RJC

RASĀYAN *J. Chem.*
Vol. 10 | No. 3 |766 - 770 | July - September | 2017
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SYNTHESIS AND CHARACTERIZATION OF HYDROXYAPATITE-COLLAGEN-CHITOSAN (HA/Col/Chi) COMPOSITE BY USING *EX-SITU* WET PRECIPITATION METHOD

Charlena[1,*], Ahmad Bikharudin[1], Setyanto Tri Wahyudi[2] and Erizal[3]
[1]Department of Chemistry, Bogor Agricultural University, Bogor-16680, (I.D.) Indonesia
[2]Department of Physic, Bogor Agricultural University, Bogor-16680, (I.D.) Indonesia
[3]Center for the Application of Isotopes and Radiation, National Nuclear Energy Agency, Jakarta-12440, (I.D.) Indonesia
*E-mail: charlena.ipb@gmail.com

ABSTRACT

Hydroxyapatite (HA) was included mineral of apatite is a bioactive ceramic material and biocompatible, which has similarity with the nature of the bone chemical structure. The synthesis of hydroxyapatite (HA) by using wet precipitation method consists of calcination, hydration, HA synthesizes, and thermal treatment. The solution 0.3 M $(NH_4)_2.HPO_4$ was dropped on the suspension of 0.5 M $Ca(OH)_2$ from tutut (*Bellamya javanica*) shells. While collagen is extracted from the waste fish (*Later calcarifer*) scales by the following acid and alkali extraction methods. Synthesis of HA/Col/Chi composite by using *ex-situ* wet precipitation method that has been formed from 2 ml collagen of 2%, 2 ml chitosan of 2% mixed with 2 gram of HA powder was dissolved in 20 ml of an ethanol solution at room temperature. The spectrum of HA/Col/Chi composite by FTIR showed the presence of functional groups of HA, collagen, chitosan, and indicate the presence of molecular interactions between -OH, -COOH, $-NH_2$ on collagen chains and -OH, $-NH_2$ on chitosan, and there is an interaction between HA, amide I, and amide II in collagen. X-Ray Diffraction Analysis of HA/Col/Chi composite showed the single phase of HA. Analysis element components of HA showed that the Ca/P ratio obtained is 1.98. In the HA analysis by scanning electron microscopy showed the formation of HA in the presence of agglomeration and has an irregular shape. In addition, has a size of 2.76 - 6.48 μm.

Keywords: *Bellamya javanica,* chitosan*,* collagen, *ex-situ*, hydroxyapatite*, Lates calcarifer*

INTRODUCTION

Hydroxyapatite (HA) was included mineral of apatite is a bioactive ceramic material and biocompatible, which has similarity with the nature of bone chemical structures and do not destroy tissue immune, not to cause swelling, and are osteoconductive.[1] Bioactive properties of hydroxyapatite serve for the formation and development of cells in the surrounding tissue.[2] HA has also been widely applied for a porous bone substitute and as a coating material for the implant. In addition to accelerating the formation of new bone, HA is also directly bound chemically to bone tissue through the formation of apatite layer, and biologically bonded interface. Hydroxyapatite-collagen composite is bone graft synthetic that is very similar to the bones of many viewpoints. Bone is composed of collagen and hydroxyapatite as a major component and a few percent of other components.[3] Hydroxyapatite-collagen composite when implanted in the human body shows osteoconductive properties better than the monolithic hydroxyapatite bone matrix and produce a similar classification.[4,5] In addition, hydroxyapatite-collagen composite proven biocompatible in humans and animals.[4,6] The combination of hydroxyapatite-collagen also helps to inhibit pathogenic bacteria that may be present during the process of implantation and the combination of hydroxyapatites also assist in inhibiting pathogenic bacteria that may be present during the implantation process.[7] The use of bone matrix collagen, as well as the mineral hydroxyapatite, also can speed up the

http://dx.doi.org/10.7324/RJC.2017.1031768

healing process of bone abnormalities, for example, when a crack or fracture. The addition of chitosan will improve the quality of the composite material. Chitosan is a polymer which is abundant in nature and is a derivative of chitin, which is biodegradable and biocompatible so widely used for medical applications. Collagen and chitosan play a role in bone tissue engineering.

