CHARACTERIZATION OF HYDROXYAPATITE-ALUMINA-ZIRCONIA BIOCOMPOSITES PREPARED FROM BIOWASTES (CLAM SHELLS)

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Abstract

Clam shells are the rich source of calcium carbonate. Calcium carbonates of clam shells are converted to hydroxyapatite (HA) by wet precipitation method, and hydroxyapatite was successfully prepared from clam shells by wet precipitation method using Ca $(NO_3)_2$, $(NH_4)_2HPO_4$, and NH_4OH as starting materials. All of the carbonate phases in shells were decomposed to calcium oxide at 900 $^{\circ}$C after 6 h heating. The calcined calcium oxide were then treated with nitric acid and crystalized to form Ca $(NO_3)_2.4H_2O$. To prepare HA powder, $(NH_4)_2HPO_4$ and prepared Ca $(NO_3)_2.4H_2O$ in ammoniacal media was kept to Ca/P stoichiometry ratio of 1.67 to produce hydroxyapatite (HA). The prepared HA powder before sintering was characterized by EDXRF, XRD, FT IR and SEM. The nano crystalline alumina and commercial zirconia were then mixed with the prepared HA in acetone medium by wet mixing method in different ratios. The prepared HA from clam shells (30 %), Alumina (10 %) and Zirconia (60 %) was designated as **CC 1** and the prepared HA from clam shell (30 %), Alumina (60 %) and Zirconia (10 %) was designated as **CC 2,** respectively. The prepared HA-Alumina-Zirconia biocomposites were characterized by EDXRF, XRD, FT IR, SEM and TEM. From EDXRF analyses, it was observed that **CC 1** had the higher relative abundance of zirconium than **CC 2**. According to XRD analyses, **CC 1** is smaller in average crystallite sizes compared to **CC 2**. Phase analyses were also carried out by XRD analysis. According to these analyses content percent of phase in **CC 2** was matched with initial ratio of composition but it was found that **CC 1** had Aluminium phase deficiency. Based on the SEM analyses, more agglomerate features were observed in **CC 1** than those in **CC 2**. From transmission electron microscope (TEM), biocomposite in 3: 1: 6 w/w ratio of HA: alumina: zirconia was found to be nano scale 20 nm. CC-2 biocomposite was chosen for further study.

Keywords: Clam Shell, Hydroxyapatite, wet precipitation method, HA-alumina-zirconia biocomposites

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Introduction

Hydroxyapatite (HA) with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ is a synthetic biomaterial. Due to its chemical and structural similarity with the mineral phase of bone and teeth, HA is widely used for hard tissues repair. As a result, this inorganic phosphate has been studied extensively for medical applications in the form of powders, composites or even coatings (Weng and Baptista, 1997). Materials which are used for the repair and reconstruction of diseased or damaged parts of the musco-skeletal system are defined as biomaterials such as hydroxyapatite (HA), bioglass, biopolymer, and Ca-phosphates etc (Piconi and Maccauro, 1999). The crystalline structure of hydroxyapatite is a hexagonal structure (Figure 1). It is not only a biocompatible, osteoconductive, nontoxic, non-inflammatory and non-immunogenic agent, but also bioactive (Agnieszka *et al*., 2009).

The composite material is usually composed of two or more components, i.e. matrix and filler called also reinforcement or more broadly dispersed phase; sometimes also additional compounds are used , mostly compatibilisers (Gergely, 2010). The matrix, known also as continuous phase, integrates filler particles and allows also shaping products appropriately and determining most of physical and chemical properties of material.

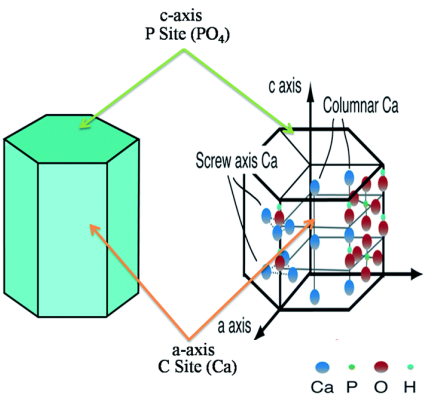


Figure 1: Structure of Hydroxyapatite (HA)

Alumina is the most widely used ceramic material due to its high hardness value, high melting point (2054 ºC), low thermal expansion and high compressive strength leading to good thermal shock resistance. Zirconia is

chemically unreactive. Zirconia is widely used in metallurgy and high temperature chemistry because of its refractory properties and chemical durability. The main use of zirconia is in the production of hard ceramics, such as in dentistry (Kim *et al.*, 1999).

