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Effect of pH condition during hydrothermal synthesis on the properties of hydroxyapatite from eggshell waste

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ABSTRACT

Hydroxyapatite (HA) powders were synthesized using eggshell waste through hydrothermal method to develop bioceramics materials for medical applications. The effects of the pH conditions during the synthesis on the phase behaviour, crystallite size, crystallinity and morphology of as-synthesized ceramic powders were evaluated. The XRD patterns showed that HA was the only main phase present in the as-synthesized powder after being calcined at 400°C. However, EDX measurement detected the presence of Mg as trace element which originated from the eggshell as starting materials. The crystallite size and crystallinity of the HA powders were increased when the powders were synthesized in acidic condition as compared to basic condition. FESEM images showed that HA powder with nano-sized rods and spherical morphologies were obtained from the powders that were synthesized at pH 5, while the powder particles synthesized at the basic condition at pH 9 produced elongated rod-shape particles. The PSD results showed that the synthesized HA was in agglomerated form which was also confirmed by FESEM images.

Keywords: Hydroxyapatite; eggshell; hydrothermal; calcination.

INTRODUCTION

Calcium phosphate bioceramic materials have been widely used in biomedical and dental applications due to outstanding biocompatibility, bioactivity and osteoconduction characteristics [1-3]. Among the various phases of calcium phosphate, the usage of hydroxyapatite [$Ca_{10}(PO_4)6(OH)_2$, HA] and bheta-tricalcium phosphate [$Ca_3(PO_4)_2$, β-TCP] have increased in clinical field due to their similar chemical composition and crystallographic structure to natural bone. These materials may be employed in various forms such as porous blocks, dense body, granular forms and hybrid composites to fill bone defects or voids [3-8].

HA is typically produced from chemical reagents such as precipitation, microwave irradiation, sol-gel, hydrothermal process, ultrasound irradiation, electrodeposition and spray pyrolysis [2, 9-10]. However, chemical synthesis is costly, has complex synthesis method, time consuming and impurity incorporation [11]. Therefore, naturally-derived HA was studied.

Recent studies have reported the production of HA from corals, cockles, eggshells, cuttlefish bone, fish bone, fish scale and bovine cortical bone [1-2, 12-16]. Biological HA obtained from these sources showed that these products are considered as natural calcium resources which contain high amount of calcium as carbonate and oxide [2, 12, 17]. Conversion of HA from these wastes not only considered as economical but also environmental friendly. Therefore, it is highly imperative to find substitute materials that are easily accessible, renewable and cost effective.

Among these natural calcium resources, eggs which are common household items are produced daily in large quantity and used extensively in food industry. Eggs have become the preferred protein source due to its availability and affordability. Being one of the top 10 food items consumed daily by Malaysians, eggs is extensively used as the main ingredient in most foods, including the dishes eaten with rice, bread, cakes, ice cream and biscuits. Usually, the eggshells are disposed to the landfills after utilizations of egg contents and its derivative. The eggshells result in microbial growth and contribute to environmental pollution. Therefore, effective utilization of the eggshell wastes is essential and must be managed to conserve a green environment. One of the reusable prospective of eggshell waste that is essentially being engrossed recently is as the calcium source to synthesize HA. Due to high demands of HA in medical and dental applications, eggshell waste can be recycled and converted into useful biomaterials as it contains high amount of calcium carbonate ($CaCO_3$) which can be used as a calcium precursor in the synthesis of HA [14]. Moreover, according to Ramesh et al. [18], eggshell contains several trace elements such as sodium, magnesium, silicon, and strontium which makes the material biocompatible to human bone when it is used as implant material.

This paper reports the utilization of eggshell waste which mainly made up of calcium carbonate for the preparation of HA powder for biomaterial applications. The effect of pH conditions of the starting solution was studied during the hydrothermal synthesis to produce HA powder. Then, the properties of the as-synthesized HA were investigated.