The combination of HA with collagen and chitosan showed a nanocomposite for bone substitution.[8] In this study, HA was synthesized from the utilization of waste of Tutut shell (*Bellamya javanica*) through wet precipitation method. Tutut shell contains a variety of minerals including high levels of calcium.[9] Collagen from aquatic by-products is generally obtained by the following acid and alkali extraction methods. The addition of chitosan as a composite to improve the quality of the material. Chitosan is one example of natural polymers that are biodegradable, non-toxic, and biocompatible.[10] The development of HA/Col/Chi composite to improve the mechanical properties and strength of its hydroxyapatite. While the manufacture of HA/Col/Chi composite done by *ex-situ* methods, namely the addition of collagen and chitosan conducted after the precipitation process is completed. The aims of this study are to form HA/Col/Chi composite by using *ex situ* wet precipitation method.

EXPERIMENTAL

Material and Methods

The reagents used tutut (*Bellamya javanica*) shells obtained from Pasar Anyar (Bogor, Indonesia), fish (*Lates calcarifer*) scales obtained from Research Center for Marine and Fisheries Product Processing and Biotechnology (Jakarta, Indonesia), surfactants (dishwashing liquid), whatman filter grade 42, $CaCO_3$ standard from Wako, $(NH_4)_2HPO_4$ from Merck, glacial acetic acid from Merck, chitosan (shrimp skin) obtained from Department of Aquatics Products Technology, Bogor Agricultural University (Bogor, Indonesia).

General procedure

Synthesis of HA

Suspension of 0.5 M $Ca(OH)_2$ from extract tutut shells as calcium precursor and solution 0.3 M $(NH_4)_2.HPO_4$ as a phosphate precursor (Ca/P 1.67). The solution 0.3 M $(NH_4)_2.HPO_4$ was dropped on the suspension of 0.5 M $Ca(OH)_2$ at rate 1.3 mL/min with temperature conditions kept to 40 °C, while stirring using a magnetic stirrer. The pH conditions were monitored and kept around 10. Then the mixture was decanted for 24 hours and sonicated for 6 hours. After that, the solution was centrifuged for 15 minutes at a speed of 4500 rpm. The pellets were filtered and rinsed with aquadest. Then it was dried in an oven at 80 °C for 8 hours, heated in a furnace at 600 °C for 2 hours.

Extraction of collagen

Fish scales obtained as byproducts of the fishing industry, the surface was washed with running water until clean. Then the fish scales immersed in the surfactant solution for 24 hours to remove grease and other impurities. Furthermore, fish scales in the surfactant solution is cleaned again by shaking it using a horizontal shaker speed of 200 rpm interval of 1 hour. Such treatment is done as much as 3 repetitions. The fish scales were then washed with distilled water to remove surfactants and dried in an oven at 60 °C for 60 minutes. Fish scales then soaked in a solution of 0.5 M CH_3COOH with a ratio of 1:10 (w/v) at 4 °C within 48 hours. Then sediment extraction results were filtered using whatman 42 paper and dried at room temperature.

Synthesis of HA/Col/Chi with *Ex-Situ* Method

Synthesis of HA/Col/Chi composite that has been formed from 2% collagen, 2% chitosan, and HA powder from tutut (*Bellamya javanica*) shells. Collagen solution of 2% made from Collagen powder (*Lates calcarifer* scales) was dissolved in 0.1 N acetic acid and 2% chitosan solution made from chitosan powder was dissolved in 0.1 N acetic acid. As much as 2 ml of collagen of 2%, 2 ml chitosan of 2% mixed with 2 gram of HA powder was dissolved in 20 ml of the ethanol solution. During the mixing

process, the solution is stirred using a magnetic stirrer for 1 hour to be homogeneous at room temperature. After that, the solution was decanted within 48 hours, sonicated within 6 hours, centrifuged for 15 minutes at a speed of 4500 rpm. The pellets were filtered using whatman 42 paper and dried at room temperature.

