International Journal of Eco-Innovation in Science and Engineering (IJEISE) Vol.05 (2), 2024 (42-49)
https://ijeise.upnjatim.ac.id/ E-ISSN: 2721-8775

Article

Optimization of Nano-Hydroxyapatite Production from Limestone with Effect of Gelatin Concentration and pH in Sol-Gel Synthesis

S. Sutiyono[a], Luluk Edahwati[b*], Mohammad Rafli Alif Wahyudi[c], Nazila Alfi Rahmah[d]

Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Pembangunan Nasional Veteran Jawa Timur

E-mail: [a]sutiyono13@yahoo.co.id , [c]21031010068@student.upnjatim.ac.id , [d]21031010082@student.upnjatim.ac.id
*Corresponding author: lulukedahwati@gmail.com | Phone number: +6287854408347

Received: 10th November 2024; Revised: 27th November 2024; Accepted: 29th November 2024; Available online: 30th November 2024; Published regularly: May and November

Abstract

Nano hydroxyapatite is one of the biomaterials that has proven to be a very potential material due to its ability to bind directly with bone tissue. Limestone with a calcium carbonate content of 99.87% was used as a calcium source for the economical and sustainable synthesis of hydroxyapatite. The sol-gel method was chosen for the in situ gelatin synthesis process due to its ability to produce nanoparticles with high homogeneity. The particle size of nanohydroxyapatite can increase the surface area to volume ratio thus affecting the interaction with surrounding cells and tissues. Gelatin was added in various concentrations of 10%, 20%, and 30% as an innovative cell growth support modifier. The synthesis was also carried out at pH 9, 10, and 11 to evaluate its effect on hydroxyapatite formation. Optimal synthesis conditions were obtained at 30% gelatin concentration and pH 10, which resulted in the highest yield of 93.95%. Product characterization included Fourier Transform Infra-Red analysis, which showed the presence of typical hydroxyapatite functional groups (PO_4^{3-}, OH-) and carbonate groups (CO_3^{2-}). Scanning Electron Microscopy characterization showed nano-sized particles in the range of 443-578 nm with a granular structure. Porosity was found to be 72.52% making it ideal for cancellous bone tissue engineering applications.

Keywords: Nanohydroxyapatite, gelatin concentration, pH, Sol gel, limestone

1. Introduction

The development of biomaterials science has brought about a revolution in the field of regenerative medicine and tissue engineering, opening up new opportunities for the healing and regeneration of damaged or lost tissues. Biomaterials, defined as materials that can interact with biological systems to replace or improve the function of tissues or organs, have undergone rapid advances in recent decades [1]. These advancements include the development of various types of biomaterials, including polymers, metals, ceramics, and composites, each of which has specific characteristics and applications in the medical field. Hydroxyapatite, with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is becoming a highly potential material for various medical applications such as dental implant materials, bone fillers, and drug delivery systems. [2]. Its unique characteristics allow it to optimally interact with biological tissues in the body.

Limestone containing large amounts of calcium carbonate ($CaCO_3$) is one of the potential sources of calcium for hydroxyapatite synthesis [3]. The use of limestone as a precursor can reduce the cost of hydroxyapatite production compared

DOI:10.4186

to the use of pure chemicals. However, hydroxyapatite synthesized from limestone often has poor mechanical properties due to large particle size and irregular morphology [4]. The limitations of these mechanical properties can be overcome by combining them with polymers [5].

Gelatin, as a protein obtained from partial hydrolysis of collagen, has the ability to support cell growth. The interaction between gelatin and hydroxyapatite can increase cell adhesion, proliferation, and differentiation of osteoblasts thereby accelerating the process of bone regeneration [6]. The interaction between gelatin and calcium ions can affect the kinetics of hydroxyapatite formation and the final characteristics of the product [5]. In this study, the concentration of gelatin and the degree of acidity will be varied in the synthesis of hydroxyapatite based on the role of gelatin as a controlling agent in the process of nucleation and growth of HA crystals.

