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FORMATION OF BIPHASIC BIOMATERIALS FROM WHITE CORAL (*ANTHOZOA CNIDRIA*) AND ITS CHARACTERISTICS

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Abstract

Bioceramics made of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$: HAP] would be good example of bioactive materials. In 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 (HAP- β-TCP) has been prepared in this work. White Coral (*Anthozoa Cnidria*) has been used to produce biphasic calcium phosphates by mechano-chemical method. 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. Crystallite size and lattice parameters at different calcination temperatures of 900°C, 1000°C and 1100°C have been calculated and compared. Fourier Transform Infrared (FTIR) spectroscopy has also been confirmed the presence of various chemical ions groups. The surface topography, morphology and agglomerated distribution of the biphasic particles have been characterized by using Scanning Electron Microscopy (SEM). Importantly, the hardness of the biphasic calcium phosphate at different calcination temperatures has been determined for future application as bone replacement.

Keywords: hydroxyapatite, beta-tricalcium phosphate, white coral, biphasic

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. However, it has been investigating to control the resorption rate of biphasic calcium phosphates (BCP) with HAP and β-TCP. Generally,

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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.

Biphasic calcium phosphate, HAP and β-TCP in powder form is frequently used in biomedical applications such as prosthetic implants and coating implants. 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.

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 can be employed in the synthesis of materials and replaced the solid state reaction at high temperature· It is a simple, environmental and low-cost technology and has been widely used in synthesis of advanced materials, covered almost all aspects of material science.

In order 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, characterization of biphasic biomaterials with HAP/β-TCP prepared from white coral (*Anthozoa Cnidaria*) has been performed.

Materials and Method

Calcium Source; White Corals

Coral is a living animal which is similar to all living things. It suffers from many environmental factors and fossilizes as inorganic component. In this research, non-living corals obtained from the Chaung Thar Beach, Ayeyarwady Division, lower part of Myanmar have been used (Figure 1). Prior to the preparation of biphasic biomaterials, the content of calcium in the white coral has been investigated.

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

Sample Preparation

Conversion of coral to calcium phosphate biphasic biomaterials was conducted 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 coral powder has also been dissolved in 300 ml of distilled water. Then, the ammonium solution has been added and gradually dropped at a rate of 2 ml min^{-1} into coral solution while heating at (80±5)°C on a hot plate with magnetic stirring. The reaction vessel has been covered with rubber cork to prevent evaporation and kept under stirring (800 rpm) for 24 hours. The pH of reaction mixture has been monitored at the end of the experiment. After that solids have been separated by centrifuging with Gallenkamp Junior Centrifuge for 1 min at 3660 rpm. The solid have been washed for three times with distilled water and separated by centrifuge for 5 min, followed by drying overnight in an oven at 80°C. Finally, the sample has been grounded with agate motor and heat-treated at 900°C, 1000°C and 1100 °C for 2 hours in each temperature. The structural analysis has been carried out by XRD analysis. The molecular vibrations have been examined by Fourier Transform Infrared Spectroscopy (FTIR). The surface morphology has been studied by Scanning Electron Microscopy (SEM). The hardness of the BCP pellets has been measured by using Hardness Tester (Mecmesin BFG-500N, Basic Force Guage). Figure 2 presents flowchart for the sample preparation and characterization of biphasic biomaterials.