Characterization Techniques

HA analyzed by XRD, FTIR, and SEM-EDS, Collagen analyzed by FTIR, and HA/Col/Chi composite analyzed by XRD and FTIR.

RESULTS AND DISCUSSION

Analysis of Functional Groups of HA/Col/Chi

The spectrum of HA/Col/Chi composite was characterized to analyze the presence of functional groups of HA, collagen, and chitosan shown in Fig.-1. The sharp absorption bands (Fig.-1b) at 569 (assignment: *v*4), 603.72 (assignment: *v*4), 962.48 (assignment: *v*1), and 1039.63 cm^{-1} (assignment: *v*3) shows the strain vibration of the PO_4^{3-} groups and assigned to P–O vibrations. The weak absorption peaks (Fig.-1b) found at 873.75 cm^{-1} indicates the presence of CO_3^{2-} groups and assigned to C–O vibrations. The Functional groups of collagen (Fig.-1b) are found at 1653 cm^{-1} (amide I), 1558.48 cm^{-1} (amide II), and 1418.32 cm^{-1} (amide III). The intensity of the ratio between the peak absorption of amide III and the absorption peak at 1450 cm^{-1} is 1.02. Ratio value close to 1.0 indicates that the collagen has a triple helix structure.[11] The absorption band (Fig.-1b) for OH^- group is found at 3404.36 cm^{-1}. The peak of –OH group indicates the characteristic of HA and chitosan. The peak of HA (Fig.-1a) and collagen (Fig.-1c) there were changes spectra (Fig.-1b) indicate the presence of molecular interactions between -OH, -COOH, $-NH_2$ on collagen chains and -OH, $-NH_2$ on chitosan, and there is an interaction between HA, amide I, and amide II in collagen.[12]

