



Impact of Alkaline Immersion Time on Nano-Hydroxyapatite Synthesis from Broiler Eggshells with Electrochemical Method Using Constant Direct Current (CDC)

Asep Muhamad Samsudin^{1,*}, Tutuk Djoko Kusworo¹, Hana Nikma Ulya¹, Muhammad Akhsanil Auladi¹

¹ Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Semarang, Indonesia

* Corresponding author: asep.samsudin@live.undip.ac.id

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Abstract

Calcium phosphate is widely used in biomaterials, especially as a bone substitute. One of the calcium phosphate compounds that is widely used is hydroxyapatite. In biomedical applications, nano-sized hydroxyapatite particles have better bioactivity than coarse crystals. Hydroxyapatite can be synthesized from various sources of calcium and phosphate. Broiler eggshells were chosen as a source of calcium in this study because they have a relatively high calcium content. The method used in the synthesis of nano-hydroxyapatite in this research is the electrochemical method. This research aims to examine the effect of varying NaOH immersion time on the size and structure of calcium phosphate particles produced using an electrochemical method made from chicken egg shells. The synthesis of nano-hydroxyapatite from chicken egg shells was carried out in 4 stages: material preparation, electrolysis, precipitate immersion, and characterization. Nanometer-sized particles can be acquired through an immersion process lasting at least four hours, and it is observed that the longer the duration of immersion, the greater the resemblance of the resulting structure to hydroxyapatite.

1. Introduction

Hydroxyapatite (HAp), with a chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and a Ca/P ratio of 1.67, constitutes a significant constituent within the inorganic mineral composition of human hard tissues, and it has found extensive utilization as a biomedical ceramic substance in both orthopedic and dental applications [1, 2, 3, 4]. Hydroxyapatite closely resembles the structure and chemical characteristics of natural bone minerals, as approximately 70% of human bone is composed of calcium phosphate, which strongly resembles hydroxyapatite. Hydroxyapatite crystals provide strength and rigidity to bone structure and play a crucial role in maintaining the integrity of human skeletal tissues [5].

Hydroxyapatite is non-toxic, non-carcinogenic, resistant to corrosion, has biocompatible properties, and is not recognized as a foreign material by the body. Hydroxyapatite is an osteoconductive substance and

belongs to the calcium orthophosphate family [6]. Additionally, it possesses bioactive properties and can be integrated into living tissue through the same active process as the normal bone remodeling process [7]. Nanoscale hydroxyapatite particles exhibit superior bioactivity compared to larger crystals in biomedical applications [8].

Many researchers have investigated the synthesis techniques and characteristics of HAp, but the procedure is costly, intricate, and time-consuming [1]. Recently, a growing interest has been in producing high-performance hydroxyapatite powders by synthesizing HAp using natural resources. However, research concentrating on the straightforward and economical processing of HAp using biowaste materials is still limited. Hence, the study of HAp preparation from biowaste possesses a high potential [1].

Several biowaste materials, including oyster and abalone shells [9], green mussel shells [10], bovine bone [11], fish bone [12], and eggshells [13], are used to synthesize hydroxyapatite. These biowaste materials are cost-effective and sustainable sources for hydroxyapatite synthesis, making the process more environmentally friendly and reducing the need for traditional mineral sources [1].

Hydroxyapatite particles can be synthesized by various techniques, including precipitation [14], solid-state reaction [15], hydrothermal [1], sol-gel method [16], microemulsion method [17], and flame spray [18]. The precipitation approach is more prevalent due to its simplicity and low expense. However, the disadvantage of this technique is that size control is challenging to achieve, and the particles produced are typically large and irregular [14, 19]. The hydrothermal process can yield hydroxyapatite with a significant level of crystallinity. However, this approach necessitates elevated temperature and pressure and generates powder that tends to aggregate with inconsistent size distributions [20]. The sol-gel method is a highly efficient technique for precisely manipulating the shape and chemical content of hydroxyapatite. However, it is characterized by a lengthy processing time [16, 21].