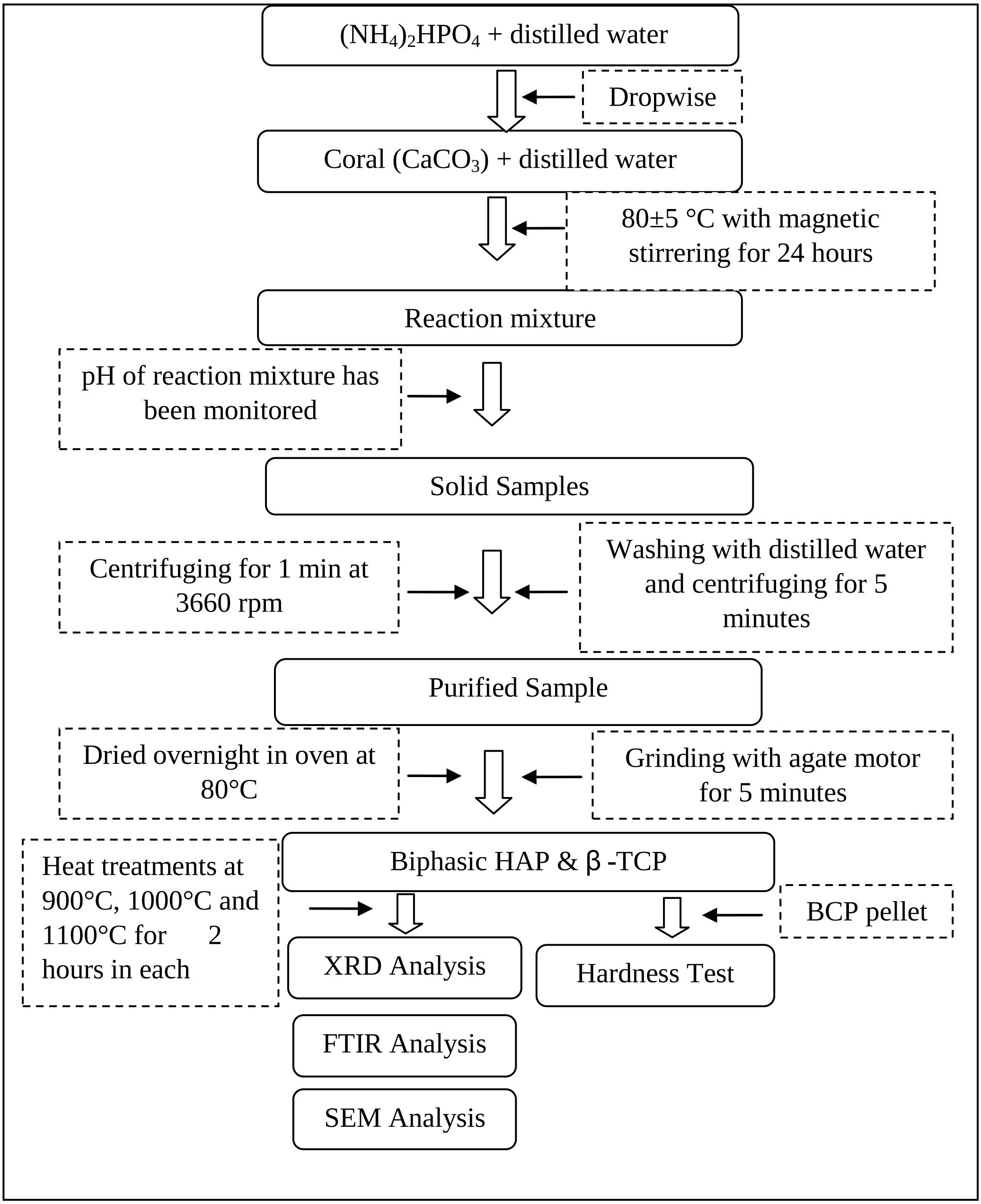


Figure 2: Flow chart of the sample preparation and characterization of biphasic calcium phosphate from white coral

Results and Discussion

Phase formation

XRD Analysis of Biphasic Biomaterials

The X-ray Diffraction has been applied to monitor the phase composition feature of the sample before and after the calcination at different temperatures of 900°C, 1000°C and 1100°C. The powder has been ground and pressed into homogeneous compacted layer in samples holder. The XRD patterns were scanned between 2θ value of 10° to 70° by using the RIGAKU, MINIFLEX600 powder X-ray Diffratometer.

