Synthesis and Characterization of Hydroxyapatite-Zinc Oxide (HAp-ZnO) as Antibacterial Biomaterial

Charlena1*, Irma Herawati Suparto1*, Eldora Kurniawan1
Department of Chemistry, Bogor Agricultural University, INDONESIA
Email: charlena@apps.ipb.ac.id, irma.suparto@gmail.com

Abstract. Hydroxyapatite (HAp) is an important biomaterial widely used as implants for bone and teeth. Biomaterial with antibacterial activities beneficial to prevent infection post implantation. Therefore, the present study aims to synthesized and characterized HAp composite with zinc oxide (ZnO) that has antibacterial properties. The HAp was synthesized through wet precipitation method using shell of Bellamya javanica as source of calcium. The zinc oxide was added with HAp using in situ method. The crystallinity of the material was decreased after added with ZnO which indicate Zn ion suppressed the crystal growth. Antibacterial abilities were tested against Eschericia coli and Staphylococcus aureus. Inhibition against bacteria increased when the HAp was composited with ZnO. The HAp-ZnO composite showed better inhibition toward Escherichia coli.

1. Introduction
Implantation technique is one of the solution to repair damaged bone and teeth. Material selection for the implant is an important factor to assure the success of the implantation. Research and studies have been done to find material that have an active interaction with the target tissue without causing undesirable reaction on the tissue. Calcium phosphate based material is one of the material which is widely studied. When implanted to the bone, it will form physicochemical bond with bone tissue [1].

Hydroxyapatite (HAp) is the most stable phase of calcium phosphate and the most studied biomaterial as bone and teeth implant. Synthetic HAp is osteoconductive and thermodinamically stable in physiological pH [2]. Natural resources with the high calcium content such as egg shell, fish bone, and the shell of tutut snail (Bellamya javanica) could be used as the precursor for HAp synthesis. Independently, HAp does not have a good antibacterial properties. It can be improve by combining inorganic ions with antibacterial properties. This can be solved by creating a composite of HAp and added with the antibacterial material. Existence of metal ions such as Ag nanoparticles, CuO and ZnO in HAp structure has been proved to increase its antibacterial material [3-5]. Tutut (Bellamya javanica) shell was chosen as starting material for this study because of its high calcium content and not yet produced commercially. Wet precipitation method was used to synthesize HAp. Zinc oxide (ZnO) was synthesized through low hydrothermal method using zinc acetate dihydrate and sodium hydroxide as the starting material. The composite was made using in situ method.

2. Methods
Zinc oxide was synthesized using Zn(CH₃COO)₂·2H₂O and NaOH as starting materials. Zn(CH₃COO)₂·2H₂O solution was made by dissolving 1.0957 g Zn(CH₃COO)₂·2H₂O in 50 mL flask with deionized water. Meanwhile, 0.2500 g NaOH was dissolved in another 50 mL flask with
deionized water. Zn(CH$_3$COO)$_2$·2H$_2$O solution was added dropwise into NaOH solution while stirred at room temperature. The mixture then heated in oven at 90 °C for 2 hours and centrifuged at 3000 rpm for 30 minutes. Supernatant that contain ZnO was seperated. [6]

The mixture of Ca(OH)$_2$ 0.5 M and (NH$_4$)$_2$HPO$_4$ 0.3 M was decantated for 120 hours. The solution of ZnO in water was added into the suspension then reheated to 80-90 °C, stirred for 1 hour, and vacuum filtered after 12 hour. Filtrate was dried at 120 °C for 4 hours [5]. The obtained powder were annealed at 900 °C, 1000 °C, 1090°C for 2 hours. Synthesized powder was characterized using X-ray diffraction, Fourier Transform Infra Red, and antibacterial activities assay.