Electrochemical methods can be used to synthesize nano-sized hydroxyapatite. This technology has emerged as the predominant technique for applying a hydroxyapatite (HAp) coating onto metal surfaces in biomedical applications. The electrochemical approach is recognized for its effectiveness and efficiency in assembling small particles with precise size and morphology. This technique can be achieved by altering the applied current or potential to the system [19, 22]. Djošić *et al.* [23] successfully produced monetite nanoparticles using electrochemical synthesis and converted them into hydroxyapatite by immersing them in a NaOH solution. They employed an electrochemical technique utilizing a constant direct current (CDC) power source. Samsudin *et al.* [24] studied the effect of pH and current density on hydroxyapatite from eggshells. HAp was identified at pH 12 [25].

In this study, broiler eggshells (*Gallus gallusdomesticus*) were used as the raw material for the synthesis of hydroxyapatite. Due to their high calcium carbonate content, broiler eggshells possess the capacity to serve as a fundamental constituent in the synthesis of hydroxyapatite [26]. Calcium oxide compounds are produced after the calcination of the calcium carbonate present in eggshells. The selection of broiler egg shells was based on their superior calcium content after calcination compared to other poultry eggshells [27]. This research aims to examine the effect of immersion time of hydroxyapatite precipitate in NaOH solution on the type and size of calcium phosphate particles produced using eggshell-based electrochemical methods.

2. Experimental

2.1. Materials

Broiler eggshells were collected from a market around Semarang, Indonesia. KH_2PO_4 , $\text{Na}_2\text{H}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$, HCl, and NaOH were obtained from Merck, Germany.

2.2. Synthesis of Nano-hydroxyapatite

The synthesis of nano-hydroxyapatite began with preparing calcium precursors from broiler eggshells. The washed eggshells were then crushed and calcined at 900°C for 5 hours. The calcium concentrations of the eggshells before and after the calcination process were analyzed using an Atomic Absorption Spectrophotometer (AAS). The calcined eggshells were then dissolved in concentrated HCl and distilled water to produce a CaCl_2 solution with the desired concentration.

Nano-hydroxyapatite was synthesized at a constant direct current (CDC) using the electrochemical method. The cathode and anode used in an electrochemical cell were platinum electrodes with a height of 10 cm, a diameter of 3 mm, and a surface area of 0.071 cm^2 . The utilized potentiostat was EZstat basic (Nuvant system, United States). The electrolyte solution consisted of CaCl_2 from egg shells, $\text{Na}_2\text{H}_2\text{EDTA}$, and KH_2PO_4 with a $\text{Ca}^{2+}/\text{EDTA}^{4-}/\text{PO}_4^{3-}$ composition of 0.25/0.25/0.15 M. Electrolysis was carried out for 6 hours galvanostatically at a current of 0.3 A (current density of 4.23 A/m^2). The precipitate was then filtered and dried in an oven at 40°C for 24 hours. The obtained dry precipitate was then weighed. Figure 1 illustrates the schematic of electrolysis equipment.

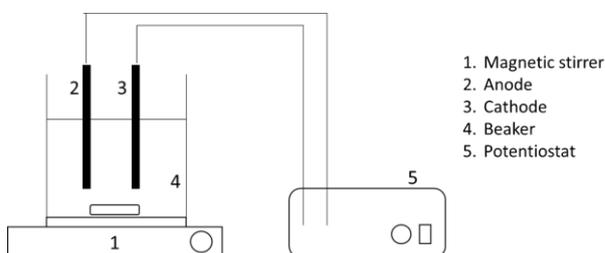


Figure 1. Schematic of electrolysis equipment

The dried precipitate was then immersed in NaOH solution at pH 12 with varying immersion times of 2, 4, 6, and 8 hours. The results were filtered and dried for further characterization using a scanning electron microscope (SEM) and X-ray diffraction (XRD).

3. Results and Discussion

3.1. Effect of Calcination on Eggshell Calcium Content

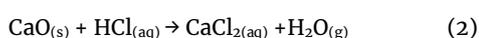
The calcination process was carried out to convert the CaCO_3 present in chicken egg shells into CaO . The reaction that occur in the calcination process is shown in Reaction (1) [28].

