Synthesis and characterization of hydroxyapatite powder derived of eggshell by precipitation method

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Abstract

Hydroxyapatite is the inorganic material with formula $Ca_{10}(PO_4)_6(OH)_2$. It is one of bioceramic was used for one repairs, fixing defects of filing voids in biomedical fields. The use of chicken eggshell is one of the natural sources to obtain the calcium phosphate compounds. The main objective of this study is to synthesize the hydroxyapatite by precipitation method from eggshell. The raw eggshell was calcined at 850°C for 2 hours following by grinding for 16 hours. The HA powder was synthesized by wet chemical method, using eggshells and phosphoric acid (H_3PO_4). X-ray diffraction spectroscopy (XRD, Scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), Fourier-transform infrared spectroscopy (FTIR) were used to characterize the morphology, composition and distribution of the particles. The Thermos gravimetric analysis (TGA-DTA) was also carried out to evaluate the stability of the synthesized HA powder. The particle-size distribution (PSD) of the powder was determined by the laser scattering particle size distribution analyzer. The results showed that the sintered at 1000°C of HA powder resembles the feature of pure and single apatite phase having favorable Ca/P ratio.

Keywords
Hydroxyapatite, Bioceramics, Synthesis, Eggshell, Precipitation.

1. Introduction

Calcium phosphate biomaterials are widely used in the medical field due to their resemblance to bone matrix (Jordan et al. 1998). To become an interesting bone substitute, calcium phosphates must have certain biological properties, themselves closely dependent on their physicochemical characteristics (Orlovskii et al. 2002).

Hydroxyapatite (HAp) is one of the most common forms of calcium phosphate with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ with a theoretical mass composition of 39.68% Ca, 18.45% P , and a Ca/P molar ratio equal to 1.667. This HA Ca/P ratio reveals excellent biocompatibility (Itoh et al. 2001, Smolen et al. 2013) and bioactivity (Noh et al. 2010, Fathi et al. 2008). It is used for filling lesions in bone tissue or as a covering. Many efforts have been made to produce good quality HAp with adequate biocompatible properties, while seeking economical and non-polluting methods (Huang et al. 2011, Sadat-shojai et al. 2013). It is characterized by its biocompatibility, non-toxicity and ose-integration (MohdPu'ad et al. 2011). This apatite can be produced by chemical or synthetic method or from natural waste.

Hydroxyapatite (HAp) can be synthesized using a variety of techniques, classified into: dry, wet and high temperature methods. The most usually used methods to obtain HAp are chemical precipitation (Dhang et al. 2014, Stipniece et al. 2014, Kimet al. 2010), biogenic sources (Huang et al. 2011, Kamalanathan et al. 2014); among others. Each of these methods results in different sizes and morphologies, as well as different crystal phases of calcium phosphate in addition to pure crystalline PHA. Therefore, these characteristics influence the bioactivity, mechanical and biological properties of PHA (Cahyaningrum et al 2018). Many apatite synthesis methods are proposed in the literature. Among them, two main categories stand out: synthesis by the dry route (Arcoset et al. 2004) and the wet route (Raynaud et al. 2002, MohdPu'ad et al. 2020). Knowing that the properties determine the application, it is interesting to develop synthetic

methods for the control of the morphology, crystallinity size and chemical composition of hydroxyapatite (PHA) (Sadat-shojai et al. 2013). It is therefore essential to be able to prepare hydroxyapatite with well-controlled properties. It is in this context that the work presented in this dissertation fits.

The objective of this research work is to prepare hydroxyapatite with the precipitation method using a natural source eggshell as a calcium precursor.