In Chemistry Department, University of Yangon, researches on hydroxyapatite have been carried out in recent years. These research concerned preparation of apatitic calcium phosphate bone cements from natural resources (Thin Thin Nwe, 2005), calcium phosphate powders from waste material (egg shell) (Than Than Khaing, 2006), bioceramics iron and zinc substituted hydroxyapatite (Khin Thu Thu Min, 2006), hydroxyapatite from fish bone (Aung Win Thant, 2014) and preparation and characterization of uncalcined cow bone hydroxyapatite (Cho Lwin Lwin Khine, 2015). In Myanmar, clam shell is readily available waste material. So, this biowaste was chosen in this study for the preparation of hydroxyapatite.

The present research is aimed to prepare clam shell hydroxyapatite and hydroxyapatite alumina zirconia composite and compare their mechanical properties for teeth implants.

Materials and Methods

Sample Collection

Clam shells were collected from Sittwe Market in the Rakhine State, Myanmar.

Sample Preparation

Shells were washed with tap water and rinsed in distilled water to remove the mud, sand and other impurities. The cleaned shells were dried in direct sunlight for 2 days. The cleaned and dried clam shells were ground in mortar and then sieved. Dry and cleaned clam shells were calcined in an electrical muffle furnace (D Lab Tech model-LEF-1055-2) at 900 $^{\circ}$C so that all organic matters and proteins escaped out. Clam shells were transformed into calcium oxide by releasing carbon of 900 ºC for 4 h. CaO obtained from clam shells was then converted into calcium nitrate [Ca $(NO_3)_2$] in concentrated nitric acid under constant stirring. To synthesize HA powder 0.5 M $(NH_4)_2HPO_4$ solution was prepared and added with continuous stirring

to a solution of 0.5 M $Ca(NO_3)_2.4H_2O$ to maintain 1.67 Ca/P ratio and vigorously stirred for 30 min. At the same time pH was observed and then adjusted to 10.5 by adding 1 M ammonium hydroxide [NH_4OH] solution drop wise. The suspension was well stirred using magnetic stirrer for 2 h and aged for overnight with ice bath. A white precipitate was formed (Wang, 2010). The precipitates were subjected vacuum filtrating using Buchner funnel, repeatedly washed to obtain pH 7 with deionized water and filtered again. The precipitates were dried at 800 ºC for 48 h. Dried lumps of powders were ground by a clean pestle and mortar; a yellowish white hydroxyapatite powder was obtained (Hasan *et al.,* 2015). The products obtained from preparation steps are shown in Figure 2.

(a) (b) (c)

Figure 2: Preparation of hydroxyapatite from clam Shells; (a) raw clam shells (b) calcium carbonate powder (c) crystalline form of HA powder

The prepared nanocrystalline hydroxyapatite (HA) powder was mixed with alumina and zirconia with various ratios in acetone medium. The samples were stirred for 1 h at room temperature and then dried in oven at 100 ºC for 1 h. The obtained samples were crushed with mortar and pestle and then pressed into pellet. The obtained pellets were sintered at 1000 ºC for 1 h. Hydroxyapatite-alumina- zirconia biocomposites were obtained (Kim *et al.,* 1999). The products obtained from preparation are shown in Figure 3. The prepared HA from clam shells (30 %), Alumina (10 %) and Zirconia (60 %) was designated as **CC-1** and the prepared HA from clam shell (30 %), Alumina (60 %) and Zirconia (10 %) was designated as **CC-2,** respectively.

Figure 3: Pellets of hydroxyapatite-alumina-zirconia biocomposite samples (CC 1 and CC 2)

Qualitative Elemental Analysis by Energy Dispersive X Ray Fluorescence (EDXRF)

Relative abundance of elements in hydroxyapatite-alumina-zirconia biocomposites were qualitatively determined by EDXRF analysis using EDX-702 spectrometer (Shimadzu Co.Ltd, Japan).

Fourier Transform Infrared (FT IR) Analysis

Fourier transforms infrared (FT IR) spectra of the hydroxyapatite-alumina-zirconia biocomposites were recorded on a FT IR spectrometer (FT IR-8400 Shimadzu, Japan). FT IR analysis was in the range of wave number from 4000 to 500 cm^{-1}.

X-Ray Diffraction (XRD) Analysis

X-Ray Diffraction pattern of the sample was recorded on X-Ray Diffractometer (Rigaku, Tokyo, Japan) using CuK_{α} radiation (λ= 1.54 A°) at 40 kV and 40 mA. The diffraction angle ranged from 10° to 70° of 2θ.

Examination of Surface Morphology by SEM

Surface morphologies of the hydroxyapatite-alumina-zirconia biocomposites were investigated by scanning electron microscope (model JEOL-JSM-5610 LV, Japan) operating at an accelerating voltage of 15 kV and 1000 X magnification.

Transmission Electron Microscope (TEM) Analysis

The morphological characteristics of the synthesized hydroxyapatite-alumina-zirconia biocomposite in 3: 1: 6 w/w ratio of HA: alumina: zirconia was examined by using TEM-HITACHI 7700 transmission electron microscope analyzer.

