Study on the Morphology and Molecular Vibration of Biphasic Biomaterials from White Coral (*Anthozoa Cnidria*) by SEM and FTIR Analysis

Khin Myat Thu[1], Aye Aye Thant[2], Win Win Thein[3]

Abstract

Bioceramics made of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$: HAP] would be good example of bioactive materials. On the other hand, bones graft made of Beta-Tricalcium Phosphate [$Ca_3(PO_4)_2$: β-TCP] appears to be an example of bioresorbable materials. Based on this background, Biphasic Calcium Phosphate comprising of Hydroxyapatite and Beta-Tricalcium Phosphate has been prepared in this work. White Coral (*Anthozoa Cnidria*) has been used to produce biphasic calcium phosphates by mechano-chemical method. Biphasic calcium phosphate has been formed at the calcination temperatures of 900° C, 1000° C and 1100° C. X-Ray Diffraction (XRD) analysis has confirmed the presence of HAP and β-TCP in major proportions along with Calcium Carbonate ($CaCO_3$) and Calcium Oxide (CaO) in traces revealing the biphasic calcium phosphate nature of synthetic powders. Fourier Transform Infrared (FTIR) spectroscopy has also been confirmed the presence of various chemical ions groups which support the XRD results. The surface topography, morphology and agglomerated distribution of the biphasic particles HAP and β-TCP has been characterized by using Scanning Electron Microscopy (SEM).

Keywords: hydroxyapatite, beta-tricalcium phosphate, white coral

Introduction

Biphasic biomaterials, hydroxyapatite and beta-tricalcium phosphate are chemically similar to the inorganic component of bone matrix. It is a class of calcium phosphate-based bioceramic. HAP products are poorly resorbable, which retain for years after the implantation. β-TCP has much faster resorption rate (Caroline Vitoria *et al.*, 2002). However, to control the resorption rate of biphasic calcium phosphates (BCP) with HAP and β-TCP which have been tried to investigate. The resorption rate of BCP depends on the molar ratio of β-TCP/HAP in the mixture. The ratio of HAP and β-TCP is higher, the resorption is faster (Zhang Xing, 2007). Biphasic calcium phosphate HAP and β-TCP in powder form is frequently used in biomedical applications such as prosthetic implants and coating implants (Teerawat Laonapakul, 2015). This is due to its excellent biocompatibility (ability of material to perform with an appropriate host response under specific condition), bioactivity (ability of material to provide an appropriate scaffold for bone formation) and osteoconductivity (formation of bone-like apatite on their surface with a strong bone-calcium phosphate biomaterial interface) and its chemical and structural similarity with natural bone mineral (Zhang Xing, 2007). Mechano-chemical reaction is a process that is strong mechanical force proceeds materials destruction and causes a formation of a different structure. Mechano-chemical method may be employed in the synthesis of materials and replaced the solid state reaction at high temperature. Mechano-chemical method has been widelyused in synthesis of advanced materials, covered almost all aspects of material science. Mechano-chemical method is a simple, environmental, low-cost technology (Hao *et al.,* 2012).

[1] Demonstrator, Department of Physics, Hinthada University
[2]Professor, Dr, Department of Physics, University of Yangon
[3]Professor and Head, Dr, Department of Physics, Hinthada University

To achieve biphasic calcium phosphate, mechano-chemical method is used in the process and the calcium carbonate skeleton of marine coral is converted into HAP and β-TCP. After the preparation process, studies on the characterization of biphasic biomaterials with HAP/β-TCP prepared from white coral (*Anthozoa Cnidaria*) have been performed.

Figure (1) The white coral *Anthozoa Cnidaria* obtained from Chaung Thar Beach, Ayeyarwady Division, lower part of Myanmar.