Various methods have been developed for hydroxyapatite synthesis, one of which is the sol-gel method which has the advantages of high purity, good homogeneity, and relatively low process temperature. The sol-gel method is expected to produce hydroxyapatite with smaller particle size and homogeneous so that it has better properties. This method involves the formation of a gel from a precursor solution, where the precipitation and drying processes can be controlled to produce the desired structure [7]. Gelatin can serve as a binding agent that helps in the formation of a better micro-network, thereby improving the stability and mechanical strength of the resulting hydroxyapatite [8]. The sol-gel method offers precise control over the particle size and porosity of the material [9].

Fig 1. Hidroksiapatit Hasil Sintesis Metode Sol-Gel

According to Monica [10] the best composite is 30% HAp-gelatin synthesized by in-situ method, with the highest degree of crystallinity (84.62%) and morphology in the form of smaller and homogeneous granules. The degree of crystallinity of hydroxyapatite increases with the addition of gelatin concentration in-situ because of the strong interaction between the C=O group of gelatin and Ca^{2+} of hydroxyapatite which shortens the distance between HAp molecules. Whereas in the ex-situ method, gelatin tends to disperse and results in a more amorphous nature at high concentrations.

The small particle size of hydroxyapatite or nanohydroxyapatite has significant advantages including being able to substantially increase the absorption efficiency of ingredients, which has the potential to improve the effectiveness of various products and formulations. In addition, this increased efficiency enables a reduction in the dose required to achieve the desired effect, which can reduce potential side effects and improve cost efficiency. The use of nanoparticles can improve product stability, which has implications for longer shelf life and better consistency of performance over time. Hydroxyapatite can be formed in nanoparticle size or called nanohydroxyapatite below 1 μm [11]

Hydroxyapatite (HA) synthesis generally achieves optimal results in the alkaline pH range, with the optimum pH for HA formation and stability being between 9.5 to 12, where at these conditions HA solubility is minimal and formation of the desired crystalline phase is most efficient. Proper pH control during the synthesis process is critical, as the pH will affect the morphology and particle size of the HA formed. Research shows that at pH below 9, there is a risk of formation of unwanted impurities such as CaO and $Ca(OH)_2$, while pH above 12 can lead to excessive incorporation of carbonate ions into the hydroxyapatite structure [12].

There have been some significant advances in the synthesis of hydroxyapatite, but there are still challenges that have not been fully solved. This research aims to address some of the critical gaps that still exist in hydroxyapatite synthesis. Previous studies have mostly focused on alternative calcium sources such as eggshells or marine resources, the utilization of limestone as a

calcium source is still less explored, particularly in terms of optimizing the synthesis parameters [13]. In addition, existing methodologies often struggle to control the particle size, morphology and mechanical properties of hydroxyapatite. The incorporation of gelatin as a bonding agent has been minimally investigated, with most studies using ex-situ methods that result in less homogeneous composites or focusing on limited variations in gelatin concentration [10]. Furthermore, comprehensive studies that simultaneously examine the effects of gelatin concentration and pH on hydroxyapatite formation are scarce.

2. Material and Method

2.1. Material

The main material used in this research is precipitated calcium carbonate from limestone (Pudak scientific, 99%). Additional chemical materials used include HNO_3, $(NH_4)_2HPO_4$ as a phosphate precursor, and NH_4OH as a pH regulator. The tools used are furnace (Thermo scientific) and hot plate magnetic stirrer (Thermo scientific).