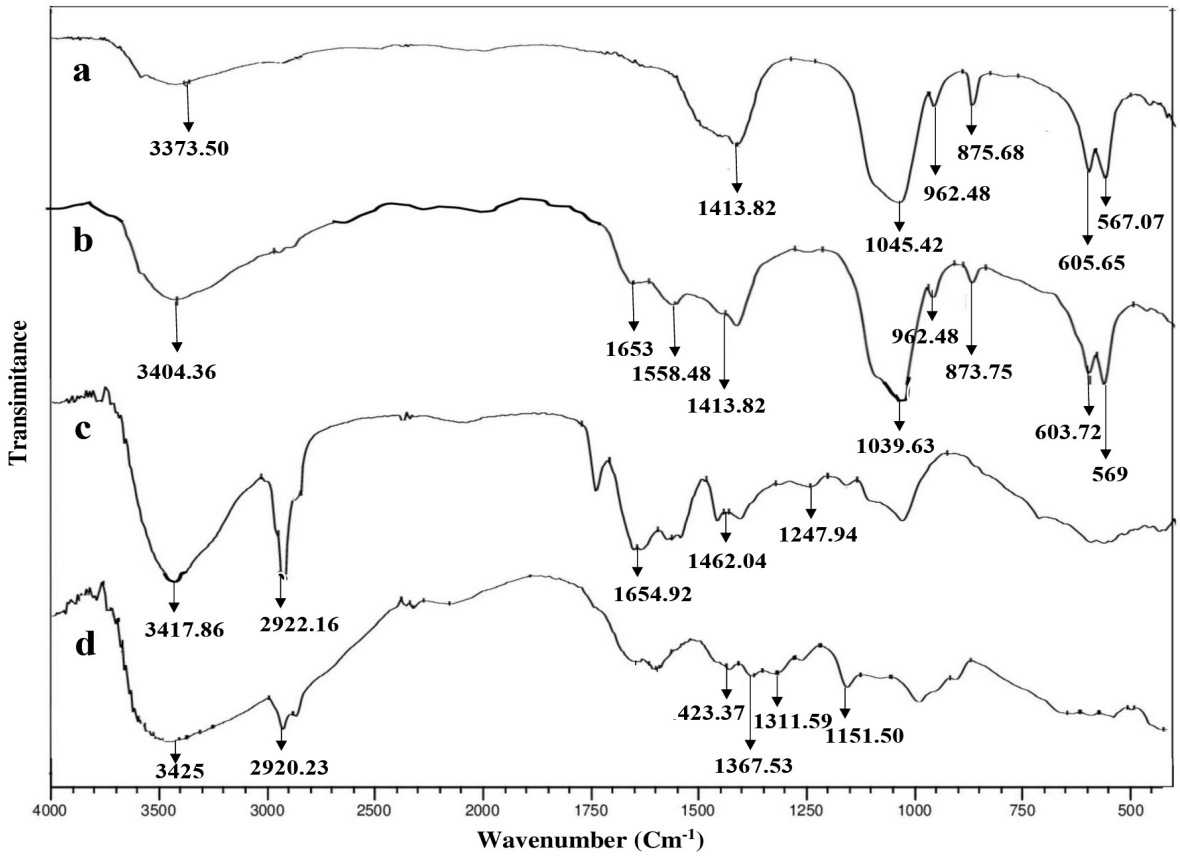


Fig.-1: The FTIR spectrum; (a) HA; (b) HA/Col/Chi; (c) Col; (d) Chi

X-Ray Diffractogram of HA/Col/Chi Composite

The XRD pattern of HA from tutut (*Bellamya javanica*) shells is shown in Fig.-2a. The phase identification was matched with the JCPDS file No. 09-432 and showed that has a single phase of HA. The degree of crystallinity of HA was obtained at 67.71 % and crystallite size of HA was found at 20.10 nm determined using Debye Scherrer equation. As shown in Fig.-2a, a sharp peak and indicating the presence of HA at 2θ = 31.94º at the diffraction plane (211), 2θ = 32.77º at the diffraction plane (300), and 2θ = 25.88º at the diffraction plane (002). The result of HA/Col/Chi powder analysis can be seen in Fig.-2b, the sharp peak is shown at 2θ = 32.01º at the diffraction plane (211), 2θ = 25.82º at the diffraction plane (002), and 2θ = 33.05º at the diffraction plane (300).