METHODS AND MATERIALS

Hydroxyapatite powders preparation derived from eggshell waste at different pH condition

The collected eggshell wastes were washed with distilled water to remove dirt and immersed in hot water to remove the inner membrane. Then, the clean eggshells were dried and ground into powder by mortar and pestle. The eggshell powder was calcined at 1000°C to convert calcium carbonate ($CaCO_3$) to calcium oxide (CaO) by removing the organic constituents. The resulting CaO powder was ball milled and sieved prior to being used as the calcium (Ca) precursor to prepare HA powder by hydrothermal synthesis. Thereafter, the CaO slurry was prepared by dispersion in distilled water and stirred with magnetic stirrer. Then, di-ammonium hydrogen phosphate ($(NH_4)_2HPO_4$, R&M Chemicals) slurry which prepared by dissolving the phosphate precursor in distilled water was stirred before being added drop-wise into the CaO slurry. The temperature of the mixed solution was maintained at ~90°C until the white paste was obtained. During the synthesis, the pH of the solution was adjusted to pH 5 and pH 9 to produce acidic solution and alkaline solution by adding acetic acid (CH_3COOH, Merck) and ammonia solution (NH_4OH, Fisher Scientific), respectively.

The obtained white paste was then dried overnight and crushed prior to calcination at 400°C. Figure 1 shows the experimental procedure to synthesis HA from eggshell waste at different pH conditions.

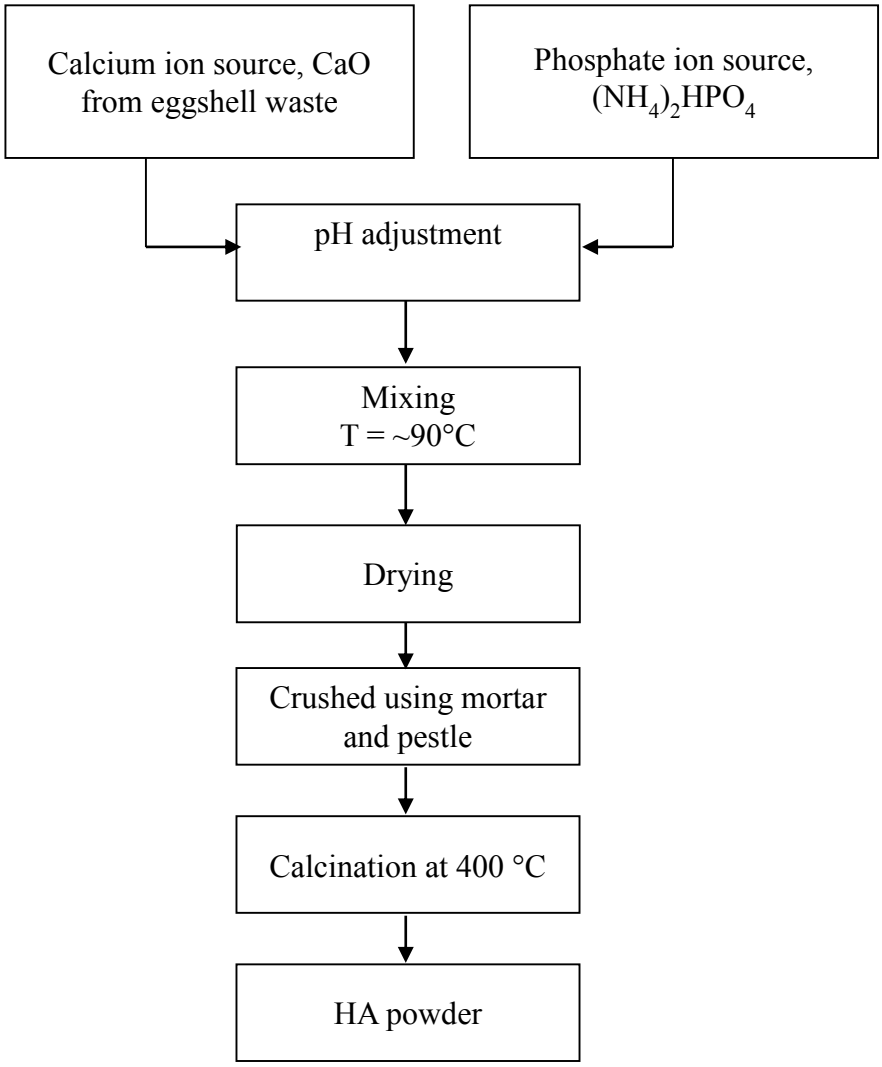

Figure 1. Synthesis of HA derived from eggshell waste at different pH condition.