Table 1. Comparison of the calcium content of eggshells before and after calcination

Eggshells	Calcium Content (%)
Before calcination	35.96
After calcination	39.93

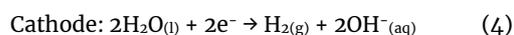
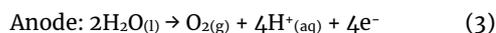


Table 1 shows a comparison of calcium levels in eggshells before and after the calcination process. The calcium content in eggshells increases from 35.96% to 39.93% following the calcination process. This increase is caused by the reduction in moisture content, the release of some gases, particularly CO₂, from reaction (1), and the evaporation of volatile organic materials [29]. During the calcination process, the reduction of organic compounds results in an elevation of calcium concentration in eggshells, increasing from 35.96% to 39.93%. The calcium concentration in the eggshell was analyzed using an Atomic Absorption Spectrophotometer (AAS). In order to produce a calcium chloride solution, which is part of the electrolyte solution, the calcium oxide present in calcined eggshells reacts with hydrochloric acid [30]. The reaction is shown in Reaction (2).



3.2. Precipitate Formation

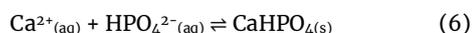
Prior to the electrochemical process, an electrolyte solution consisting of CaCl₂, Na₂H₂EDTA, and KH₂PO₄ was clear and transparent. Gas bubbles were generated at the platinum cathode and anode during the electrochemical process. Water electrolysis occurs due to the presence of a platinum electrode as an inert electrode. The reactions at the anode and cathode are shown in Reactions (3) and (4) [31].



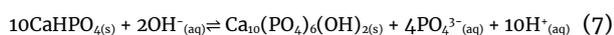
Following Reaction (4), the cathode facilitates the generation of hydroxide ions by the reduction process of water. The production of hydroxide ions at the cathode had an impact on the dissociation of phosphoric acid, as demonstrated by the equilibrium processes (Reaction (5)).



HPO₄²⁻ ions, which are generated from Reaction (5), then react with Ca²⁺ ions to produce CaHPO₄ (monetite) (Reaction (6)).



Monetite exhibits thermodynamic stability solely at pH levels lower than 4.8. The presence of hydroxide ions played a significant role in the stabilization process of monetite, leading to its conversion into hydroxyapatite. The reaction of monetite conversion to hydroxyapatite due to the presence of OH⁻ ions from water reduction and NaOH immersion is shown in Reaction (7).



The immersion process resulted in the partial dissolving at the interface between monetite and the solution. The interaction between calcium and phosphate ions leads to hydroxyapatite formation and rearrangement of the monetite structure, resulting in the precipitation of hydroxyapatite crystallites [32].

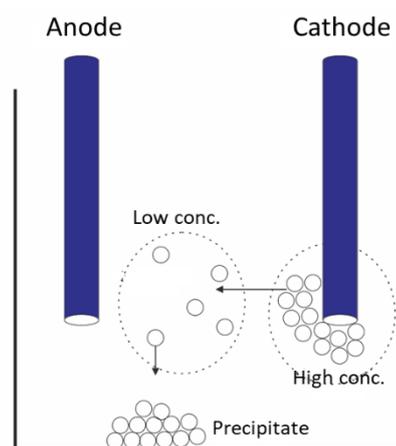


Figure 2. Precipitate formation mechanism

The pH could affect the stability of calcium phosphate. Dicalcium phosphate (CaHPO₄·2H₂O), as one type of calcium phosphate, exhibits the highest stability at a pH below 4.2. When pH is greater than 4.2, the stable phase for calcium phosphates at high temperatures (up to 1,000°C) is in the form of Hap [33].

Figure 2 illustrates the process of precipitate formation in the electrolysis process. Particles are initially formed around the cathode. The number of particles created around the cathode will enhance with the increase in the duration of electrolysis. Then, these particles will move from the cathode to the body of the solution due to the difference in concentration. The concentration gradient between the cathode and the bulk solution leads to the diffusion of particles from the cathode to the bulk solution. As the electrolysis duration extends, the settling of particles in the solution intensifies, influenced by their specific gravity.

3.3. Morphology of HAP Precipitates

The HAP precipitates obtained from the electrolysis process with various NaOH immersion times were then characterized using scanning electron microscopy (SEM). The results of SEM characterization can be seen in Figure 3.