2.2. Hydroxyapatite Gelatin Synthesis

The sol-gel method was used to synthesize hydroxyapatite by mixing Precipitated Calcium Carbonate and $(NH_4)_2HPO_4$. 5 g of Precipitated Calcium Carbonate (PCC) was dissolved in 0.5 M HNO_3, while 6.68 g of $(NH_4)_2HPO_4$ was dissolved in the same volume of distilled water to make a 0.3 M solution. The $(NH_4)_2HPO_4$ solution was then added to the PCC solution at a rate of 1.3 mL/min. Gelatin was incorporated with concentrations (m/v) varying from 10%, 20% and 30%. The mixing process lasted for 1 hour at 60°C. The pH of the solution was adjusted using NH_4OH 33% until it reached various pH values of 9, 10 and 11 while stirring at 500 rpm for 30 minutes. This concentration and degree of acidity were chosen based on previous research which states that the synthesis of hydroxyapatite in situ is optimal at a concentration of 30% and pH 10. So this study was made by varying the concentration of gelatin by 10%, 20% and 30%, while the pH was varied at 9, 10 and 11 [10]. The solution was then allowed to age for 24 hours at room temperature for gel formation and maturation. After aging, the precipitate was separated through filtration and washed to neutral pH. Drying was carried out at 110°C for 2 hours until constant weight. The sintering process was carried out at 900°C for 2 hours. This is based on previous research which states that at this temperature, the sintering process is able to induce significant transformations, facilitating the formation of a stable and homogeneous hydroxyapatite crystal phase [14]. The final stage of the product in the form of HAp-gelatin powder was allowed to reach room temperature before being sieved using a 200 mesh sieve.

2.3. Yield Analysis of Gelatin Nano-hydroxyapatite

Yield is an important parameter in determining the efficiency of the synthesis process of a nano-hydroxyapatite material. Yield analysis is the first step to evaluate the ability of the production process to produce nano-hydroxyapatite from the raw materials used. The yield determination was carried out by weighing the hydroxyapatite-gelatin produced three times to ensure consistency of measurement. The yield percentage was calculated using the following formula:

$$yield = \frac{product\ mass}{mass\ of\ PCC}\ x\ 100\% \quad (1)$$

The greater the percentage yield obtained, the higher the conversion rate of hydroxyapatite-gelatin produced from the initial precipitated calcium carbonate mass.

2.4. Characterization of Gelatin Nano-hydroxyapatite

Characterization of hydroxyapatite-gelatin synthesis was carried out through a series of comprehensive tests, mainly using FTIR (Fourier Transform Infrared Spectroscopy) and SEM-EDX (Scanning Electron Microscopy with Energy Dispersive X-ray spectroscopy) techniques. FTIR analysis plays a role in identifying characteristic functional groups, verifying the presence of hydroxyapatite components such as phosphate (PO_4^{3-}) and carbonate (CO_3^{2-}), as well as hydroxyl (OH^-) groups. The resulting spectra provide important information regarding the molecular interactions between hydroxyapatite and gelatin in the composite. Meanwhile, SEM provides a detailed picture of the morphology and surface topography of the sample, revealing the granular crystal

structure and particle size distribution. These observations allow evaluation of the effect of synthesis parameters on the physical characteristics of the material. As a complement, EDX analysis integrated with SEM enables the determination of the elemental composition of the sample.

3. Results and Discussion

3.1. Results of calcium carbonate content in Precipitated Calcium Carbonate Raw Material

The results of the $CaCO_3$ content test on limestone showed a very high value, indicating the quality and purity of the raw material which is very good for hydroxyapatite synthesis. The analysis showed that the limestone used had a $CaCO_3$ content of 99.87%. The high content of $CaCO_3$ allows better control over the stoichiometry of the reaction. Limestone is composed of Ca (96.38%), Si (1.47%), Sr (1.44%), Fe (0.595%), Ti (0.060%), Nb (0.0222%), Sn (0.0123%), Sb (0.0123%), and In (0.0120%) [15]. This near 100% $CaCO_3$ content also indicates that the limestone has few impurities, which can minimize the risk of contamination and stoichiometric variations in the final hydroxyapatite product.

3.2. Yield results of in situ synthesis of hydroxyapatite gelatin

The hydroxyapatite yield analysis of the results of the in-situ synthesis of gelatin hydroxyapatite showed significant variability in the concentration and pH parameters as shown in the following table:

Table 1. Yield of Gelatin Hydroxyapatite

Gelatin Concentration	pH	Mass of PCC	Product mass				Yield	Average yield
			Mass 1	Mass 2	Mass 3	Average		
10%	9	5	4,1569	4,0987	4,2136	4,1564	83,13%	85,44%
	10	5	4,2997	4,3776	4,2889	4,3221	86,44%	
	11	5	4,2837	4,4007	4,3278	4,3374	86,75%	
20%	9	5	4,3567	4,3611	4,3091	4,3423	86,85%	85,18%
	10	5	4,3001	4,2665	4,2797	4,2821	85,64%	
	11	5	4,1097	4,2003	4,1478	4,1526	83,05%	
30%	9	5	4,5672	4,292	4,5427	4,4673	89,35%	86,97%
	10	5	4,7423	4,6589	4,6919	4,6977	93,95%	
	11	5	3,9631	4,0008	3,6788	3,8809	77,62%	

Based on the data shown, in the hydroxyapatite synthesis process with variations in gelatin concentration and pH, the yield produced shows a diverse pattern. At 10% gelatin concentration, a fairly consistent yield was obtained with a range of 83.13% to 86.75%, with an average yield of 85.44%. The highest yield value at this concentration was achieved at pH 11 of 86.75%. At 20% gelatin concentration, the yield produced was in the range of 83.05% to 86.85%, with an average yield of 85.18%. The highest yield at this concentration was obtained at pH 9, which was 86.85%. Increasing the pH to 11 caused a decrease in yield to 83.05%. For the 30% gelatin concentration, there was a more significant yield variation with a range of 77.62% to 93.95%, resulting in an average yield of 86.97%. The highest average yield was achieved at this concentration. This indicates that at 30% gelatin concentration, pH 10 provides optimal conditions for hydroxyapatite formation. Overall, the optimal synthesis conditions were achieved at 30% gelatin concentration with pH 10 which resulted in the highest yield of 93.95%. This indicates that the combination of gelatin concentration and pH provides a favorable environment for the formation of hydroxyapatite crystals.

3.3. Fourier Transform Infrared Spectroscopy Analysis

Analysis of the FTIR spectra of hydroxyapatite synthesized with gelatin at pH 10 and 30% gelatin concentration showed some important characteristic peaks.