2. Methods

2.1 Samples synthesis by precipitation method

Calcium phosphate was synthesized at room temperature by precipitation method reported by Marlaet al (Marla et al. 2019). The hen Eggshell pretreatment was consisted to the washing with water an dimmersed in boiling water for a few minutes to remove the surface contaminants. To eliminate the organic materials and to transform the $CaCO_3$ to Cao a heat treatment was used at 850°C for 2 hours. The grinding is carried out with planetary grinding machine for 8 hours. The calcium phosphate was prepared with 31 g of CaO, dissolved in 500 ml distilled water at 60°C for 30 minutes under stirring. Later, the acid solution (H_3PO_4) at 0.6 M was mixed with the hydrolysis of CaO in a rate of 3.33 ml/min. The mixture is stirred at 80°C and the pH was adjusted with HCl to 9.5. The precipitate was washed by water, filtered and dried at 200°C for 4h. It is important to note that the suspension was aged for 2 h at 80°C (Figure1).

Figure 1. Samples preparation by precipitation method

2.2 Samples characterization

Several techniques have been utilized for the preparation of hydroxyapatite (HA). In order to determine the weight loss, approximately 10 mg of powders were characterized by thermal analysis from ambient temperature up to 1200º C at heating rate of 10ºC / min using SDT Q 600 DSC/TGA equipment. The particle size distribution of the powder was determined with a Partica LA960 type laser particle size analyzer in the wet process.The X-ray diffraction (XRD) analysis was carried out with a RIGAKU Ultima IV diffractometer using CuKα radiation from 10 ° to 90 ° in order to determine the chemical structure and phase purity of the powder. Fourier-transform infrared spectroscopy (FTIR) spectral analysis of powder was obtained from a thermo scientific model Nicolet iS10 FT-IR spectrometer equipped with smart ATR accessory without preparation. The IR spectrum range of powder is 4000 - 600 cm^{-1} with 32 scans at a resolution of 1 cm^{-1}. The morphology and qualitative chemistry of the powder were analyzed on a scanning electron microscope (SEM/EDS) type QUANTA 250.

3. Results and Discussion

3.1 ATD /ATG analysis

In order to assess the calcination temperature, the eggshell was studied by thermogravimetric analysis. Figure 2 shows thermogram of powder samples of waste eggshell. It can be seen that the analysis of weight loss can be sorted into three different regions. From room temperature to 1200°C, the curve exhibited three domains of weight loss (25°C-600°C), (600°C-800°C) and (800°C-1200°C). From room temperature to 600 °C, no weight loss was detected. Then in the range of 600 to 800°C, there is a significant weight loss due to the transformation of calcium carbonate into calcium oxide and the evaporation of carbon dioxide (CO_2). Finally, no weight loss was therefore observed when the temperature exceeds 800°C, assuming a complete transformation. So we selected the calcination temperature that exceeds 800 °C to obtain calcium oxide from the eggshell. Without forgetting to mention that the percentage of weight loss is estimated at more than 50%.

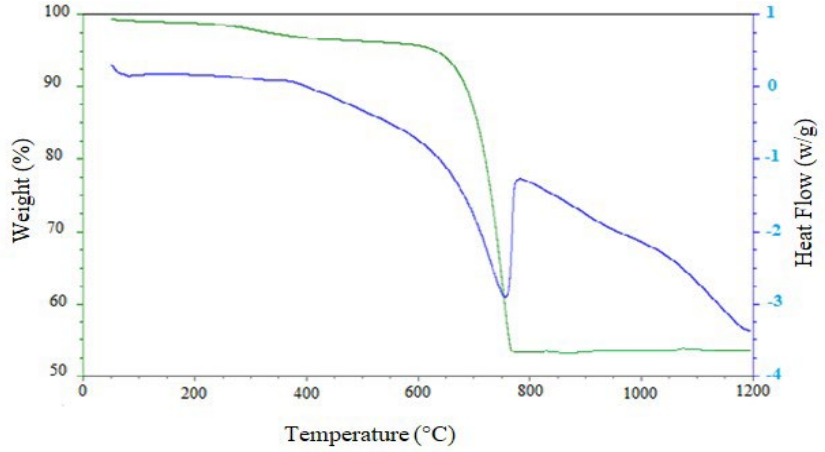

Figure 2. Differential thermal analysis of raw eggshell

3.2. Particle size analysis

The particle size distribution of powder calcined at 850°C for 2 hours obtained after planetary grinder for 16 hours is shown in figure 3.It can be seen that the powder has an average particle size of 8.20327 microns. It should be noted that the largest quantity of particles has an average size of (0.5-1) µm that is to say of the order of a few nanometers. This size is recommended for obtaining a hydroxyapatite with higher physicochemical and microstructural properties (Kweh et al. 1999).