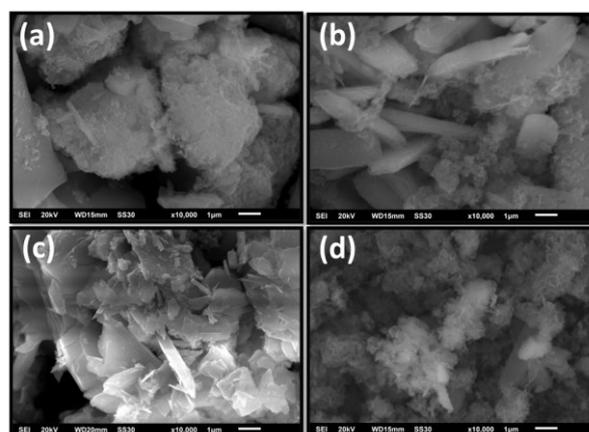


Figure 3. SEM images at 10,000× magnification with immersion times of (a) 2 hours, (b) 4 hours, (c) 6 hours, and (d) 8 hours

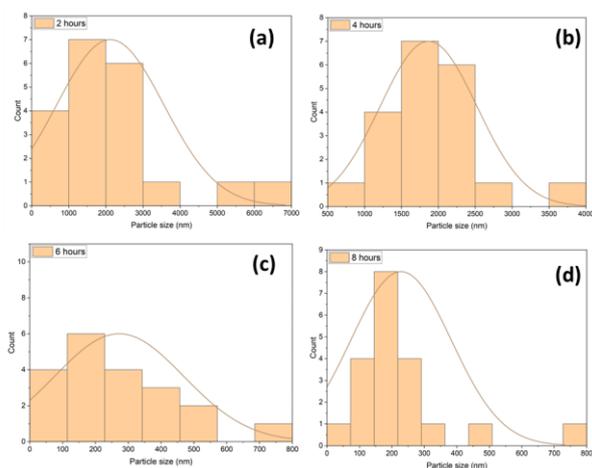


Figure 4. Histogram of particle size distribution at various immersion times of (a) 2 hours, (b) 4 hours, (c) 6 hours, and (d) 8 hours

According to the SEM results depicted in Figure 3, the precipitate exhibits a plate-like morphology. The observed morphology has similarities to the findings of previous studies [34, 35]. The plate-like hydroxyapatite (HAp) exhibits a favorable morphology due to its resemblance to the inorganic phase of biological tissue. Moreover, its suitability and ease of usage in tissue engineering further enhance its potential [36]. The structure of plate-shaped HAp can be explained as an inheritance from either an amorphous calcium phosphate phase or a precursor octacalcium phosphate (OCP) [19].

Figure 4 shows the particle size distribution at various immersion times of precipitate in NaOH. According to Figure 4, an immersion time of 2 hours in NaOH yields a particle size distribution ranging from 0.92 to 6.08 μm , with an average particle size of 2.12 μm . When the immersion of NaOH was increased to 4 hours, the particle size distribution tended to fall between 0.75 and 3.10 μm , with an average particle size of 1.87 μm . At an immersion time of 6 hours, the size of the particle achieves nano-size with an average size of 272 nm falling within the range of 90 to 775 nm. Meanwhile, at an immersion time of 8 hours, particles reach a range and the smallest average size of 87–763 nm and 228 nm, respectively. The result indicates that an extension of the NaOH immersion time correlates with a reduction in particle size. Specifically, the particle size achieves the nano-size range when immersed in NaOH for 6–8 hours.

3.4. Chemical Properties

The chemical characteristics of the resulting powder were investigated by determining the phase composition using X-ray diffraction (XRD). In order to examine the degree of crystallinity, the synthesized hydroxyapatite (HAp) was subjected to analysis using powder X-ray diffraction (XRD). The obtained XRD pattern was subsequently compared to the Joint Committee on Powder Diffraction Standards (JCPDS) data based on the 2θ angle values.