DOI:10.4186

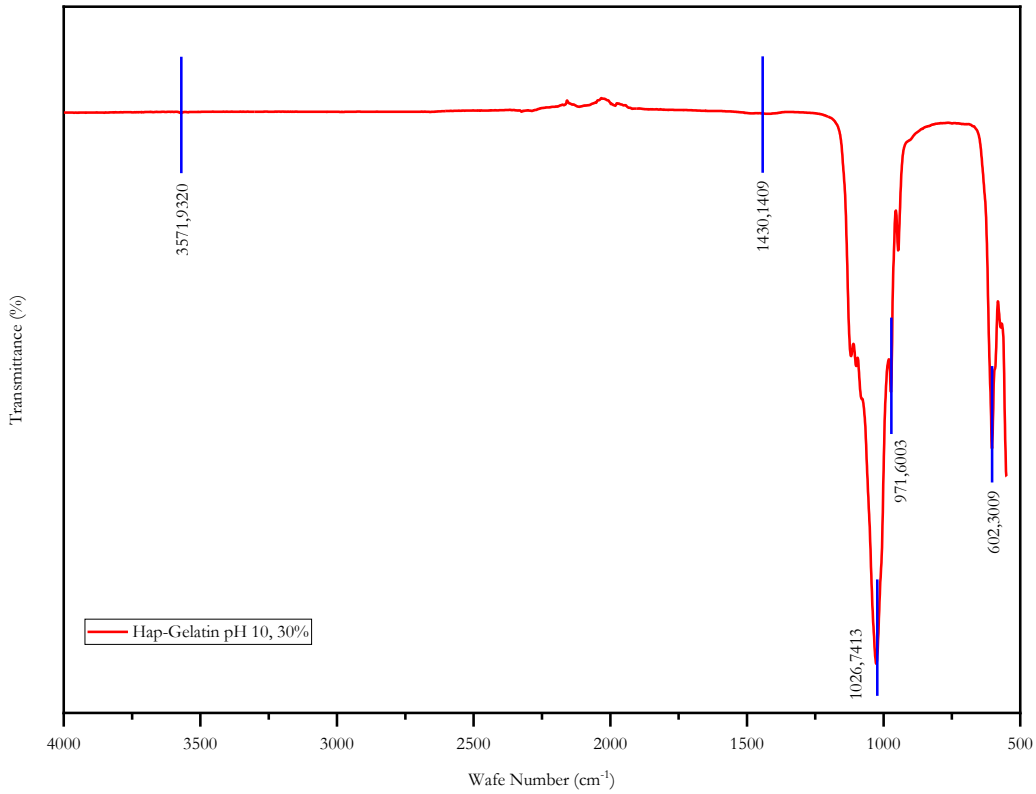

Fig 2. Fourier Transform Infra-Red analysis results

The sharp absorption peak at wave number 1026.7413 cm^{-1} indicates the stretching vibration of PO_4^{3-} group which is the main characteristic of hydroxyapatite. The peaks at 602,3009 cm^{-1} and 971,6003 cm^{-1} also show vibrations of the PO_4^{3-}, group, further confirming the formation of the hydroxyapatite structure. The broad peak at 3571,9320 cm^{-1} indicates the stretching vibrations of OH^- groups derived from hydroxyapatite. Another group detected was the carbonate group (CO_3^{2-}) in the wave number range of 1430,1439 cm^{-1}. No significant extraneous peaks were observed, indicating that the hydroxyapatite-gelatin synthesis at pH 10 with 30% gelatin concentration has been successfully carried out. The functional groups in the synthesized hydroxyapatite showed similarities with the functional groups of hydroxyapatite in human bone, characterized by the presence of OH^-, CO_3^{2-}, dan PO_4^{3-} groups. arbonate groups do not actually have to form during the hydroxyapatite nano-synthesis process. However, this is not detrimental because isomorphic substitution between carbonate and phosphate ions is a common phenomenon and plays a role in bone mineral homeostasis [16]

3.4. Scanning Electron Microscopy Analysis

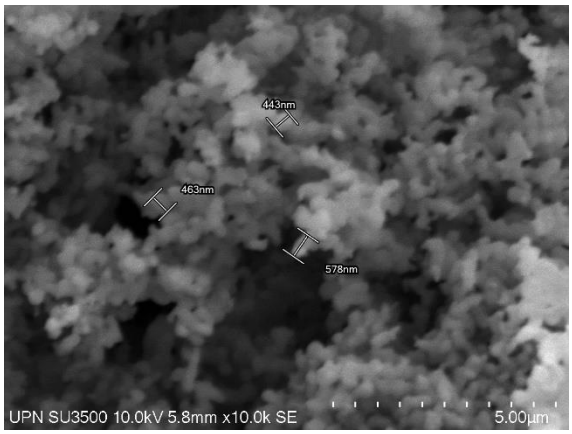

Fig 3. Scanning Electron Microscopy Results of Hydroxyapatite-Gelatin pH 10 30% Concentration with 10,000x Magnification.

Characterization results using Scanning Electron Microscope (SEM) showed the morphology of hydroxyapatite-gelatin synthesized in situ via sol-gel method. Microstructural analysis revealed the distribution of particles with dimensions varying in the range of 443-578 nm, indicating the formation of nano-hydroxyapatite structures. The surface morphology showed agglomeration of particles with granular shapes

that were scattered inhomogeneously, which is a typical characteristic of the interaction between the inorganic phase of hydroxyapatite and the organic matrix of gelatin. The formation of the nano-hydroxyapatite structure can be confirmed through the measurement of particle dimensions that are on the submicron scale, where clusters with the smallest size of 443 nm to the largest reaching 578 nm are visible.