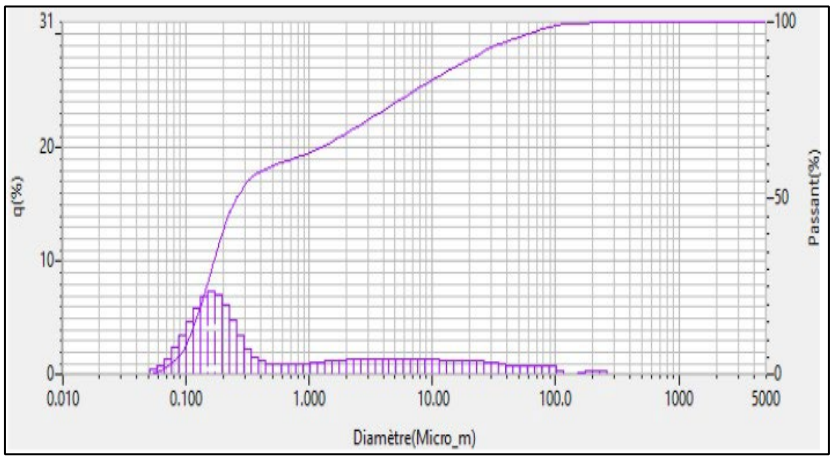

Figure 3. Particles size distribution of the powder

3.3 XRD analysis

The XRD patterns obtained before and after heat treatment of the HA powder synthesized are identified by comparison with the references of the International Center for Diffraction Data - Powder Diffraction Files (ICDDPDF) file (Figure 4). Figure 4a shows the diffraction patterns of the eggshell waste sample. The results indicated the presence of calcite ($CaCO_3$) peaks. This spectrum is composed of a single chemical phase, which crystallizes in the monoclinic system. The results are in good agreement with the literature (Freire et al. 2006, Jenniffer et al. 2018, Tomasell et al. 2014). A change in the crystallographic structure was observed after calcination at 850°C / 2h (Figure 4b). X-ray analysis showed the presence of calcium oxide (CaO) (Kweh et al. 1999). The results of synthesis of the powder by precipitation showed that the obtained spectra are composed of the diagram of powders with hydroxyapatite structure (Figure 4c) (Marla et al. 2019).

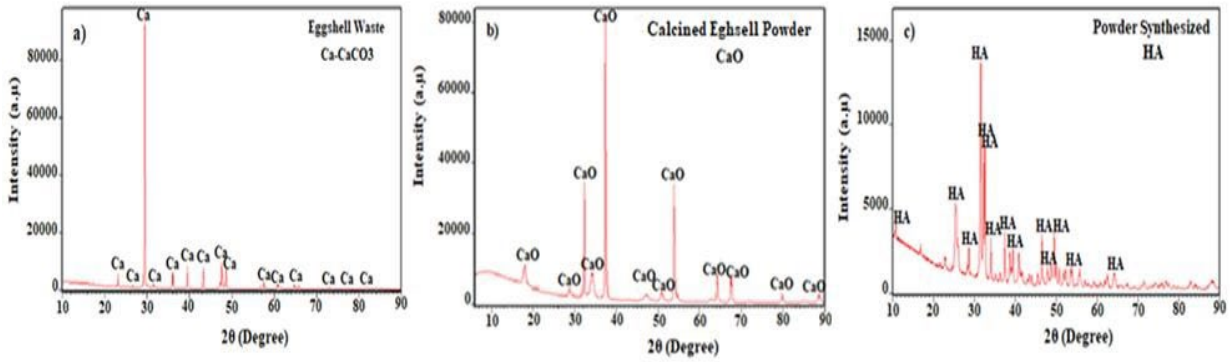

Figure 4. X-ray diffraction pattern for the HA synthesized a) Eggshell waste sample, b) Calcined eggshell powder, c) Powder synthesized