Sample characterization

The thermal analysis of the crushed eggshell waste was studied by using a TG/DTA instrument (Diamond, Perkin Elmer). Differential scanning calorimetry (DSC) analysis was conducted concurrently with this analysis. The crystallinity and phase identification was analyzed using XRD (X-Ray Diffractometer System X'Pert Pro, PANalytical). Field Emission Scanning Electron Microscope– Energy Dispersive X-Ray (FESEM, JSM-6701F, Jeol) was used to observe the morphology of the as-synthesized HA powder. The peaks of the XRD diffraction pattern were used to estimate the crystallite size (*D*) using the Scherer's formula (Eqn. (1)) [1]:

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (1)$$

where D obtained is in nanometer, λ is the wavelength of the incident radiation, K is the Scherer's constant which equals to 0.94, θ is the diffraction angle and B is the full width at half maximum (FWHM) of X-ray reflection in radians. The crystallinity (X_c) can be evaluated by the following relation (Equation 2)[9]:

$$X_c = 1 - \frac{V_{112/300}}{I_{300}} \quad (2)$$

$V_{112/300}$, which represents the intensity of the valley between peaks (112) and (300), is divided with intensity of peak (300) [20].

RESULTS AND DISCUSSION

Figure 2 shows the TG/ DTA curve of eggshell which composed mainly of $CaCO_3$. The weight loss observed as the temperature was approaching 100°C was due to evaporation of physically absorbed water. A further weight loss of 4% occurred over the range of temperature from ~230°C to ~340°C due to degradation of organic substance. A gradual drop of the TGA at temperature between ~340°C - 550°C was corresponded to loss of dehydrated water in the lattice [18]. Moreover, a sharp drop of mass which contribute to the major weight loss was observed at temperature between ~630°C to ~800°C with an endothermic peak at 783°C which indicates the decomposition of $CaCO_3$ [17]. This results also in good agreement with a study conducted by Singh and Mehta [19]. Moreover, since TG/ DTA curve shows that the weight loss was only stabilized after at 807°C, the calcination temperature to completely transform the $CaCO_3$ in eggshell to CaO was set at 1000°C.

Figure 3 shows the TGA/ DSC curve of HA synthesized using solution with pH 5 and pH 9 during the hydrothermal synthesis. The result illustrates that at ~50 °C to 200°C for both pH conditions was attributed to loss of absorbed water. During this stage, TGA curve for HA powder synthesized from the solution of pH 9 experienced higher weight lost which was approximately at 11% when compared to HA powder that was synthesized in acidic condition, which was about 6.95%. The higher weight loss was supported by the presence of clearer endothermic peak at around 200°C. At the temperature of ~200°C to 400°C, crystallization of water has contributed to further weight loss of 1.4% and 2.53% of the as-prepared HA powder synthesized using a solution of pH 5 and pH 9, respectively. Furthermore, a significant mass transition at 700 - 750°C shows that the mass lost was attributed to the crystallization process [20]. The TGA curve depicts an abrupt change has occurred in HA powders that was synthesized in acidic condition with pH 5. This result explains why this type of HA powder having a high degree of crystallinity which was revealed in the XRD pattern. A study conducted by Wang et al. [20] also reported the decomposition of HA to β-TCP phase has occurred at this temperature. Moreover, this study found that the pH condition during the synthesis of HA using eggshell waste as the starting precursor influence the mass loss of the as-synthesized HA. Furthermore, HA powder that was synthesized using the solution with low pH did not exhibit significant mass loss as compared to HA powder that was synthesized in the starting solution with higher pH condition.

