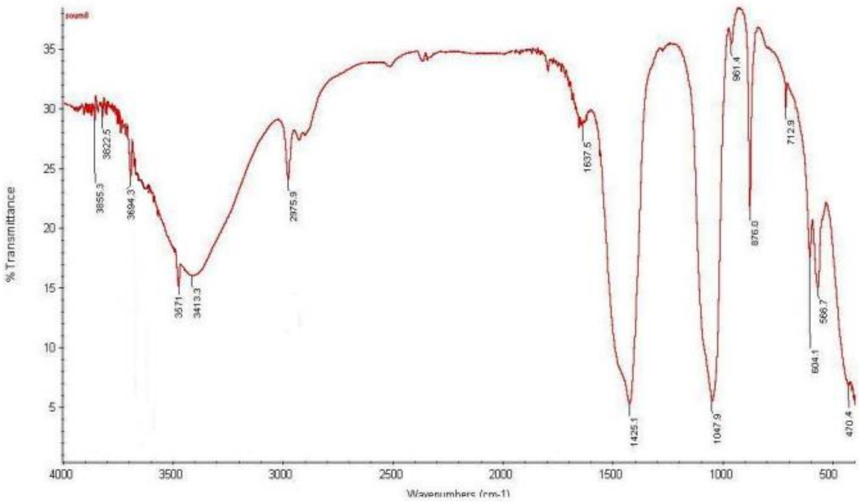
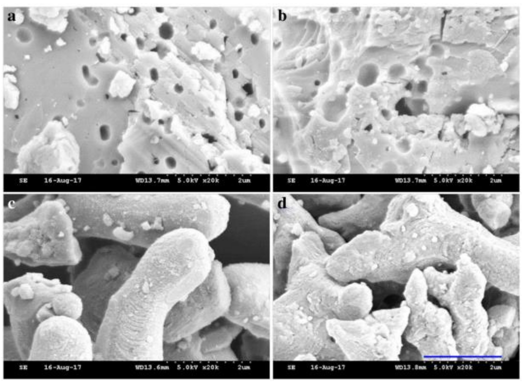
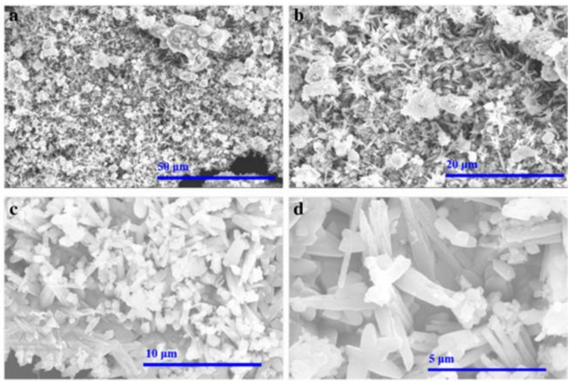
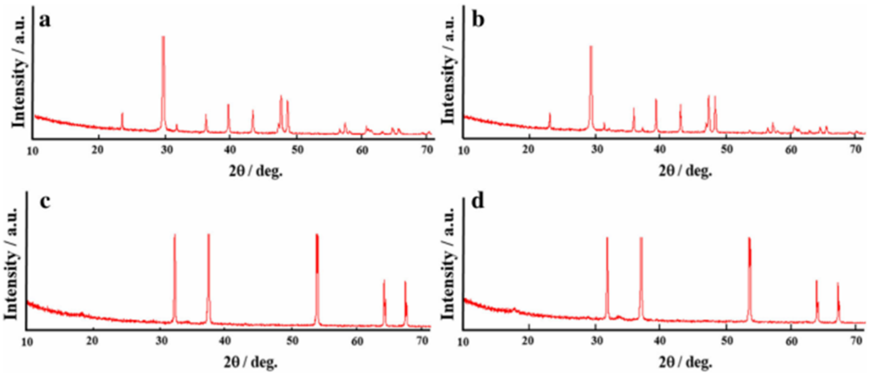


Figure 3.6: The result reported by (Hui et al., 2010)

In the research by (Patel et al., 2019), the calcination temperature used are 600, 800, 1000 and 1200 °C. At lower temperature which are 600 and 800 °C, the peak existence at 2θ of 24° and at high temperature, the peak existence at 2θ of 32°. This shows that degradation of calcium carbonate into crystalline calcium oxide is not feasible with heat treatment (Patel et al., 2019). The synthesized HA showed a needle-like shape, indicating that it is crystalline. The carbon and oxygen concentrations were lower in samples treated at high temperatures (1000 and 1200 °C) than in samples treated at low temperatures (600 and 800 °C). Furthermore, the calcium content of the samples that had been heated to a high temperature was increased significantly.







Based on these result, I am suggested the best result reported by (Le et al., 2012) because it can detect hydroxyapatite nanoplates which are similar to the aragonite crystals found in seashell nacre. It can prove a new nanomaterial for fabrication of artificial nacre that has the potential to enhance the strength of biomaterials used in bone implants.

4. Conclusion

Based on the analysis, it can conclude that hydroxyapatite (HA) extracted from the wasted eggshells was successful by combining the methods of precipitation and calcination. The study indicates that wasted eggshell suitable to be used for application as a coating material on the metal implant due to feasible and inexpensive grease material. The study of effect of pH and calcination temperature of hydroxyapatite (HA) to determine the compatibility the extraction hydroxyapatite (HA) from the wasted eggshells or application as a coating material on the metal implant. Based on the results of the analysis, it can be determined that the study's objectives can be achieved.

The HA powder is strongly influenced by the solution conditions such as pH and temperature. For the effect of pH during precipitation process, the best pH is 11 from the finding of review because it has been prove that hydroxyapatite nanoplates are successfully formed. While, for the effects of calcination temperature, many researchers use a calcine temperature range from 700 until 1200 °C. The crystallinity, microstructure and chemical bonding differed according to the samples at different temperatures of calcination. In my opinion, the best calcine temperature is 1200 °C. Due to the increasing of calcined temperature such as 1200 °C, the purer and more crystallized the synthesised of HA. According to the finding result and various characterizations conducted, the presence of hydroxyapatite in the prepared samples can be determined. The current study shown that bio-waste eggshell may be used to make high-purity HA.

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