3.3 FTIR analysis

Figure 5 shows the FTIR absorption spectra of the synthesized HA powders before and after heat treatment. It can be seen that infrared absorption spectra of powder indicated the presence of the CO_3^{-2} at 1794 cm^{-1}, the $CaCO_3$ at 715 cm^{-1} and 1396 cm^{-1}(Figure 5a) (Pelin et al. 2019). The calcined eggshell powder at 850°C for 2 hours showed a substitutional group at 1415 cm^{-1} and 886 cm^{-1}corresponding to (C-O) and OH^- groups respectively (Figure 2b). These bands are the resulting of the transformation of $CaCO_3$ to CaO (Rey et al. 2014, Rey et al. 2011). The groups formed after the synthesis of calcium phosphate have been demonstrated (Figure 5c). Indeed, the infrared spectrum (FTIR) of the synthesized powder shows the existence of bands characteristic of apatitic calcium phosphate. Indeed, the bands at 3756.9 cm^{-1} correspond to the group of OH^-ions (Kamalanatha et al. 2014). Then the bands at 580.71cm^{-1} are the PO_4^{3-} phosphate groups. The bands at 2354.3 cm^{-1}feature of carbonates vibrational spectra carbonate CO_3 ions have also been revealed.

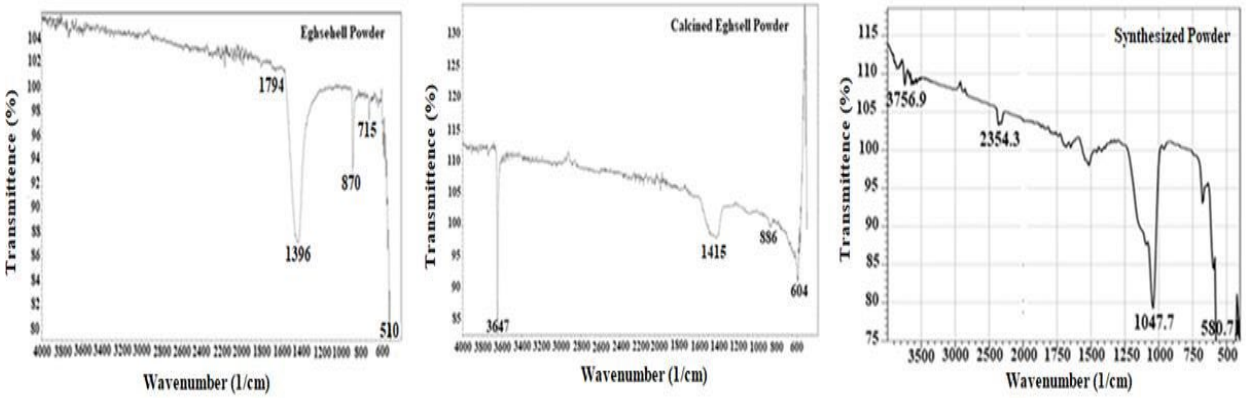

Figure 5. FTIR spectra of the HA synthesize a) Eggshell powder, b) Calcined eggshell powder, c) Powder synthesized

3.4 SEM analysis

The morphology of particle powder prepared by precipitation method is studded by Scanning Electron Microscopy. The observations revealed that the eggshell powder sample presents a heterogeneous size and shape (Figure 6a). After calcination treatment, the powder becomes denser. Therefore, the morphology of the calcined eggshell powder at 850 ° C/2h is in good agreement with the literature (figure 6b) (Rey et al. 2014, Rey et al. (2011)]. Figure 6c shows the SEM micrographs of synthesized powder after sintering at 1000°C / 2h. It can be seen are markable change in structure. Indeed, the particle samples present a spherical form with irregular form characteristic of hydroxyapatite powder (Hamidi et al. 2017).