Biphasic Hydroxyapatite and Beta-Tricalcium Phosphate are prepared at an aging time of 24 hours, aging temperature of 80°C followed by calcination at temperatures of 900°C, 1000°C and 1100°C. The XRD patterns of the sample without heat-treatment and those calcined at 900°C, 1000°C and 1100°C are shown in Figure 3. From the XRD analysis, diffraction peaks have been identified as the hexagonal phase of the HAP and β-TCP (ICDD- PDF Release 2015 RD). From the XRD data, weight percentages of HAP and β-TCP, lattice parameters and mean crystallite size has been calculated. The sample without heat treatment exhibits the presence of $CaCO_3$ in the spectrum. Starting from the calcination temperature of 900°C, biphasic biomaterial of HAP/β-TCP has been formed involving CaO peaks in the spectrum. However, the number of CaO peaks is gradually increased at the calcination temperatures of 1000°C and 1100°C.

From the XRD data, lattice parameters and mean crystallite size have been calculated and tabulated in Table 1 and Table 2. Crystallite size has been found to be 21.52 nm for HAP and 29.52 nm for β-TCP with poor crystallinity before calcination. After calcination at 900°C, the crystallite size of HAP and β-TCP has been increased to 56.74 nm and 44.95 nm. Again, the crystallite size of HAP and β-TCP has been gradually decreased with the higher calcination temperatures to 35.06 nm and 23.21 nm respectively at 1100°C as shown in Figure 4. The relative intensity ratio (RIR) of HAP/β-TCP in the biphasic calcium phosphate has been calculated by using the formula, $RIR = I_{\beta\text{-}TCP} / (I_{\beta\text{-}TCP} + I_{HAP})$. The obtained RIR follow the same trend as the decreasing value of crystallite size with increasing calcination temperature. Based on the XRD analysis, it has been confirmed that white coral can be utilized for preparing biphasic biomaterials, HAP/β-TCP

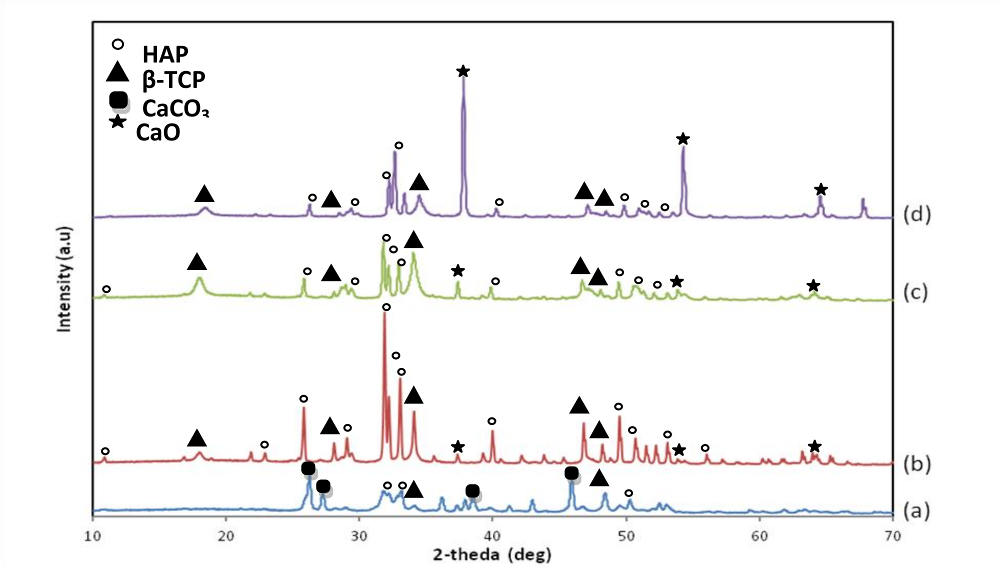


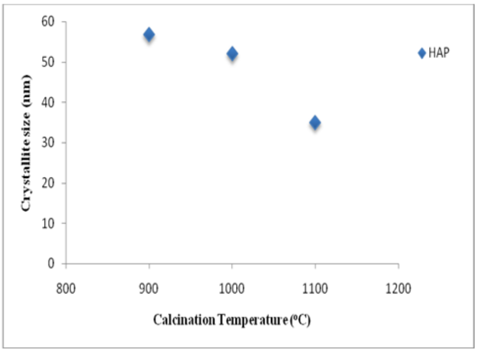
Figure 3: XRD spectra of biphasic calcium phosphate (HAP and β-TCP) with different calcination temperatures (a) before calcination, calcination at (b) 900°C, (c) 1000°C and (d) 1100°C