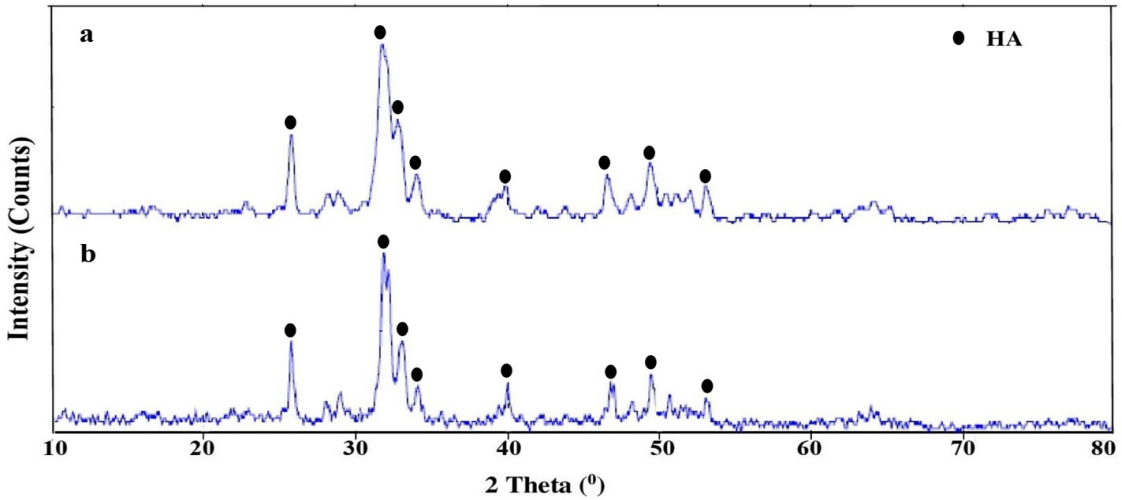


Fig.-2: X-ray diffraction patterns: (a) HA; (b) HA/Col/Chi

Elemental Composition and Surface Morphology of HA

Analysis element components of HA (Fig.-3) was investigated using energy dispersive spectroscopy. The ratio of Ca/P molar obtained is 1.98 and that the product obtained was calcium-rich HA. This value according to the literature[13], HA compounds will form at a Ca/P ratio of about 1.2-2.0. In the calcium content, the increased concentration of carbonate in the mixing reaction causes the absorption of phosphate ions to be inhibited so that the ratio of Ca/P increases.

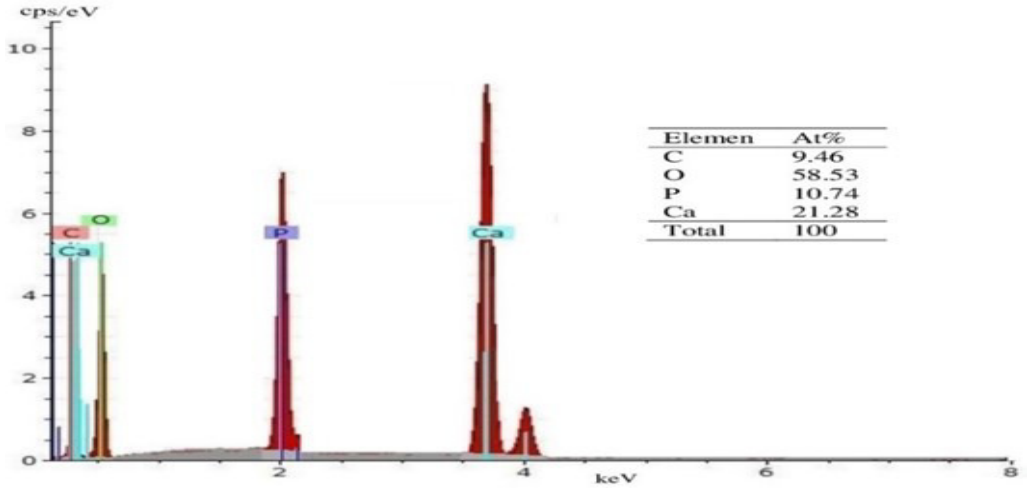
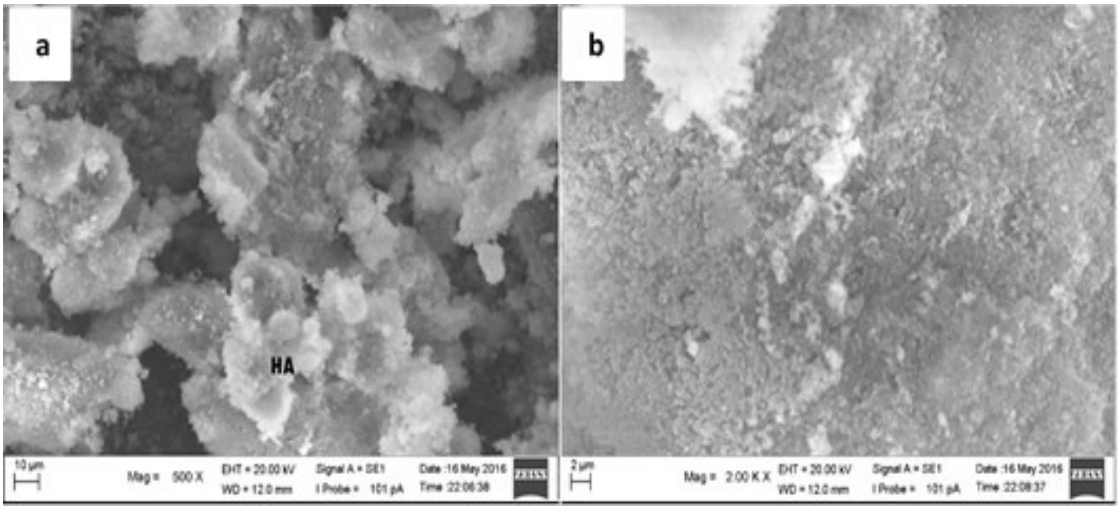