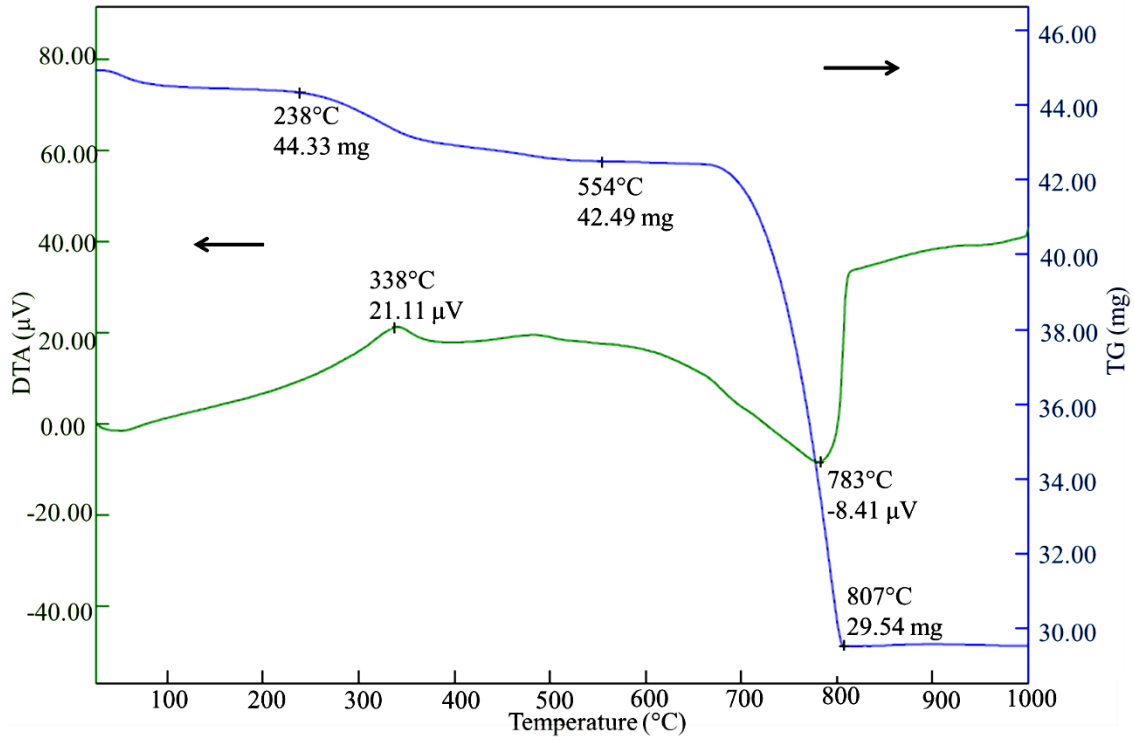

Figure 2. TG/ DTA analysis of eggshell powder.

The XRD patterns of the as-synthesized HA calcined at 400°C at both pH conditions are shown in Figure 4. The XRD pattern is in good agreement with JCPDF Card Number 09-432 for HA which indicates the presence of single phase HA. As observed from the XRD patterns, there is no apparent difference was observed from the XRD pattern of as-synthesized HA at different pH conditions. The results obtained in this study has also confirmed the absence of CaO originated from the thermally treated eggshell at 1000°C in the produced HA for both conditions. The crystallite size values from both samples were 78.53 nm and 58.92 nm for HA powder synthesized from the solution of pH 5 and pH 9, respectively obtained from the highest peak (211). Similar value of crystallite size was also obtained in the study reported by Adeogun et al. [1] which also used eggshell as the raw material to produce HA. Moreover, this result showed that eggshell-derived HA synthesized at pH 5 or at acidic conditions produced bigger crystallite sizes.

These results illustrated higher crystallinity for samples prepared at a more acidic condition [21]. The same peak which was used to calculate the crystallite size was also used to calculate the crystallinity (Xc), where 66.9 % and 60.8 % were obtained for samples synthesized from solutions of pH 5 and pH 9, respectively. These results illustrated higher crystallinity for samples prepared at a more acidic condition. However, the crystallinity of these powders was considered lower when compared to previous study conducted by Wu et al. [9] due to the lower heat treatment temperature during the calcination used in this study. Table 1 summarizes the crystallite size and degree of crystallinity of the HA derived from eggshell waste which was synthesized at different pH conditions.