Table 1: Lattice constant of biphasic calcium phosphate before and after calcination

Type	Lattice Parameter	Before calcination	900°C	1000°C	1100°C
Hydroxyapatite $(Ca)_{10}(PO_4)_6(OH)_2$	a (Å)	9.413	9.384	9.405	9.321
	b (Å)	9.413	9.384	9.405	9.321
	c (Å)	6.807	6.893	6.897	6.780
β-Tricalcium Phosphate $(Ca_3)(PO_4)_2$	a (Å)	10.428	10.180	10.150	10.413
	b (Å)	10.428	10.180	10.150	10.413
	c (Å)	36.647	36.912	37.570	36.552
Aragonite $(CaCO_3)$	a (Å)	5.735	-	-	-
	b (Å)	4.948	-	-	-
	c (Å)	7.974	-	-	-

Table 2: Crystallite size of biphasic calcium phosphate before and after calcination

Type	Before calcination	900°C	1000°C	1100°C
Hydroxyapatite $(Ca)_{10}(PO_4)_6(OH)_2$	21.518 nm	56.735 nm	52.021 nm	35.056 nm
β-Tricalcium Phosphate $(Ca_3)(PO_4)_2$	29.516 nm	44.944 nm	41.944 nm	23.206 nm



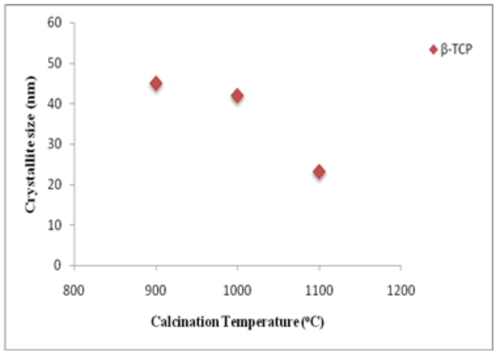


Figure 4: The Variation of crystallite size of HAP and β-TCP in biphasic calcium phosphate with different calcination temperatures

Table 3: Relative intensity ratio of biphasic biomaterials before and after calcination

Condition	Relative Intensity Ratio
Before calcination	22% HAP : 18% β-TCP: 60% CaCO3
Calcination at 900°C	70% HAP : 20% β-TCP: 10% CaO
Calcination at 1000°C	65% HAP : 13% β-TCP: 23% CaO
Calcination at 1100°C	59% HAP : 9% β-TCP: 32% CaO

Table 4: Comparison of phase analysis of white coral and biphasic calcium phosphate

Condition	Sample	Phase	Phase ID	Crystal Structure
Raw	White Coral powder	Single phase	-Aragonite ($CaCO_3$)	Ortho-rhombic
After preparation	Calcium phosphate biomaterials	Bi-phase	-Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) -Beta-tricalcium phosphate ($Ca_3(PO_4)_2$)	Hexagonal

FTIR Analysis of Biphasic Calcium Phosphate

The FTIR spectra of biphasic calcium phosphate has been recorded in the wave number region 2000-400 cm^{-1} by using Fourier Transform Infrared spectrometer (FTIR-8400 Shimadzu) and shown in Figure 5. It is found that FTIR analysis strongly supported the XRD result. The most characteristic vibrational chemical groups in the FTIR spectrum of synthesized biphasic biomaterials are found to be PO_4^{3-}, OH^-, CO_3^{2-} and HPO_4^{2-}. The absorption peak of O-H bending mode is evidence of the presence of absorbed water that appeared after the calcinations 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. 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 at 560-610 cm^{-1} for 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 stronger than those in the other calcination temperatures. This finding again confirmed the formation of biphasic calcium phosphate from white coral by using mechano-chemical method. The type of molecules and vibration mode of biphasic HAP/β -TCP with different calcination temperature have been described in Table 5.