Experimental Procedure

Sample Preparation

Conversion of coral to calcium phosphate biphasic biomaterials followed the following procedure by using mechano-chemical method. Stoichiometric amount of 3.3 g of $(NH_4)_2HPO_4$ has been dissolved in 25 ml of distilled water. Then, required amount of 6 g of coral powder has also been dissolved in 300 ml of distilled water. And then the ammonium solution has been added and gradually dropped at a rate of 2ml min-1 into coral solution while heating at 80 ± 5° C on a hot plate with magnetic stirrer. This action vessel has been covered with rubber cork to prevent evaporation. The reaction has been kept under stirring (800 rpm) for 24 hours. After heating the mixture for 24 hours, pH of reaction mixture has been monitored with pH paper at the end of experiment. After that solids have been separated by centrifuging with Gallenkamp Junior Centrifuge for 1 min at 3660 rpm. The solid have been washed triple times with distilled water and separated by centrifuge for 5 min, then dried overnight in an oven at 80° C. After that the sample has been ground with agate motor and heat treatments at 900° C, 1000° C and 1100° C for 2 hours in each. The molecular vibrations have been examined by Fourier Transform Infrared Spectroscopy (FTIR). The surface morphology has been studied by Scanning Electron Microscopy (SEM). Figure (2) describes flowchart for the sample preparation and characterization of biphasic biomaterials.