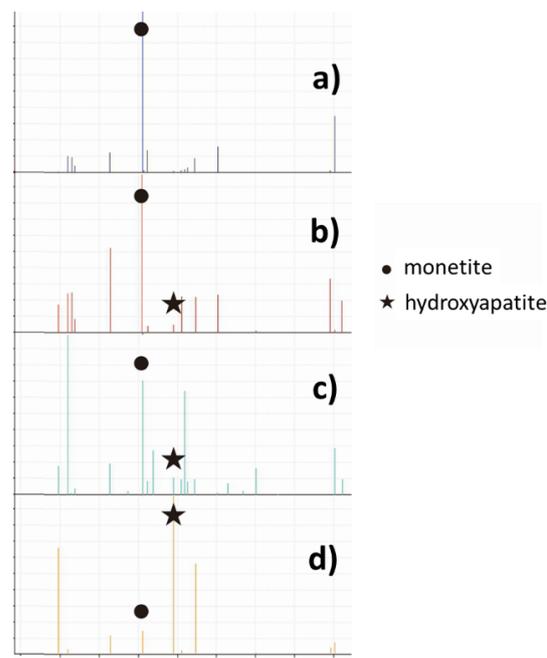


Figure 5. X-ray diffraction results of particle samples with varying NaOH immersion times of (a) 2 hours, (b) 4 hours, (c) 6 hours, and (d) 8 hours

Figure 5 shows the results of the diffraction peaks in the samples resulting from electrolysis by immersion time in NaOH at pH 12. The peaks tend to be sharp and narrow form. High-intensity sharp peaks in XRD spectra typically denote the presence of well-ordered crystalline material [37].

When the immersion time was set to 2 hours, the characteristic 2θ peaks of the precipitate exhibit closer proximity to monetite (CaHPO_4) than hydroxyapatite. As the duration of immersion was extended to 4, 6, and 8 hours, the observed properties of the peaks tended to resemble those of hydroxyapatite, particularly in the 2θ peaks of 32, as determined by X-ray diffraction (XRD). The immersion of the precipitate in NaOH for 8 hours results in a higher quantity of comparable peaks, corresponding with the JCPDS data of HAp no 09-0432. Monetite compounds can be converted into hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) by immersion in an alkaline solution [32]. According to Reaction (7), there is a positive correlation between the duration of immersion time and the efficiency of converting monetite to hydroxyapatite.

4. Conclusion

Hydroxyapatite can be synthesized from broiler eggshells with an electrochemical process. Using a calcination temperature of 900°C can increase the calcium content in eggshell powder. According to the SEM analysis results, the precipitate exhibited a plate-like morphology. Increasing the immersion time of the precipitate in NaOH solution reduces the particle size. By immersion time of more than 6 hours, precipitate with a size of nanometers was obtained. The smallest size, with an average of 228 nm, was achieved after immersing for 8 hours. Referring to the XRD spectra, the immersion of the precipitate in NaOH for 8 hours leads to the closest structure to hydroxyapatite.