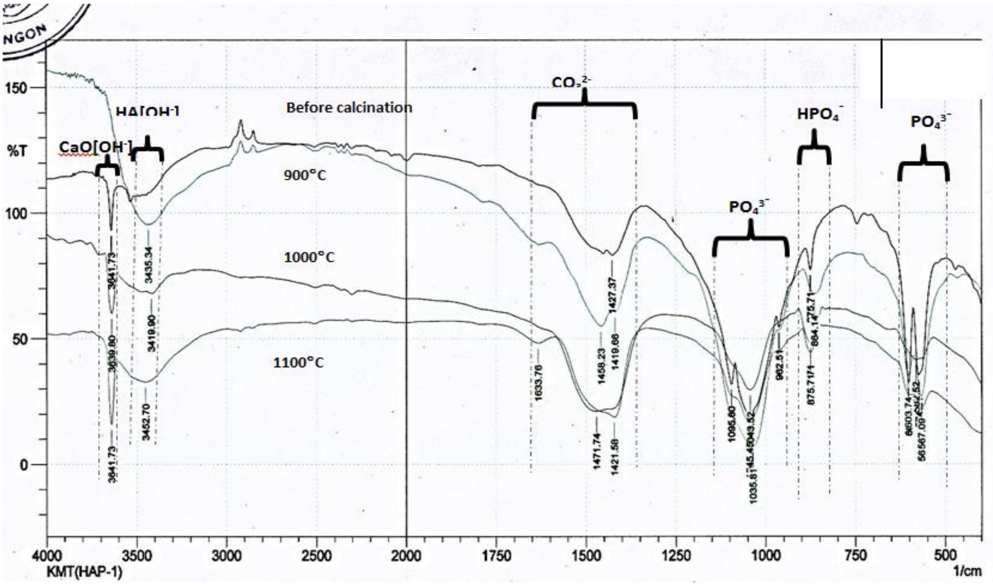


Figure 5: FTIR spectra of biphasic HAP/ β-TCP with different calcination temperatures (a) before calcination, (b) 900°C, (c) 1000°C and (d) 1100°C

Table 5:The type of molecules and vibration mode of biphasic HAP/□-TCP with different calcination temperatures

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

SEM Analysis of Biphasic Calcium Phosphate

Morphological properties of biphasic biphasic calcium phosphate has been determined by using SEM (model: JEOL-JSM 5610LV) in this work. The SEM micrographs of biphasic HAP/β-TCP at different calcination temperatures are as shown in Figure 6. The SEM images of all powders exhibit agglomerated nature with irregular grains. It has observed that, the grain size of the sample is gradually increased from 1.7 μm to 3.7 μm for HAP and 1.1 μm to 2.4 μm for β-TCP with increasing calcination temperature. It is essential to investigate the porosity of the sample and determine for the circulation of the physiological fluid in biomedical purpose. The grain size of the sample at different calcination temperatures is shown in Table 6.

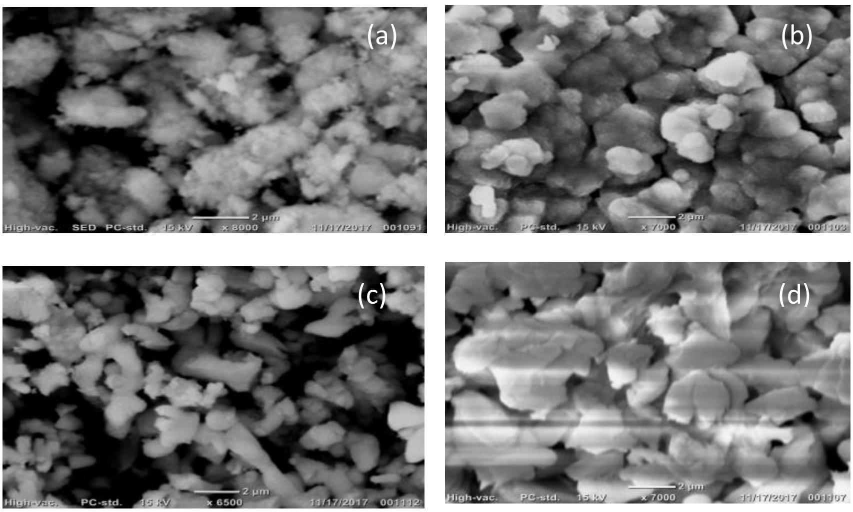


Figure 6: The SEM micrographs of HAP and β-TCP with different calcination temperatures (a) before calcination, (b) 900°C, (c) 1000°C and (d) 1100°C