The observed agglomeration phenomenon is likely due to the electrostatic interaction between the functional groups of gelatin and calcium and phosphate ions during the sol-gel process, which results in a hierarchical structure at the nanometer scale. The agglomeration phenomenon observed in the synthesized hydroxyapatite can be attributed to the particle segregation process through the sieving technique with a specification of 200 mesh. This process results in a heterogeneous particle size distribution below the sieve threshold, resulting in non-uniformity. This morphological heterogeneity contributes to an increase in the surface energy between particles, which further facilitates van der Waals interactions and promotes the formation of agglomerated clusters with inhomogeneous morphology [17]. In addition, an increase in extraction temperature results in the agglomeration process occurring. Hydroxyapatite will reach the agglomeration point at 750^{o}C and at higher temperatures causes the hydroxyapatite crystal size to decrease and shrink. Therefore, there are several ways to overcome the agglomeration phenomenon in hydroxyapatite synthesis through optimizing the sieving technique by using a finer mesh to produce a more even size distribution. In addition, setting a more precise sintering temperature, where lower temperatures can reduce consolidation and improve particle morphology [18]

The sol-gel method has succeeded in creating the expected nano-hydroxyapatite that is below 1 μm in size. However, the scalability and effectiveness of the sol-gel method has limitations. The sol-gel process often requires very tight control of the experimental conditions. In addition, the addition of gelatin can increase the total cost of synthesis. Therefore, the process of optimizing process parameters such as temperature, reaction time and the reactor used needs to be carried out in order to allow production in larger quantities without compromising the quality of the material produced.

3.5. Nano-Hydroxyapatite Porosity Analysis

Porosity analysis of hydroxyapatite-gelatin (HAp-gelatin) was implemented using an intensity-based threshold method with ImageJ software. The analysis procedure was carried out through selection of region of interest (ROI) from SEM micrographs, followed by porosity quantification using particle analysis features.

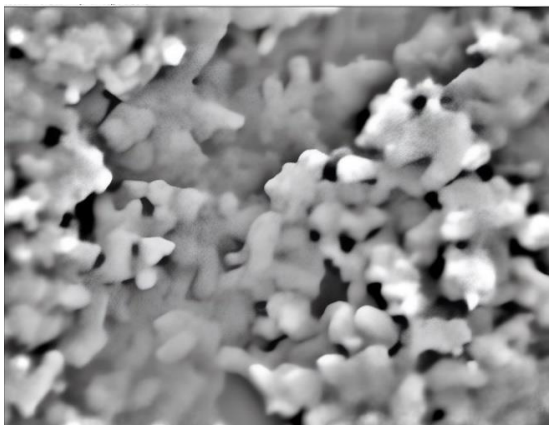

Fig 4. Scanning Electron Microscopy Result Section for Image J Analysis

The dark areas in Figure 4 were identified as pore structures, which were then calculated for their porosity percentage using the Analyze function in ImageJ. The analysis results showed a porosity value of 72.52%. The porosity value of HAp-gelatin indicates a highly porous material structure. This porosity level is within the ideal range for bone tissue engineering applications, as reported by Bemis [16] the porosity characteristics of implant material designs must meet biomimetic standards according to the target tissue. Trabecular bone structure requires porosity in the range of 70-95%, while dental tissue requires moderate porosity in the range of 40-60%. As for applications in cortical bone (compact bone), lower porosity characteristics in the interval of 5-30% are required.

Differentiation of porosity values is essential to ensure mechanical compatibility and optimal integration of the implant material with the target biological tissue. The porosity obtained corresponds to the range of requirements of cancellous bone structure. Cancellous bone has unique structural characteristics that enable it to act as a natural shock absorber in the body. Due to its porous and non-dense internal structure, this type of bone is able to efficiently absorb and distribute external forces, such as impacts or mechanical stresses received by the body. This

vibration absorption mechanism is essential in protecting the bone structure and surrounding tissues from direct damage due to sudden mechanical forces [19]

4. Conclusions

This study successfully optimized the synthesis of nano-hydroxyapatite from limestone using the sol-gel method with the main focus on exploring the effect of gelatin concentration and pH. Through a series of systematic experiments, this study successfully identified the optimal synthesis conditions that produced the highest yield of 93.95% at 30% gelatin concentration and pH 10. The characteristics of the resulting material indicate the successful synthesis of nano-hydroxyapatite with particle dimensions ranging from 443-578 nm, which is highly suitable for bone tissue engineering applications. FTIR analysis confirmed the presence of typical functional groups similar to human bone, while porosity analysis showed a value of 72.52%, which is ideal for cancellous bone structure. A significant contribution of this research lies in the utilization of limestone as an economical and sustainable source of calcium. The applied sol-gel method proved its effectiveness in producing nano-hydroxyapatite with desired characteristics. As a prospect for further research, it is suggested to explore alternative biopolymers other than gelatin, such as chitosan or alginate, which could potentially provide additional characteristics to nano-hydroxyapatite. In addition, future research could consider alternative synthesis methods such as hydrothermal or chemical precipitation to compare the performance and characteristics of the resulting materials.