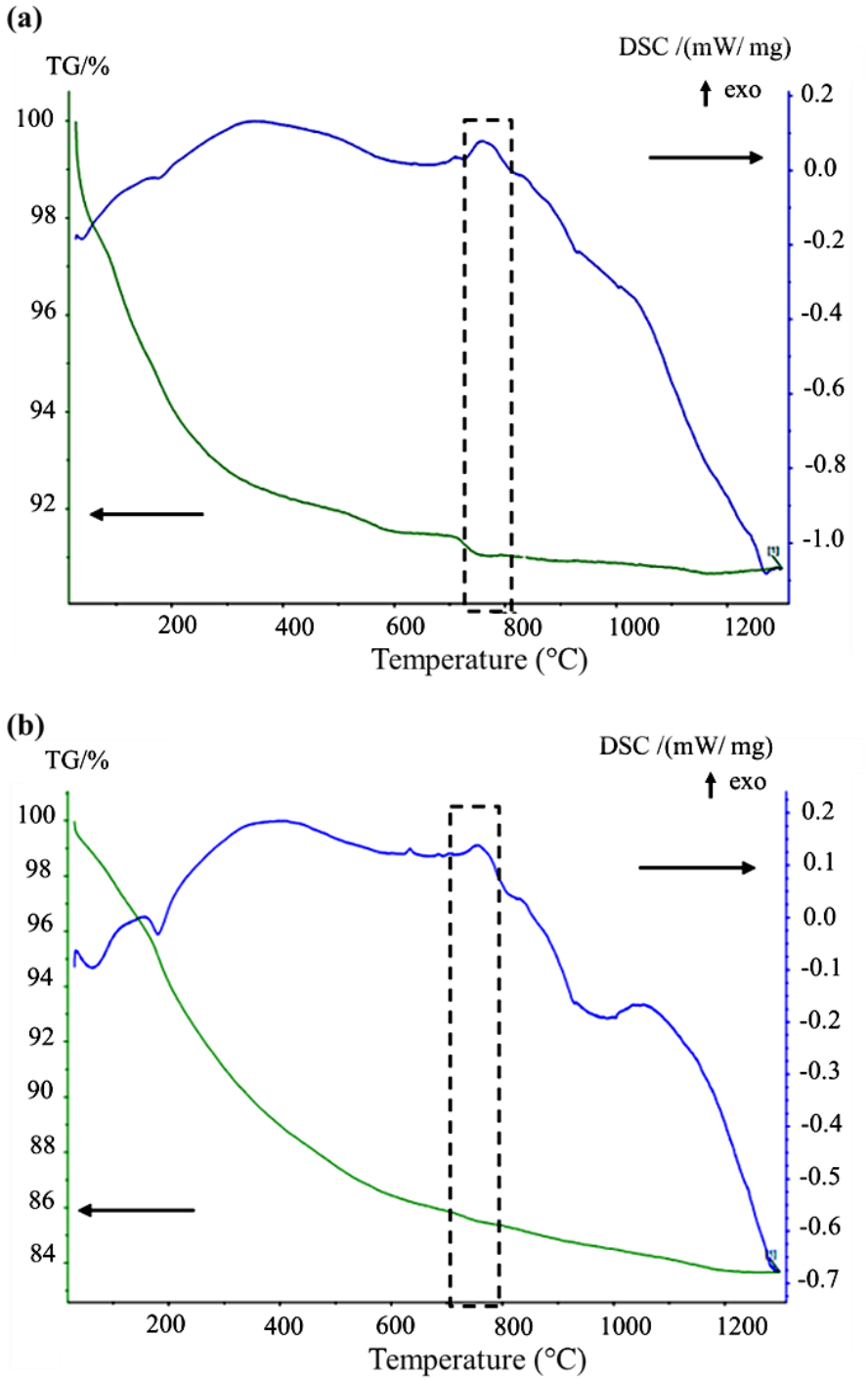

Figure 3. TGA/ DSC Curve of Eggshell-derived HA ad different pH condition during the synthesis; (a) pH 5 and (b) pH 9.