Fig.-3: Elemental Composition of HA

In the HA analysis by scanning electron microscopy (Fig.-4) showed the formation of HA in the presence of agglomeration and has an irregular shape. In addition, has a size of 2.76 - 6.48 μm and homogeneous. HA particle shape has a granular shape resembling a sphere.



.

Fig.-4: Morphology of SEM of HAp: (a) 500x, (b) 1000x

CONCLUSION

Synthesis of HA/Col/Chi composite has been successfully performed by using *ex situ* wet precipitation method. X-ray diffraction patterns of HA/Col/Chi composite showed that has a single phase of HA. The spectrum analysis of HA/Col/Chi composite showed the presence of HA, collagen, chitosan and showed the presence of molecular interactions between -OH, -COOH, $-NH_2$ on collagen chains and -OH, $-NH_2$ in chitosan, and there was an interaction between HA and amide I and amide II in collagen.

REFERENCES

1. M. Mahabole, M. Bahir, N. Kalyankar, R. Khairnar, *Journal of Biomedical Science and Engineering*, **5**, 396 (2012).
2. M. Javidi, S. Javadpour, M. E. Bahrololoom, J. Ma, *Materials Science and Engineering C*, **28(8)**, 1509 (2008).
3. A.R. Vaccaro, *Orthopedics*, **25(11)**, 571 (2002).
4. C. M. Serre, M. Papillard, P. Chavassieux, G. Boivin, *Biomaterials*, **14(2)**, 97 (1993).
5. R. Z. Wang, F. Z. Cui, H. B. Lu, H. B. Wen, C. L. Ma, H. D. Li, *Journal of Materials Science Letters*, **14(7)**, 490 (1995).
6. A. Scabbia, L. Trombelli, *Journal of Clinical Periodontology*, **31(5)**, 348 (2004).
7. D. A. Wahl, J.T. Czernuszka, *European Cells and Materials*, **28(11)**, 43 (2006).
8. P. Wang, L. Zhao, J. Liu, M. D. Weir, X. Zhou, H. H. K. Xu, *Bone Research*, **2**, 4017 (2014).
9. R. L. Baby, I. Hasan, K.A. Kabir, M.N. Naser, *J. Sci. Res.*, **2(2)**, 390 (2010).
10. P. Kim, S. Wakai, S. Matsuo, T. Moriyama, T. Kirino, *Journal of Neurosurgery*, **88(1)**, 21 (1998).
11. K. Matmaroh, S. Benjakul, T. Prodpran, A. Encarnacion, H. Kishimura, *The Journal of Food Chemistry*, **129(3)**, 1179 (2011).
12. XL. Wang, XD. Li, XM. Wang, J. Lu, HC. Zhao, XD. Zhang, ZW. Gu. *Key Engineering Materials*. **332**, 415 (2007).
13. S. Larsen, A. E. Widdowson, *Experientia*, **27(4)**, 483 (1971).

[RJC-1768/2017]