The EDX analysis of powder is also revealed the reactions during powder synthesis. First, the chemical composition of eggshell before calcination contains a large amount of calcium and oxygen (Figure 6d).The calcium oxide can be attributed to calcium carbonate ($CaCO_3$) principal source of eggshell (Freire et al. 2006). The spectrum also contained phosphorus, magnesium and aluminum. On the other hand, the results of EDX analysis after calcination showed the presence only the calcium and the oxygen (Figure 6e). This confirms that the decarbonation reaction has taken place. Figure 6f shows the presence of calcium, phosphorus and oxygen. As the result, the powder of hydroxyapatite was obtained. It should be noted that the ratio of Ca/P was found as 1.69.

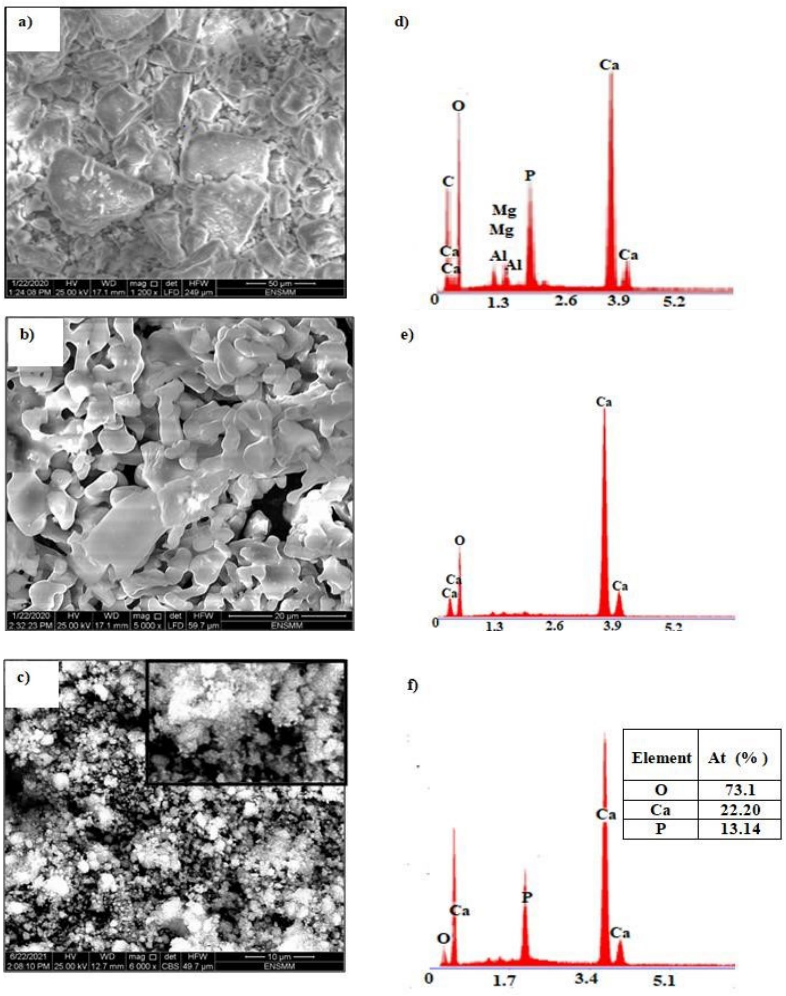

Figure 6. SEM /EDX of the HA synthesize a) MEB Eggshell powder, b) MEB Calcined eggshell powder, c) MEB Powder synthesized, d) EDX Eggshell powder, e) EDX Calcined eggshell powder, f) EDX Powder synthesized