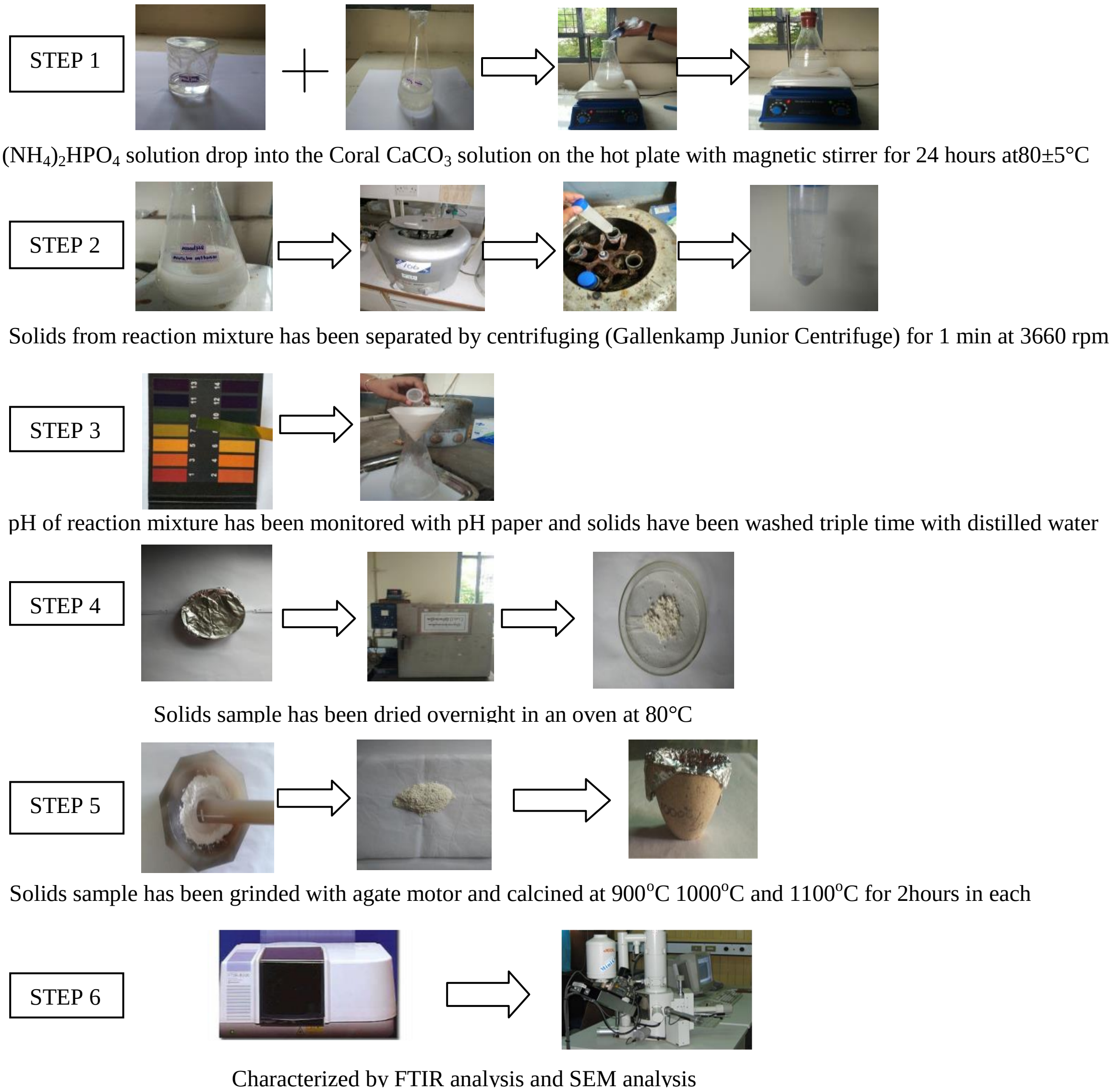

Figure (2) Procedure for the sample preparation of the biphasic biomaterials from white coral.

Results and Discussion

FTIR Analysis of Biphasic Biomaterials

It is found that FTIR analysis strongly supported the XRD result (Hanno, 2001). The most characteristic chemical groups in the FTIR spectrum of synthesized biphasic biomaterials are PO_4^{3-}, OH^-, CO_3^{2-} and HPO_4^{2-} (Berzina-Cimdina, *et al.*, 2012). The FTIR spectra has been recorded in the wave number region 2000-400 cm^{-1} by using Fourier Transform Infrared spectrometer (FTIR-8400 Shimadzu) (Berzina-Cimdina, *et al.*, 2012).

The absorption peak of O-H bending mode is the evidence of the presence of absorbed water that appear after the calcination temperature of 900° C, 1000° C and 1100° C. The other vibration modes of structural OH group indicate the existence of a Ca-O phase in the structure (Liga Berzina *et al.,* 2012). In the FTIR analysis, it has been confirmed that phosphate groups represent biphasic biomaterials, HAP/β-TCP. The bending vibration of PO_4^{3-} has been formed by band located at 560-610 cm^{-1} all the calcination temperatures. According to the FTIR analysis, PO_4^{3-}group of HAP and β-TCP at calcination temperature of

900° C is sharper than the other calcination temperatures. This again confirmed that the biphasic calcium phosphate can be produced in the calcination temperature of 900° C. FTIR Absorption band cm^{-1} and type of molecules and vibration mode of biphasic HAP/β-TCP with different calcination temperature has been described in table (1).

Table (1) FTIR Absorption band cm^{-1} and type of molecules and vibration mode of biphasic HAP/β-TCP with different calcination temperatures.

T°C / Functional Groups	Before Calcination	900°C	1000°C	1100°C
	Absorption band, cm^{-1}			
CO_3^{2-} (O-C-O)	1419.66, 1458.23	1427.37	1471.74	1421.58, 1633.76
HPO_4^-	864.14	875.71	875.71	875.71
OH^-(HAP)	-	3485.49	3419.90	3462.70
OH^- (Ca-O)	-	-	3639.80	3641.73
PO_4^{3-}	1035.81	1043.52, 1095.60	1043.52	1045.45
PO_4^{3-} (HAP)	567.09, 603.76	574.81, 601.81, 962.51	582.52	567.09