References

[1] I. Sukmana, A. Y. Eka Risano, M. Arif Wicaksono, and R. Adi Saputra, "Perkembangan dan Aplikasi Biomaterial dalam Bidang Kedokteran Modern: A Review," *Insologi: Jurnal Sains dan Teknologi*, vol. 1, no. 5, pp. 635–646, Oct. 2022, doi: https://doi.org/10.55123/insologi.v1i5.1037

[2] A. R. Noviyanti, H. Haryono, R. Pandu, and D. R. Eddy, "Cangkang Telur Ayam sebagai Sumber Kalsium dalam Pembuatan Hidroksiapatit untuk Aplikasi Graft Tulang," *Chimica et Natura Acta*, vol. 5, no. 3, p. 107, Dec. 2017, doi: https://doi.org/10.24198/cna.v5.n3.16057.

[3] M. Sirait, K. Sinulingga, N. Siregar, and R. S. D. Siregar, "Synthesis of hydroxyapatite from limestone by using precipitation method," in *Journal of Physics: Conference Series*, Institute of Physics Publishing, Mar. 2020. Accessed: Nov. 10, 2024. [Online]. Available: https://iopscience.iop.org/article/10.1088/1742-6596/1462/1/012058

[4] A. E. Wardiana, G. Shalli, C. Saputra, and S. E. Cahyaningrum, "Pemanfaatan Batu Kapur sebagai Bahan Baku Hidroksiapatit," *Journal of Chemistry*, vol. 8, no. 2, pp. 62–66, May. 2019, Accessed: Nov. 10, 2024. [Online]. Available: https://ejournal.unesa.ac.id/index.php/unesa-journal-of chemistry/article/view/30920

[5] M. R. Nawafi, M. Masruroh, and D. J. D. H. Santjojo, "Morphological and Mechanical Study of Gelatin/Hydroxyapatite Composite based Scaffolds for Bone Tissue Regeneration," *Indonesian Journal of Applied Physics*, vol. 12, no. 2, p. 235, Nov. 2022, doi: https://doi.org/10.13057/ijap.v12i2.59365

[6] I. Wahda, "Sintesis dan Karakterisasi Komposit HIdroksiapatit/SiO2/Gelatin sebagai Kandidat Scaffold Tulang," Thesis, Dept. Matematika dan Ilmu Pengetahuan Alam., Universitas Hasanuddin., Makassar., 2024

[7] P. H. Yanti and Y. Gandi, "Pengaruh Waktu Kalsinasi Terhadap Sifat Fisika-Kimia Hidroksiapatit dari Cangkang Geloina Coaxans," *Chemistry Progress*, vol. 13, no. 2, Nov. 2020, doi: https://doi.org/10.35799/cp.13.2.2020.31473

[8] L. Sitepu, "Karakterisasi Gelatin Hasil Ekstraksi dari Tulang Sapi Melalui Proses Perlakuan Basa NaOH," *Jurnal Kartika Kimia*, vol. 5, no. 1, Nov. 2022, doi: https://doi.org/10.26874/jkk.v5i1.106

[9] M. Baladi *et al.*, "Green sol–gel synthesis of hydroxyapatite nanoparticles using lemon extract as capping agent and investigation of its anticancer activity against human

cancer cell lines (T98, and SHSY5)," *Arabian Journal of Chemistry*, vol. 16, no. 4, Apr. 2023, doi: https://doi.org/10.1016/j.arabjc.2023.104646

[10] S. Monica, "Sintesis dan Pencirian Komposit Hidroksiapatit-gelatin dengan Presipitasi Basah secara In-situ dan Ex-situ," skripsi, Departemen kimia., Institut Pertanian Bogor., Bogor., 2015.