4. Conclusion

In the present study, the synthesis of hydroxyapatite powder by the precipitation method using phosphoric acid from the eggshell has been investigated. The main results were summarized as follows:

- Eggshell was once found to be a suitable calcium source
- Biogenic sources present sources without any type of contamination which allows to obtain HA powderwith high crystallinity and respectful of the environment
- The properties, phase purity and particle size distribution from a natural resource source (eggshell) obtained by different experimental techniques (DRX, FTIR, MEB, ...) confirmed the synthesis of hydroxyapatite
- Hydroxyapatite HA prepared by precipitation method from the eggshell is of interest for biomedical applications.

Acknowledgements

This work is supported by the research of URMA-CRTI.

References

Jordan, D.R., Munro, S.M., Brownstein, S., Gilberg, S.M., Grahovac, S.Z, A synthetic hydroxyapatite implant: the so-called counterfeit implant, *Ophthalmic Plastic and Reconstructive Surgery*, Vol. 14, pp 244-2449, 1998.

Orlovskii, VP., Komlev, VS., Barinov, SM. Hydroxyapatite and hydroxyapatite-based ceramics, *Inorganic Materials*, Vol. 38, pp. 973-984, 2002.

Itoh, S., Kikuchi, M., Takakuda, M., Koyama, Y., Matsumoto, HN., Ichinose, S., Tanaka, J., Kawauci, T., Shinomiya, K., The biocompatibility and osteoconductive activity of a novel hydroxyapatite, collagen composite biomaterial, and its function as a carrier of rhBMP-2, *Journal of Biomedical Materials Research*, Vol. 54, pp.445- 453, 2001.

Smolen, D., Chodoba, T., Iwona, M., Kedzierska, K., Lojkowski, W., Swieszkowski, W., Kurzydlowski, KJ., Mierzynska, MK., Szumiel, ML., Highly biocompatible, nanocrystalline hydroxyapatite synthesized in a solvothermal process driven by high energy density microwave radiation, *International Journal Nanomedecine*, Vol. 8, pp.633-668, 2013.

Noh. KT., Lee. H.Y., Shin. U.S., Kim. H.W., Composite nanofiber of bioactive glass nanofiller incorporated poly(lactic acid) for bone regeneration, *Materials Letters*, Vol. 64, pp.802-805, 2010.

Fathi. M.H., Hanifi. A., Mortazavi. V., Preparation and bioactivity evaluation of bone-like hydroxyapatite nanopowder, *Journal Materials Process Techology*, Vol. 202, pp. 536-542, 2008.

Huang. Y.C., HSIAO P.C., CHAI H.J.,Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells, *Ceramics International*, V. 37, pp. 1825-1831, 2011.

Sadat-shojai. M., KhorasanI, M.T., dinpanah-khoshdargi, E., et al.,Synthesis methods for nanosized hydroxyapatite with diverse structures,*Acta Biomaterialia*, V. 9, pp. 7591-7621, 2013.

MohdPu'ad. N.A.S., P. Koshy, H.Z., Abdullah, M.I. Idris. Lee.T.C., Syntheses of hydroxyapatite from natural sources, *Heliyon*, Vol. 5, pp. 1-13, 2019.

Dhang. V., Rhee K.Y., Park S.-J., The facile and low temperature synthesis of nanophase hydroxyapatite crystals using wet chemistry, *Materials Science and Engineering: C*, Vol. 36, pp. 152-159, 2014.

Stipniece. L., Salma-ancane. K., Borodajenko., N., et al., Characterization of Mg substituted hydroxyapatite synthesized by wet chemical method, *Ceramics International*., V. 40, pp. 3261-3267, 2014.

Kim D.W., CHO I.-S., Kim J.Y., et al., Simple large-scale synthesis of hydroxyapatite nanoparticles: in situ observation of crystallization process, *Langmuir*, V. 26, pp. 384-388, 2010.