3. Results and Discussion
 Calcium source that was used in this study was from the shell of tutut (*Bellamya javanica*). Calcium that obtained from tutut shell was in form of calcium carbonate. After calcination process, CaCO$_3$ was converted to CaO according to this reaction:

\[ \text{CaCO}_3(s) \rightarrow \text{CaO}(s) + \text{CO}_2(g) \]

CaO powder was left in open air for one week and covered to Ca(OH)$_2$. Calcium content of the Ca(OH)$_2$ powder from tutut shell was 32.97%. Hydroxyapatite was synthesized through precipitation method [7] using Ca(OH)$_2$ and (NH$_4$)$_2$HPO$_4$ as precursor.

\[ 10\text{Ca(OH)}_2 + 6(\text{NH}_4)_2\text{HPO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{H}_2\text{O} + 12 \text{NH}_4\text{OH} \]

Hydroxyapatite was characterized using XRD. HAp has characteristic peaks at 2θ 31.80°- 34.00° [8]. XRD spectrum of synthesized HAp powder showed strong peaks at 2θ 31.80°, 32.95°, 32.21° (Fig 1).

Zinc oxide was obtained in the form of white powder. The powder was characterized using XRD and showed strong peaks at 2θ 36.22°, 33.01°, 31.72°. These peaks are characteristic peaks of ZnO. XRD spectrum also showed a peak at 2θ 13.28°. This peak indicated that there was zinc acetate dihydrate which was left after synthesis. (Fig.2)
HAp-ZnO composite was characterized using XRD. The crystallinity of the material was decreasing after the addition of ZnO. Synthesized HAp had crystallinity of 75.44% meanwhile HAp-ZnO composite had crystallinity of 60.02%. The decrease of crystallinity could be caused by the substitution of calcium ions by zinc ions. The substitution would suppress the crystal growth [9].

In this study, composite materials were calcinated at 3 different temperature. The degree of crystallinity was increased when the material was calcinated at higher temperature. The degree of crystallinity of HAp-ZnO composite that was calcinated at 1000 °C was 76. 5849%, while the one that
calcinated at 1090 °C had a crystallinity of 86.6479%. XRD pattern showed that material that was calcinated at higher temperature had narrow peak width (Fig. 3).

Hydroxyapatite and its composite was characterized using FTIR. The spectrum showed absorption bands at 1400-1500 cm\(^{-1}\) indicated the existence of functional group \(-\text{CO}_3\). Absorption bands that showed the presence of \(-\text{OH}\) was observed at wavenumber 630, 1600, 1900, and 3500 cm\(^{-1}\). Wide absorption bands at wavenumber 3440 cm\(^{-1}\) showed the presence of adsorbed water molecule [10]. The functional group of \(-\text{PO}_4\) showed by the absorption band at 470.63, 567.07, 960.55, 1050 cm\(^{-1}\).

Zinc oxide showed vibration at the 400-600 cm\(^{-1}\)[11]. FTIR spectrum of HAp-ZnO composite showed absorption band at 401-416 cm\(^{-1}\) (Fig.4). This absorption band did not appear at HAp FTIR spectrum so it could be concluded that ZnO was successfully composited with HAp. FTIR spectrum of the composite that was calcinated at 3 different temperatures showed the difference at the absorption bands at 3500 cm\(^{-1}\). The intensity of the bands was decreasing which was caused by the interaction of ZnO with OH group from HAp[5]. High calcination temperature also affected interaction of ZnO with PO\(_4\) group (Fig. 5). The absorption peaks of PO\(_4\) at 560 cm\(^{-1}\) decreased when material was calcinated at 1000 and 1090 °C. This was caused by interaction of ZnO with PO\(_4\) from hydroxyapatite with bidentate coordination of Zn[5].

**Figure 4.** FTIR spectrum of HAp and HAp-ZnO composite that was calcinated at 900 °C.

**Figure 5.** FTIR spectrum of HAp-ZnO composite which was calcinated at different temperature.
Synthesized ZnO, HAp, and HAp-ZnO were tested to observe the antibacterial capability. The result showed the increase of antibacterial activity after ZnO addition.

Table 1. Inhibition Index of HAp and HAp-ZnO for E.coli and S.aureus

|                | E.coli | S.aureus |
|----------------|--------|----------|
|                | Inhibition Index | Inhibition Diameters (mm) | Inhibition Index | Inhibition Diameters (mm) |
| ZnO            | 0.56   | 14       | 0.67   | 15       |
| HAp            | 0.22   | 11       | 0.11   | 10       |
| HAp-ZnO 900    | 0.44   | 13       | -      | -        |
| HAp-ZnO 1000   | 0.78   | 16       | 0.33   | 12       |
| HAp-ZnO 1090   | 0.56   | 14       | 0.33   | 12       |

The increase of antibacterial ability was proved by the widening of its inhibition zone (Fig.6).