[11] A. B. Hanura, W. Trilaksani, and P. Suptijah, "Characterization OF Nanohydroxyapatite from Tuna's Thunnus sp Bone as Biomaterials Substance," *Jurnal Ilmu dan Teknologi Kelautan Tropis*, vol. 9, no. 2, pp. 619–629, Jan. 2018, Accessed: Nov. 10, 2024. [Online]. Available: https://www.neliti.com/id/publications/223062/characterization-of-nanohydroxyapatite-from-tunas-thunnus-sp-bone-as-biomaterial.

[12] Siswanto, D. P. Sormin, D. Hikmawati, Aminatun, and R. Apsari, "Effect of pH condition during sol-gel synthesis on the volume fraction of hydroxyapatite from sea coral," in *Journal of Physics: Conference Series*, IOP Publishing Ltd, Mar. 2021. doi: https://doi.org/10.1088/1742-6596/1825 /1/012045.

[13] F. T. Prayoga, S. L. Aprilianty, and L. Edahwati, "Synthesis of Hydroxyapatite from Precipitated Calcium Carbonate of Thorny Shells (Murex trapa) with Variable Ca/P Mole Ratio and pH," *Jurnal Rekayasa Kimia & Lingkungan*, vol. 19, no. 1, pp. 116–124, Jun. 2024, doi: https://doi.org/10.23955/rkl.v19i1.36177.

[14] D. Supangat and S. E. Cahyaningrum, "Synthesis and Characterization of Hydroxyapatite of Crabs Shell (scylla serrata) by Wet Application method," 2017. *Journal of Chemistry*, vol. 6, no. 3, pp. 143–149, Sept. 2017, Accessed: Nov. 26, 2024. [Online]. Available: https://ejournal.unesa.ac.id/index.php/unesa-journal-of-chemistry/article/view/21999.

[15] M. Megawati, A. Alimuddin, and L. Abdul Kadir, "Komposisi Kimia Batu Kapur Alam dari Indutri Kapur Kabupaten Kolaka Sulawesi Tenggara," *Saintifik*, vol. 5, no. 2, pp. 104–108, Jul. 2019, doi: https://doi.org/10.31605/saintifik.v5i2.230.

[16] R. Bemis, H. Heriyanti, R. Rahmi, R. D. Puspitasari, and D. Imawati, "Pengaruh Variasi Konsentrasi Alumina Pada Sintesis Nanokomposit Hidroksiapatit/ Alumina dari Udang Papai Menggunakan Metode Hidrotermal," *Journal of The Indonesian Society of Integrated Chemistry*, vol. 15, no. 1, pp. 56–66, Jun. 2023, doi: https://doi.org/10.22437/jisic.v15i1.21309.

[17] I Irhamni *et .al.,* "Utilization of oyster shell waste (crassostrea gigas) as a source of calcium in hydroxyapatite synthesis," *Jurnal Geuthèë,* vol. 7, no. 2, pp. 96–104, 2024, doi: https://doi.org/10.52626/jg.v7i2.357.

[18] N. I Made and S I Nengah, "Karakteristik Hidroksiapatit Hasil Ekstraksi TERMAL dari Tulang Limbah dan Aplikasinya untuk Adsorpsi Ion Selektif Biru Metilen," *Indonesian Journal of Applied Chemistry*, vol. 6, no. 2, pp. 123-130, Des. 2024, Accessed: Nov. 26, 2024. [Online]. Available: https://jurnal.harianregional.com/cakra/full-46704

[19] K. G. Waruwu, A. Suprihanto, gunawan dwi Haryadi, and rilo Berlin, "Analisis Pengaruh Larutan Infus Saline Water dan Ringer Lactate terhadap Laju Korosi Paduan Titanium TI-6AL-4V yang digunakan untuk Implan Tulang," *Jurnal Teknik Mesin S-1*, vol. 11, no. 4, pp. 179–186, Oct. 2023, Accessed: Nov. 27, 2024. [Online]. Available: https://ejournal3.undip.ac.id/index.php/jtm/article/view/41865