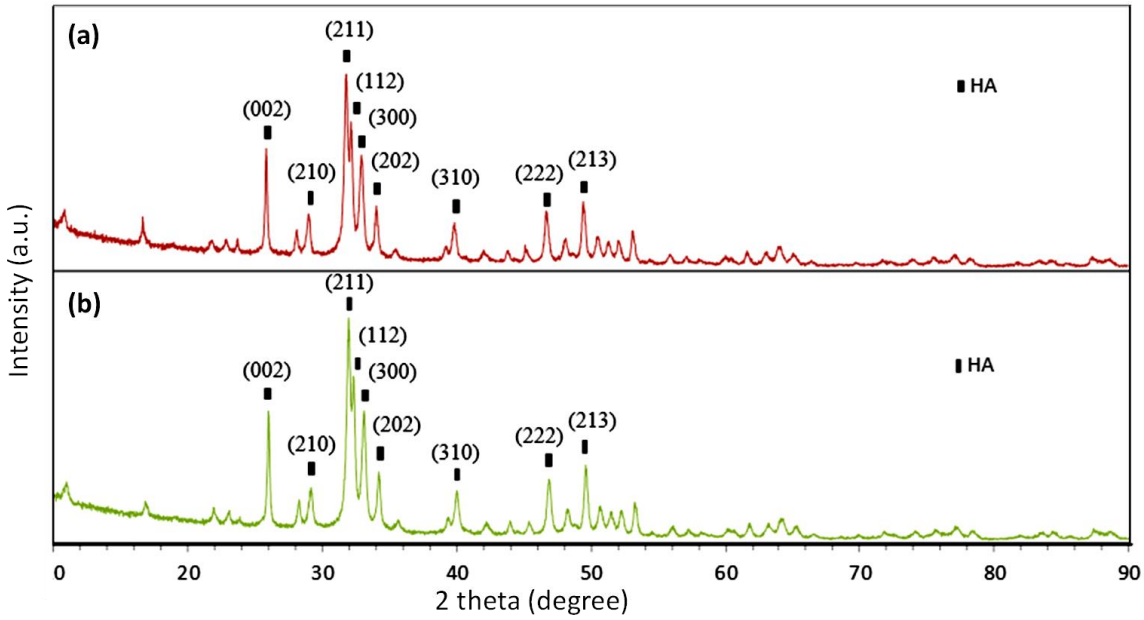

Figure 4. XRD of eggshell-derived HA at different pH condition during the synthesis; (a) pH 5 and (b) pH 9.

Table 1 Crystallite Sizes and Degree of Crystallinity of HA Powders

Sample	Crystallite Size, Xs (nm)	Degree of crystallinity, Xc (%)
HA-pH 5	78.53	66.9
HA-pH 9	58.92	60.8

Figure 5 shows the FESEM image of CaP powder samples synthesized at different pH conditions. It was found that the as-synthesized HA powder synthesized using starting solution of pH 5 exhibited a more spherical and rod-like morphology as shown in Figure 5 (a). Hui et al. [22] and Zhou and Lee [21] also found that the acidic conditions during the hydrothermal synthesis of HA powder produced spherical-shaped particles. According to Le et al.[23], increase in pH of the raw materials leads to the increase of the anisotropic growth, which also increases the crystal aspect ratio resulting in a higher nucleation rate for HA crystals in alkaline conditions. The corresponding morphology would be the elongation of the particles. This argument is in good agreement with the results obtained in this study as revealed by the FESEM image (Figure 5 (b)). The FESEM micrograph of the HA derived from eggshell waste synthesized at pH 9 exhibited elongated rod-shaped particles as shown in Figure 5 (b). The formation of rod-like morphologies are reported to be commonly used in the human hard tissue [14]. Moreover, both HA powders showed particles in nanometer scale when observed at high magnification using the FESEM as shown in Figure 5 (c) and (d).