![Figure 6](image-url)  
*Figure 6. Antibacterial Test on Staphylococcus aureus on (A)HAp (B)ZnO (C)HAp-ZnO 1000 (D)HAp-ZnO 900 (E)HAp-ZnO 1090 and Antibacterial Test on Eschericia coli on (F)ZnO (G)HAp (H)HAp-ZnO 1000 (I)HAp-ZnO 900 (J)HAp-ZnO 1090.*

Inhibition index of the HAp-ZnO composite towards E.coli was increased until 0.56 higher than HAp without ZnO addition. Antibacterial test towards S.aureus was also increased. The test showed that HAp-ZnO composite inhibited E.coli more effectively than S.aureus. But HAp-ZnO that synthesized in this study had lower inhibition index that HAp-CuO composite which was done by Yuliandari (2016).
4. Conclusion
HAp-ZnO composite was synthesized through low hydrothermal method. Zinc oxide addition caused the decrease of the sample crystallinity. Higher calcination temperature caused the increase of crystallinity and the narrowing of the XRD peak width. Antibacterial test of HAp-ZnO composite towards *E.coli* and *S.aureus* showed the increase of inhibition index. Composite material with the best inhibition index was the composite that was calcinated at 1000 °C, which was 0.78.

5. References
[1] Bagambisa FB, Joos U 1990 Preliminary studies on the phenomenological behaviour of osteoblast cultured on hydroxyapatite ceramics *Biomaterials* 11 50-56
[2] Nayak AK 2010 Hydroxyapatite synthesis methodologies: An overview. *Int J. ChemTech Res* 2(2)903-907
[3] Charlena, Nuzulia NA, Handika 2017 Synthesis and characterization of composite hydroxyapatite-silver nanoparticles *IOP Conf. Series: Earth and Environmental Science* 58 1-9
[4] Yuliandari E. 2016. Sintesis dan pencirian komposit hidroksiapatit-tembaga(II) oksida (HAp-CuO) sebagai biomaterial antibakteri [skripsi]. Bogor (ID): Institut Pertanian Bogor.
[5] Molodovan M, Prodan D, Popescu V, Prejemerean C, Sarosi C, Saplontai M, Talu S, Vasile E. 2015 Structural and morphological properties of HA-ZnO powders prepared for biomaterials *Open Chemistry* 13 725-733
[6] Osman DAM, Mustafa MA 2015 Synthesis and characterization of Zinc Oxide Nanoparticles using zinc acetate dihydrate and sodium hydroxide *Journal of Nanoscience and Nanoengineering* 1 248-251
[7] Santos MH, de Oliveira M, Souzal PF, Mansur HS, Vasconcelos WL 2004 Synthesis control and characterization of hydroxyapatite prepared by wet precipitation process. *Materials Research* 7(4) 625-630
[8] Elhadad AA, Barranco V, Jiménez-Morales A. Peon E, Galvan JC. 2007. Multifunctional sol-gel derived thin film based on nanocrystalline hydroxyapatite powders. *Journal of Physics: Conference Series* 252(1) 1-8
[9] Galindo TGP, Kataoka T, Tagaya M 2015 Morphosynthesis of Zn-substituted stoichiometric and carbonate hydroxyapatite nanoparticles and their cytotoxicity in fibroblasts. *Journal of Nanomaterials* 2015 1-8
[10] Esfahani H, Salahi E, Tayebifard A, Rahimipour MR, Keyanpour-Rad M 2016 Structural and morphological analysis of zinc incorporated nonstoichiometric hydroxyapatite nano powders *Revista Materia* 21 569576
[11] Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A 2012 Antimicrobial activity of metal oxide nanoparticles against gram-positive and gram-negative bacteria:a comparative study. *International Journal of Nanomedicine* 7 6003-6009