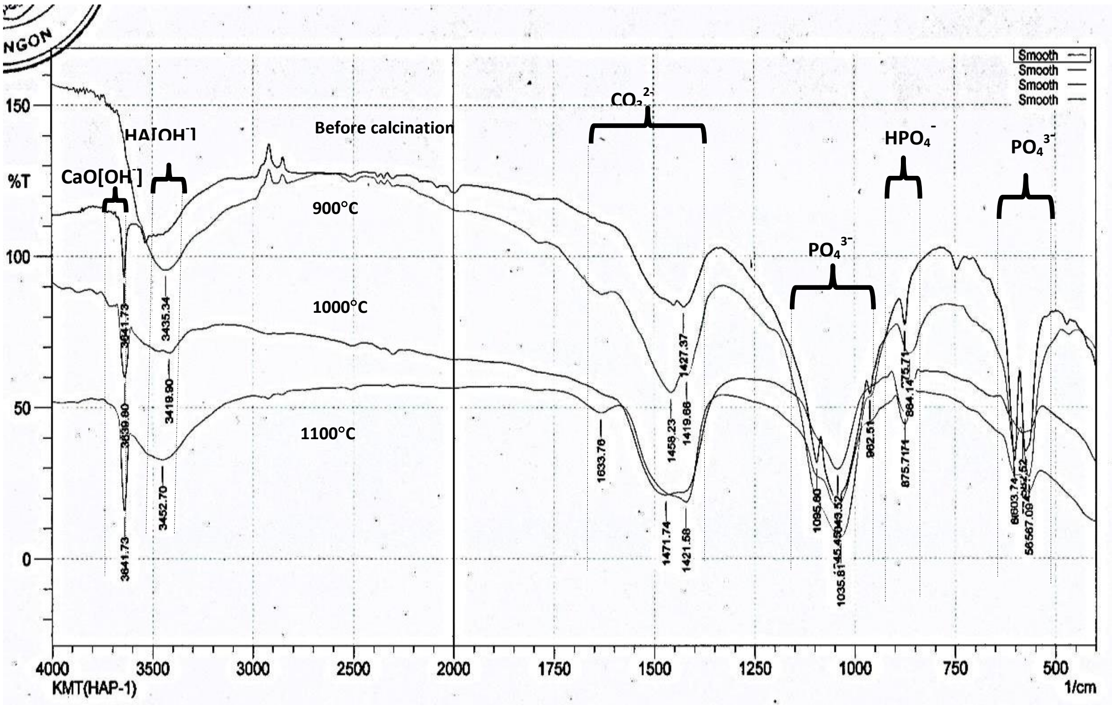

Figure (3) FTIR spectra of biphasic HAP/ β-TCP with different calcination temperature (a) before calcination (b) 900°C (c) 1000°C (d) 1100°C.

SEM Analysis of Biphasic Biomaterials

Morphological properties of biphasic biomaterials have been determined by using SEM (model: JEOL-JSM 5610LV) in this work (Innocent *et al.*, 2015). The SEM micrographs of HAP and β-TCP with different calcination temperatures are as shown in figure (4). The SEM images of all powders exhibit in agglomerated nature of irregular grain. It has observed that, the grain size of the sample is gradually increased from 1.7μm to 3.7 μm for HAP and 1.1 μm to 2.4 μm for β-TCP respectively due to the different calcination temperatures. It is important information to investigate the porosity of the sample and determine for the circulation of the physiological fluid in biomedical purpose (Maninder Singh *et al.*, 2014). The grain size of the sample with different calcination temperature is shown in table (2).

Table (2) The average grain size of the Biphasic Calcium Phosphate HAP and β-TCP.

Condition	**Grain size of HAP (μm)**	**Grain size of β-TCP (μm)**
Before calcination	1.70	1.11
Calcination at 900°C	2.06	1.20
Calcination at1000°C	3.00	2.22
Calcination at 1100°C	3.70	2.35