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References

- [1] Shih-Ching Wu, Hsueh-Chuan Hsu, Hsueh-Fang Wang, Shu-Ping Liou, Wen-Fu Ho, Synthesis and Characterization of Nano-Hydroxyapatite Obtained from Eggshell via the Hydrothermal Process and the Precipitation Method, *Molecules*, 28, 13, (2023), 4926 <https://doi.org/10.3390/molecules28134926>
- [2] Rebaz Obaid Kareem, Omer Kaygili, Tankut Ates, Niyazi Bulut, Suleyman Koytepe, Ali Kuruçay, Filiz Ercan, Ismail Ercan, Experimental and theoretical characterization of Bi-based hydroxyapatites doped with Ce, *Ceramics International*, 48, 22, (2022), 33440–33454 <https://doi.org/10.1016/j.ceramint.2022.07.287>
- [3] Dewantoro Dewantoro, Margareta Novian Cahyanti, Sri Hartini, Effect of Phosphate Addition and Exposure of Micro Waves on Comparatives Ca/F in Gypsum Waste: Preliminary Study of Hydroxyapatite Synthesis (HAP) from Ceramics Gypsum Industry Waste, *Jurnal Kimia Sains dan Aplikasi*, 21, 4, (2018), 218–223 <https://doi.org/10.14710/jksa.21.4.218-223>
- [4] Nur Akbar, Asril Pramutadi Andi Mustari, Atiek Rostika Noviyanti, New Chemicals and Routes for the Preparation of Gelatin/HA Composites using the Wet Precipitation Method, *Jurnal Kimia Sains dan Aplikasi*, 23, 2, (2020), 46–50 <https://doi.org/10.14710/jksa.23.2.46-50>
- [5] Himanshu Khandelwal, Satya Prakash, Synthesis and characterization of hydroxyapatite powder by eggshell, *Journal of Minerals and Materials Characterization and Engineering*, 4, 2, (2016), 119–126 <http://dx.doi.org/10.4236/jmmce.2016.42011>
- [6] Fatih İsen, Omer Kaygili, Niyazi Bulut, Tankut Ates, Fatih Osmanlioğlu, Serhat Keser, Beyhan Tatar, İmren Özcan, Burhan Ates, Filiz Ercan, Experimental and theoretical characterization of Dy-doped hydroxyapatites, *Journal of the Australian Ceramic Society*, 59, (2023), 849–864 <https://doi.org/10.1007/s41779-023-00878-8>
- [7] Sergey V. Dorozhkin, Bioceramics of calcium orthophosphates, *Biomaterials*, 31, 7, (2010), 1465–1485 <https://doi.org/10.1016/j.biomaterials.2009.11.050>
- [8] Yoshiki Oshida, in, Momentum Press New York [New York] (222 East 46th Street, New York, NY 10017), New York [New York] (222 East 46th Street, New York, NY 10017), 2015,
- [9] Saranya R., Aruna Devi B., Meenakshi Sundaram N., Preparation of ciprofloxacin-loaded oyster shell derived hydroxyapatite composite film for biomedical applications, *Journal of the Australian Ceramic Society*, 59, (2023), 621–632 <https://doi.org/10.1007/s41779-023-00861-3>
- [10] M. Sari, Y. Yusuf, Synthesis and characterization of hydroxyapatite based on green mussel shells (*perna viridis*) with the variation of stirring time using the precipitation method, *IOP Conference Series: Materials Science and Engineering*, 432, (2018), 012046 <https://doi.org/10.1088/1757-899X/432/1/012046>