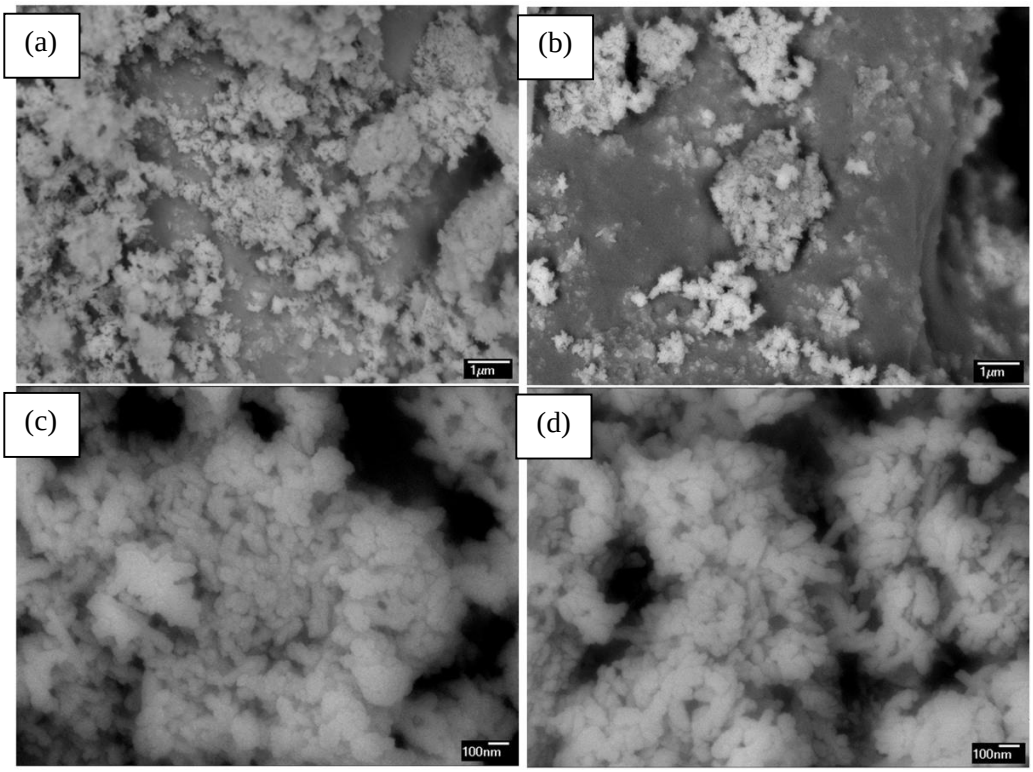

Figure 5. FESEM image of HA powders synthesized from eggshell waste at different pH at low and high magnification, respectively: (a &c) HA-pH 5 and (b & d) HA-pH 9.

Elemental analysis was conducted to define the elemental composition of the eggshell waste and the HA synthesized in acidic and basic conditions. According to the results obtained, both powders are mainly composed of calcium, phosphorus and oxygen as illustrated in Figure 6. The EDX analysis has also confirmed the presence of Mg as the minor elements in the as-synthesized HA which was originated from the starting materials; eggshell waste [1-2, 14]. The ratio of the calcium and phosphorus (Ca/P) was estimated to be 1.55 and 1.48 for HA that were synthesized from the solution of pH 5 and pH 9, respectively. Due to the presence of the trace element in the as-prepared HA, the HA derived from the eggshell waste are considered as a non- stoichiometric HA. Human bone itself is considered as non-stoichiometric HA due to the presence of trace elements such as Mg, Sr and Na in its HA lattice [12, 14]. Therefore, the presence of Mg in the as-synthesized HA in this study for both pH conditions indicated that the HA powders were comparable with composition to human bone. Thus, it will minimize the risk of rejection of the artificial bone from these as-synthesized HA materials after the implantation in the human body. Moreover, the presence of trace elements such as Mg^{2+} can reduce the percentage of bone loss and fragility and may induce bone regeneration [9]. Besides that, the result found in this study is in agreement with the finding reported by Goloshchapov et al. [24]. The study reveals that when the Ca/P molar ratio was decreased, the degree of crystallinity of the HA powder will also decrease. This is shown in the XRD results which was confirmed by the calculation of the degree of crystallinity.

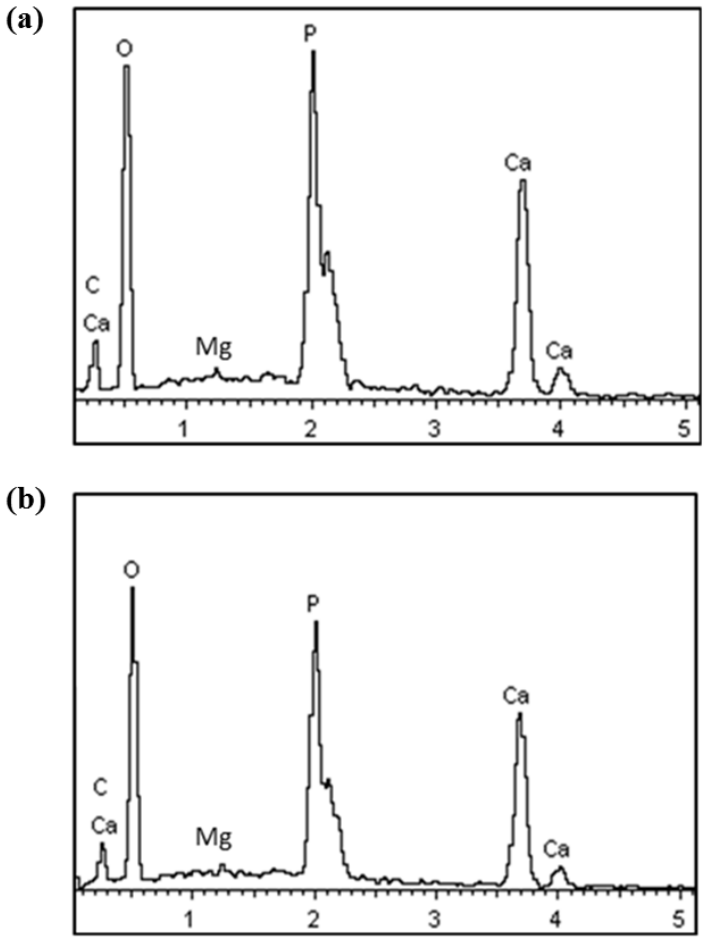

Figure 6. EDX of eggshell-derived HA at different pH condition during the synthesis; (a) pH 5 and (b) pH 9.