Kamalanathan. P., Ramesh S., Mang L.T., et al., Synthesis and sintering of hydroxyapatite derived from eggshells as a calcium precursor, *Ceramics International*, 40, pp. 16349-16359, 2014.

Cahyaningrum, S. E., Herdyastuty, N., Devina, B., Supangat, D., Synthesis and characterization of hydroxyapatite powder by wet precipitation method, *IOP Conference Series: Materials Science and Engineering*, Vol. 299, pp. 1-5, 2018.

Arcos, D., Rodriguez-Carvajal, J., Vallet-Regí, M., Silicon incorporation in hydroxylapatite obtained by controlled crystallization, *Chemistry of materials*, Vol.16, pp. 2300-2308, 2004.

Raynaud, S., Champion, E., Bernache-Assollant, D., Thomas, P., Calcium phosphate apatites with variable Ca/P atomic ratio I. Synthesis, characterisation and thermal stability of powders, *Biomaterial*, Vol.23, pp. 10651072, 2002.

Boanini, E., Gazzano, M., Bigi, A., Ionic substitutions in calcium phosphates synthesized at low temperature, *Acta Biomaterialia*, Vol.6, . pp. 1882-1894, 2010.

MohdMohd Pu'ad. N.A.S., Abdul Haq R.H., Mohd Noh. H, Abdullah. H.Z., Idris. M.I., Lee. T.C., Synthesis Method of hydroxyapatite: A Review, *Materials Today: Proceedings*, Vol.29, pp.233–239, 2020.

Sadat-shojai, M., Khorasani, M.T., dinpanah-khoshdargi, E., et al.,Synthesismethods fornanosized hydroxyapatite with diverse structures, *Acta Biomaterialia*, V. 9, pp. 7591-7621, 2013.

Marla H., Marilza. A., Francisco. M., José. C., Vitor. R., AdilsonQ.,Synthesis and characterization of green nanohydroxyapatite from hen eggshell by precipitation method, *Materials Today: Proceedings*, Vol. 14, pp. 716-721, 2019.

Kweh. S.W., Khor.K..A, Cheang. P., Production and characterization of hydroxyapatite (HA) powders, *Journal Materials Process Techology*, pp. 373-377, 1999.

Freire.M. N., Holanda.J. N. F., Characterization of avian eggshell waste aiming its use in a ceramic wall tile paste, *Cerâmica*, Vol.52, pp. 240-244, 2006.

Jenniffer.S. de O.S, André. G de S.G., Bioceramic composite: hen's eggshell characterization and main applications, *Revist Ifes Ciência*, Vol.4, 2018.

Tomasell.i M.G., Síntese caracterização de hidroxiapatita a partir de casca de ovo de galinha, 2014.

Pelin. Y., Eliföztürk. Er., Sezgin. B.,Kutlu.Ü, Belma. Ö., Application of supercritical gel drying method on fabrication of mechanically improved and biologically safe three-component scaffold composed of graphene oxide/chitosan/hydroxyapatite and characterization studies, *Journal of Materials Research and Technology* , Vol.8, pp. 5201-5216, 2019.

Kamalanathan.P., Ramesh.S.,BangL.T., NiankanA., Tan.C.Y., Purbolaksono.J., Synthesis and sintering of hydroxyapatite derived from eggshell as a calcium precursor, *Ceramics International* , Vol.40, pp. 16349-59, 2014.

Rey. C.Combes. C., Drouet. C., Grossin. D., Bioactive Ceramics: Physical Chemistry, Comprehensive Biomaterials, *Elsevier Ltd. C*, pp. 187-210, 2011.

Hamidi.A.A.,Salimi.M.N, Yusoff. A.H.M, Synthesis and Characterization of Eggshell-Derived Hydroxyapatite via MechanochemicalMethod: A Comparative Study, *AIP Conference Proceedings: Advanced Materials Engineering and Technology*, pp. 1-12, 2017.

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