- [11] Jamiu K. Odusote, Y. Danyuo, Abdulazeez D. Baruwa, Akeem A. Azeez, Synthesis and characterization of hydroxyapatite from bovine bone for production of dental implants, *Journal of Applied Biomaterials & Functional Materials*, 17, 2, (2019), <https://doi.org/10.1177/2280800019836829>
- [12] Renata Neves Granito, Ana Claudia Renno, Hirochi Yamamura, Matheus Cruz de Almeida, Pedro Luiz Menin Ruiz, Daniel Araki Ribeiro, Hydroxyapatite from Fish for Bone Tissue Engineering: A Promising Approach, *International Journal of Molecular and Cellular Medicine*, 7, 2, (2018), 80–90 <https://doi.org/10.22088/ijmcm.Bums.7.2.80>
- [13] Huda S. Alhasan, Nadiyah Alahmadi, Suhad A. Yasin, Mohammed Y. Khalaf, Gomaa A. M. Ali, Low-Cost and Eco-Friendly Hydroxyapatite Nanoparticles Derived from Eggshell Waste for Cephalexin Removal, *Separations*, 9, 1, (2022), 10 <https://doi.org/10.3390/separations9010010>
- [14] N. A. S. Mohd Pu'ad, J. Alipal, H. Z. Abdullah, M. I. Idris, T. C. Lee, Synthesis of eggshell derived hydroxyapatite via chemical precipitation and calcination method, *Materials Today: Proceedings*, 42, (2021), 172–177 <https://doi.org/10.1016/j.matpr.2020.11.276>
- [15] Parisa Taghipour, Farhad Zolfagharpour, Hamideh Daneshvar, Synthesis of hydroxyapatite through solid-state reaction method and study of its thermoluminescence dosimetric properties against gamma rays, *Radiation Physics and Engineering*, 3, 2, (2022), 7–10 <https://doi.org/10.22034/rpe.2022.335334.1062>
- [16] M. S. AlHammad, Nanostructure hydroxyapatite based ceramics by sol gel method, *Journal of Alloys and Compounds*, 661, (2016), 251–256 <https://doi.org/10.1016/j.jallcom.2015.11.045>
- [17] V. Collins Arun Prakash, I. Venda, V. Thamizharasi, Synthesis and characterization of surfactant assisted hydroxyapatite powder using microemulsion method, *Materials Today: Proceedings*, 51, (2022), 1788–1792 <https://doi.org/10.1016/j.matpr.2021.05.059>
- [18] Jung Sang Cho, Yun Chan Kang, Nano-sized hydroxyapatite powders prepared by flame spray pyrolysis, *Journal of Alloys and Compounds*, 464, 1, (2008), 282–287 <https://doi.org/10.1016/j.jallcom.2007.09.092>
- [19] Adrian Nur, Alifah Rahmawati, Noor Izzati Ilmi, Samsudin Affandi, Arief Widjaja, Electrochemical synthesis of nanosized hydroxyapatite by pulsed direct current method, *AIP Conference Proceedings*, 1586, (2014), 86–91 <https://doi.org/10.1063/1.4866736>
- [20] Shahid Hussain, Kazi Sabiruddin, Synthesis of eggshell based hydroxyapatite using hydrothermal method, *IOP Conference Series: Materials Science and Engineering*, 1189, (2021), 012024 <https://doi.org/10.1088/1757-899X/1189/1/012024>
- [21] Adrian Nur, Heru Setyawan, Arief Widjaja, I Wuled Lenggono, Electrochemical processes for the formation of hydroxyapatite powders, *Bulletin of Chemical Reaction Engineering & Catalysis*, 9, 3, (2014), 168–175 <https://doi.org/10.9767/bcrec.9.3.6686.168-175>