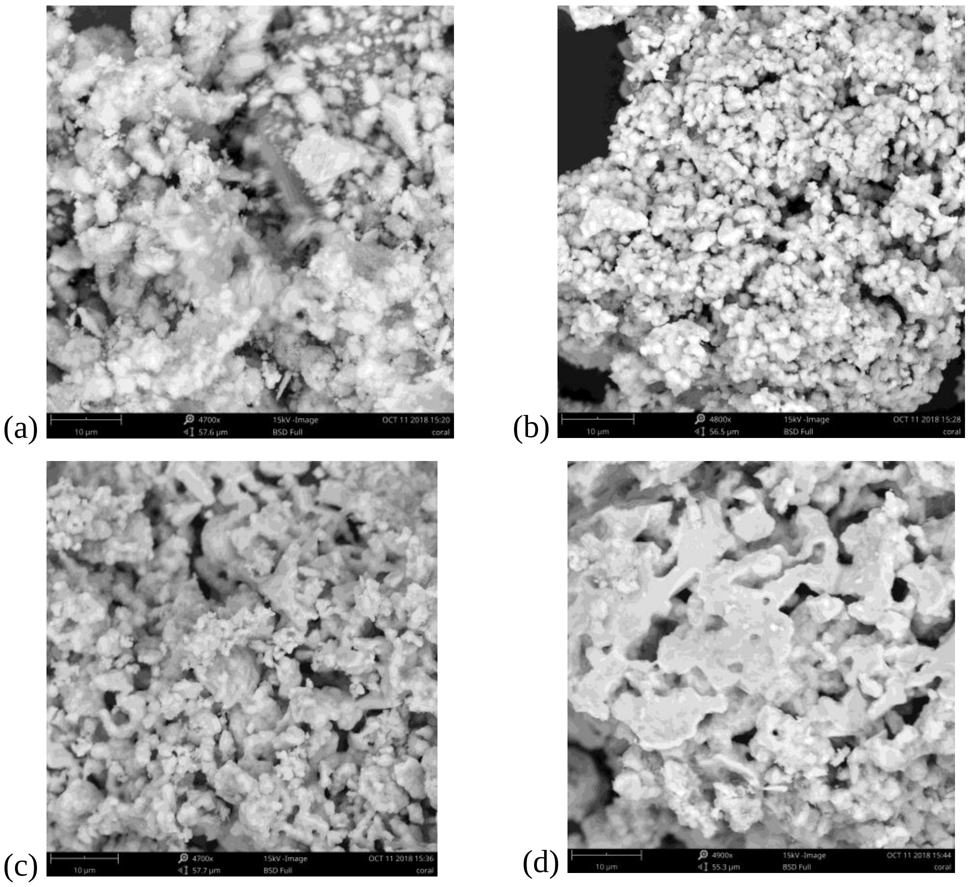

Figure (4) The SEM micrographs of HAP and β-TCP with different calcination temperatures (a) before calcination, (b) 900°C, (c) 1000°C, (d) 1100°C.

Conclusion

Biphasic calcium phosphate has been prepared from white coral and diammonium hydrogen phosphate using mechano-chemical method. The FTIR result of biphasic calcium phosphate has shown the presence of PO_4^{3-} group in HAP and β-TCP. From the SEM micrographs, the grain shape of the sample has been found to the non-uniform circular shape and grain sizes are actually increased and some pores have been formed in the sample. It has been suggested that white coral can be successfully converted to biphasic calcium phosphate by studying on their morphology and molecular vibration of biphasic biomaterial HAP and β-TCP.

Acknowledgements

We would like to express our gratitude to Dr Tin Htwe, Rector of Hinthada University, and Dr Mar Lar, Pro-Rector of Hinthada University, for allowing us to submit this research paper. Our sincerest gratitude also goes to the Research Committee members of Hinthada University, for organizing the journals and giving us a chance to get involved in this research journal. Last but not least, we are grateful to all the staff in Universites' Research Center for helping us to do this research.

References

Berzina-Cimdina L., and Borodajenko, N., (2012). " Infrared Spectroscopy", Material Science, Engineering and Technology, Intech publisher, Croatia.

Caroline Vitoria, E. and Gnanan, F. D., (2002). "Synthesis and Characterization of Biphasic calcium phosphate", Center of Ceramic Technology, Department of Physics, Anna University.

Hanno Zur Loye, (2001). "X-Ray Diffraction, How it works what it can and what it cannot tell us", University of South Carolina.

Innocent Meachal, J.,Upsorn Booyang, Sophie Cazalbou, Besim Ben-Nissan, Cedric Charvillat, Faik N Oktar and David Grossin, (2015). "Comparative study of Coral Conversion, Part 2: Micro structural evolution of calcium phosphate", Department of Chemistry and Forensic Science, University of Technology Sydney.

Maninder Singh Mehta and Ravinder Pal Singh, (2014). " Effect of Aging Time and Sintering temperatures on the Structral, Morphological and Thermal properties of Coralline Hydroxyapatite", Sri Guru Granth Sahib World University, Fatehgarh Sahib, Punjab, India.

Liga Berzina-Cimdina and Natalija Borodajenko, (2012)."Research of Calcium Phosphates Using Fourier Transform Infrared Spectroscopy", Riga Technical University, Institute of General Chemical Engineering, Latvia.

Hao WU and Qiang Li, (2012). "Journal Advanced ceramic", Department of Chemistry, East China National University, Shanghai 200062, China.

Teerawat Laonapakul, (2015). " Synthesis of hydroxyapatite from biogenic wastes", Department of Industrial Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand.

Zhang Xing, (2007). "Preparation and characterization of Calcium Phosphate Ceramics and Composite as bone substitutes", Electronic Thesis and Dissertation, UC San Diego.