Figure 7 shows the particle size distribution (PSD) of each HA powder which were synthesized at different pH. The size distribution of HA powder obtained from the solution of pH 5 and pH 9 is summarized in Table 2. The results reveal that the PSD measurement of the synthesized HA powders was in agglomerated form which were confirmed and proven by the FESEM results. Moreover, there is no significant difference in the PSD of the particles for both pH conditions. The finding suggested that the as-synthesized HA powder at these conditions are widely distributed indicating that smaller particles which have been observed by FESEM images were agglomerated together due to the high surface area of the particles to volume ratio [25]. It is believed that small particle especially in the nanometer range provides better circumstances for good affinity with living cells in the bone metabolism which may also be ideal to be used for coating of biomedical implants [25].

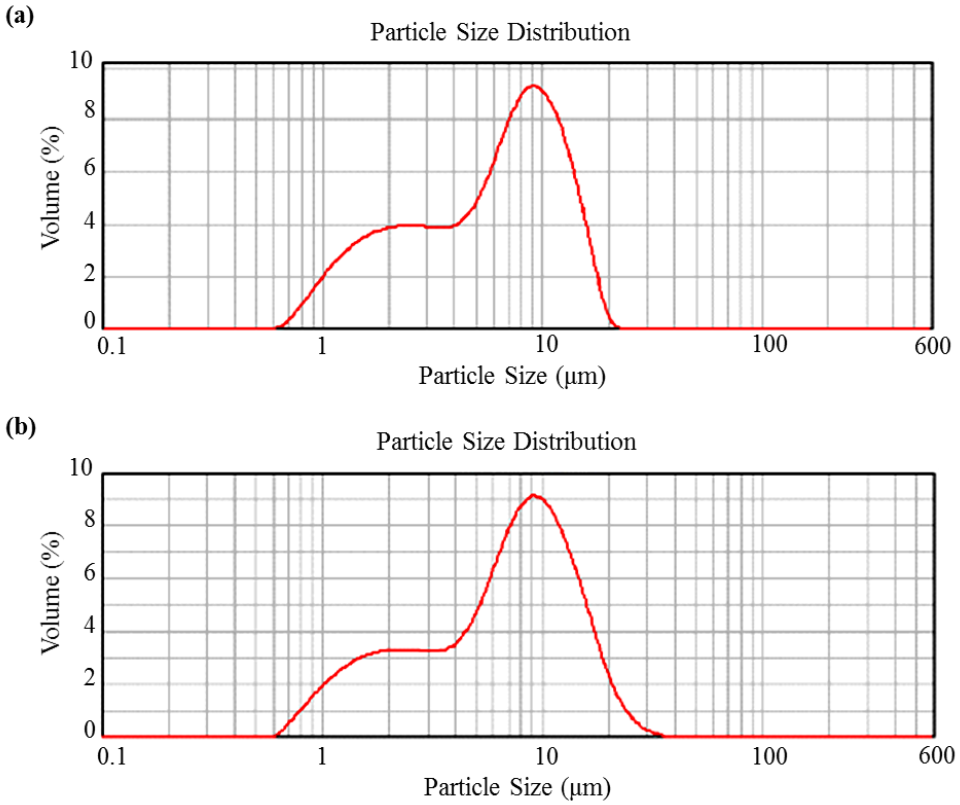

Figure 7. Particle size distribution of HA powders synthesized at different pH conditions; (a) pH-5 and (b) pH-9.

Table 2 Particle Size Distribution of HA Powders.

Samples of HA	d_{10}	d_{50}	d_{90}
HA-pH 5	1.478	6.860	15.070
HA-pH 9	1.552	7.125	15.103

CONCLUSIONS

In conclusion, pure HA phase was successfully synthesized by utilizing the eggshell waste as the Ca precursor using the hydrothermal method. Crystallite size, crystallinity, morphology and temperature of crystallization were affected by the pH condition of the starting precursor. The crystallite size and crystallinity of the HA powders were increased with decreased of pH in the starting solution. The HA powders that were synthesized in acidic condition of pH 5 resulted in HA powder with spherical and rod-like shaped particles, while the powders synthesized using starting solution of pH 9 resulted rod-shaped particles. EDX analysis confirmed the existence of Mg as the trace element which contributes to the formation of non-stoichiometric HA which is also found in the human bone.

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