- [22] Fauziatul Fajaroh, Heru Setyawan, W. Widiyastuti, Sugeng Winardi, Synthesis of magnetite nanoparticles by surfactant-free electrochemical method in an aqueous system, *Advanced Powder Technology*, 23, 3, (2012), 328–333
<https://doi.org/10.1016/j.apt.2011.04.007>
- [23] M. S. Djošić, V. B. Mišković-Stanković, Z. M. Kačarević-Popović, B. M. Jokić, N. Bibić, M. Mitrić, S. K. Milonjić, R. Jančić-Heinemann, J. Stojanović, Electrochemical synthesis of nanosized monetite powder and its electrophoretic deposition on titanium, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 341, 1, (2009), 110–117
<https://doi.org/10.1016/j.colsurfa.2009.03.046>
- [24] Asep Muhamad Samsudin, Hargono, Kristinah Haryani, Hantoro Satriadi, Hana Nikma Ulya, M. Akhsanil Auladi, Effect of pH on Nano-Hydroxyapatite Synthesis from Chicken Eggshell Prepared by Electrochemical Method, *Advanced Science Letters*, 23, 6, (2017), 5733–5735
<https://doi.org/10.1166/asl.2017.8816>
- [25] Asep Muhamad Samsudin, Norman Iskandar, Didik Setiyo Widodo, Hana Nikma Ulya, M. Akhsanil Auladi, Effect of Current Density on Nano-Hydroxyapatite Synthesis from Chicken Eggshell Using Electrochemical Method with Constant Direct Current (CDC), *Advanced Science Letters*, 23, 6, (2017), 5736–5738
<https://doi.org/10.1166/asl.2017.8817>
- [26] Idris Abdulrahman, Hamzat Ibiyeye Tijani, Bashir Abubakar Mohammed, Haruna Saidu, Hindatu Yusuf, Mohammed Ndejiko Jibrin, Sulaiman Mohammed, From Garbage to Biomaterials: An Overview on Egg Shell Based Hydroxyapatite, *Journal of Materials*, 2014, (2014), 802467
<https://doi.org/10.1155/2014/802467>
- [27] Setyanto Tri Wahyudi, Setia Utami Dewi, Ajeng Anggraeni, Kiagus Dahlan, Akhmaloka, Muhammad Ali Zulfikar, Rukman Hertadi, Simple and easy method to synthesize chicken eggshell based hydroxyapatite, *Advanced materials research*, 896, (2014), 276–279
<https://doi.org/10.4028/www.scientific.net/AMR.896.276>
- [28] Mohammad Zulhasif Ahmad Khiri, Khamirul Amin Matori, Norhazlin Zainuddin, Che Azurahaman Che Abdullah, Zarifah Nadakkavil Alassan, Nur Fadilah Baharuddin, Mohd Hafiz Mohd Zaid, The usability of ark clam shell (*Anadara granosa*) as calcium precursor to produce hydroxyapatite nanoparticle via wet chemical precipitate method in various sintering temperature, *SpringerPlus*, 5, (2016), 1206
<https://doi.org/10.1186/s40064-016-2824-y>
- [29] Nadia Razali, Nurriswin Jumadi, Adlin Yasmin Jalani, Nurhanim Zulaikha Kamarulzaman, Khairul Faizal Pa'ee, Thermal decomposition of calcium carbonate in chicken eggshells: study on temperature and contact time, *The Malaysian Journal of Analytical Sciences*, 26, 2, (2022), 347–359
- [30] Duygu Ağaoğulları, Özge Balcı, M. Lütfi Öveçoğlu, İsmail Duman, Preparation of LaB₆ Powders via Calciothermic Reduction using Mechanochemistry and Acid Leaching, *KONA Powder and Particle Journal*, 33, (2016), 203–218
<https://doi.org/10.14356/kona.2016001>
- [31] Alex Gu, Canis Li, Jamin Xie, Daisy Zeng, Kingshuk Daschowdhury, Leon Yee, Yitian Li, Austin Chiang, Emeka Okekeocha, Effects of microconvection on bubble displacement during water electrolysis under microgravity, *Journal of Emerging Investigators*, 6, (2023), 1–6
- [32] M. H. Prado Da Silva, J. H. C. Lima, G. A. Soares, C. N. Elias, M. C. de Andrade, S. M. Best, I. R. Gibson, Transformation of monetite to hydroxyapatite in bioactive coatings on titanium, *Surface and Coatings Technology*, 137, 2, (2001), 270–276
[https://doi.org/10.1016/S0257-8972\(00\)01125-7](https://doi.org/10.1016/S0257-8972(00)01125-7)
- [33] Rebaz Obaid Kareem, Niyazi Bulut, Omer Kaygili, Hydroxyapatite Biomaterials: A Comprehensive Review of their Properties, Structures, Medical Applications, and Fabrication Methods, *Journal of Chemical Reviews*, 6, 1, (2024), 1–26
<https://doi.org/10.48309/jcr.2024.415051.1253>
- [34] M. S. Djošić, V. B. Mišković-Stanković, S. Milonjić, Z. M. Kačarević-Popović, N. Bibić, J. Stojanović, Electrochemical synthesis and characterization of hydroxyapatite powders, *Materials Chemistry and Physics*, 111, 1, (2008), 137–142
<https://doi.org/10.1016/j.matchemphys.2008.03.045>
- [35] Adrian Nur, Heru Setyawan, An Experimental and Theoretical Investigation of the Formation of Hydroxyapatite Particles Prepared by an Electrochemical Method, *Journal of Chemical Engineering of Japan*, 49, 2, (2016), 144–151
<https://doi.org/10.1252/jcej.15we130>
- [36] K. Ioku, S. Yamauchi, H. Fujimori, S. Goto, M. Yoshimura, Hydrothermal preparation of fibrous apatite and apatite sheet, *Solid State Ionics*, 151, 1, (2002), 147–150
[https://doi.org/10.1016/S0167-2738\(02\)00593-3](https://doi.org/10.1016/S0167-2738(02)00593-3)
- [37] Sohrab Nasiri, Marzieh Rabiei, Arvydas Palevicius, Giedrius Janusas, Andrius Vilkauskas, Venkatramaiah Nutalapati, Ahmad Monshi, Modified Scherrer equation to calculate crystal size by XRD with high accuracy, examples Fe₂O₃, TiO₂ and V₂O₅, *Nano Trends*, 3, (2023), 100015
<https://doi.org/10.1016/j.nwnano.2023.100015>