Table 6: The average grain size of the Biphasic Calcium Phosphate of 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

Hardness of Biphasic Calcium Phosphate

Hardness test has been performed by Hardness Tester (Mecmesin BFG-500N, Basic Force Guage) in Department of medical research, Innsein, Myanmar. The maximum applied force of the Hardness Tester is 51 kg (1 kg = 9.80665 N). Figure 7 shows the variation of hardness with calcination temperature

It has been investigated that biphasic HAP/β -TCP pellets calcined at temperature 1100^{o}C has very low hardness than compared to those calcined at 900 ^{o}C and 1000 ^{o}C. During pelletization process, biphasic HAP/β -TCP powder calcined at temperature 1100^{o}C are difficult to make pellet as it has non-interconnected grains which is observed in SEM image. Therefore, it is worth to note that calcination temperatures of 900 ^{o}C and 1000 ^{o}C can be utilized to form dense BCP structure where the calcination temperature of 1000 ^{o}C can produce BCP with the high hardness value of 41.23 N.

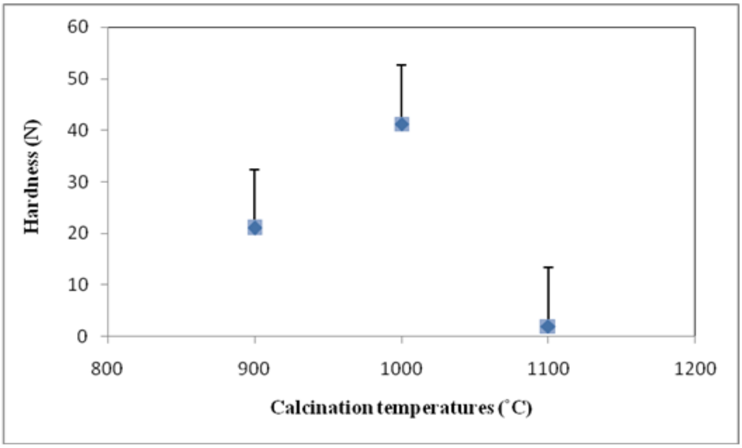


Figure 7: The variation of Hardness with calcination temperature

Conclusion

Biphasic calcium phosphate (BCP) has been prepared from white coral and diammonium hydrogen phosphate using mechano-chemical method. In present study, biphasic calcium phosphate has been crystallized at the calcination temperatures of 900°C, 1000°C and 1100°C. X-Ray Diffraction (XRD) has been 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. The FTIR result of BCP shows the presence of PO_4^{3-}group in HAP and β-TCP. The SEM micrographs proved the formation of grains in the non-uniform circular grain shape and grain size are increased with calcination temperature and some pore has been formed in the sample. Importantly, this work pointed out that dense BCP structure could unified at calcination temperatures of 900 °C and 1000 °C and the BCP with relatively higher hardness can be obtained at the calcination temperature of 1100 °C.

Based on the results obtained, it has suggested that white coral can be successfully converted to biphasic calcium phosphate (HAP/β-TCP) with optimized synthesis parameters and interesting characterizes.

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References

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

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

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

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.

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.

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.

Rebea-Naemi Rosa Cegla, Innocent MeachaJ., Besim Ben-Nissan, David Grossin, Greg Heness and Ren-Jei Chung (2014). "Comparative Study of Conversion of Coral with Ammonium Dihydrogen Phosphate and Orthophosphoric Acid to Produce Calcium Phosphates", Department of Materials Engineering Glass and Ceramics, Westerwald Campus, University of applied science, Koblenz, German.

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.