Results and Discussion

Elemental composition of biocomposites by EDXRF Analysis

Figures 4 and 5 show the relative percentages of elements present in hydroxyapatite-alumina-zirconia biocomposites prepared CC-1 and CC-2 from clam shells determined by EDXRF analysis (Table 1). It can be seen that calcium, zirconium, aluminium and phosphorus were found as main components.

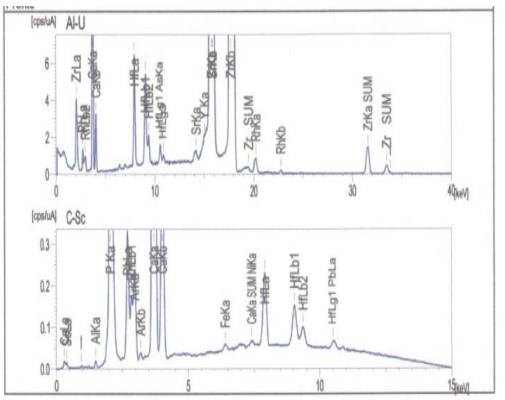


Figure 4: EDXRF spectra of biocomposites from clam shells (CC 1)

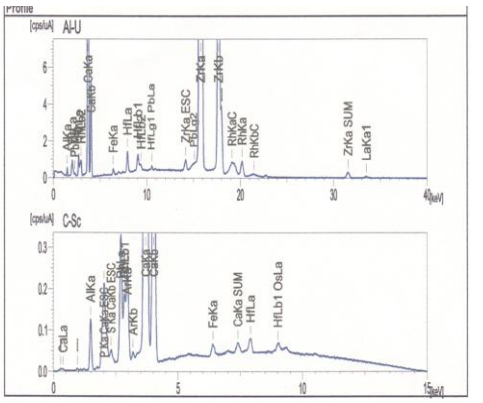


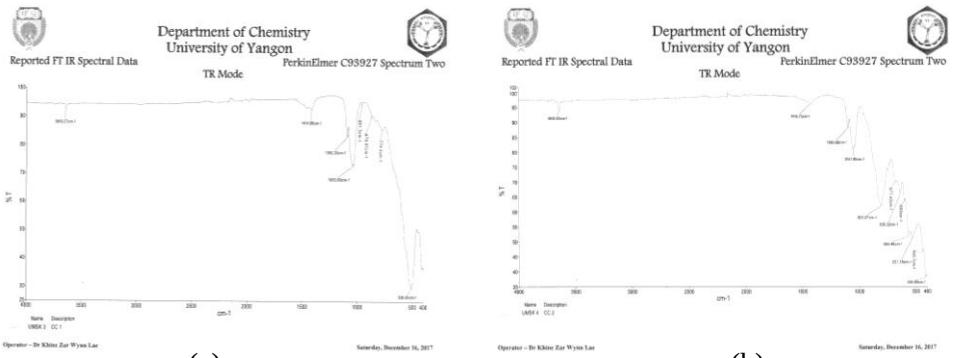
Figure 5: EDXRF spectra of biocomposites from clam shells (CC 2)

Table 1: Relative Abundance of Elements in Prepared Samples (CC 1 and CC 2) from EDXRF Analysis

Elements	Relative Abundance of elements in the Sample (%)	
	CC 1	**CC 2**
Ca	24.060	53.540
Al	1.617	22.224
Zr	55.561	18.655
P	16.833	4.091
Fe	-	0.114
Hf	1.796	0.797

FT IR Analysis

Figures 6 (a) and 6 (b) show FT IR spectra of the prepared hydroxyapatite-alumina-zirconia biocomposite samples CC-1 and CC-2. The peaks at 3643 and 3642 cm^{-1}were appeared due to stretching of O-H from $Ca_{10}(PO_4)_6(OH)_2$. The peaks at 1092, 1093, 1033, 1047,565 and 563 cm^{-1} were assigned to PO_4^{3-} group. The peaks at 806 $cm^{-1}$was assigned to stretching of Al-O bond. The peaks at 507 and 531 cm^{-1}were assigned to stretching of Zr-O bond. The possible band assignments of the bands are listed in Table 2.



(a) (b)

Figure 6: FT IR spectra of hydroxyapatite-alumina-zirconia biocomposite (a) CC 1 (b) CC 2

Table 2: FT IR Spectral Band Assignment Data of Hydroxyapatite-Alumina-Zirconia Biocomposite Samples (CC 1 and CC 2)

No	Wavenumber (cm^{-1})		Reported Values	Assignment
	CC 1	CC 2		
1	3643	3642	3500-3100*	Stretching of O-H from $Ca_{10}(PO_4)_6(OH)_2$
2	1414	1416	1629-1400*	Stretching of C-O from CO_3^{2-}
3	1092	1093	1200-1100*	Stretching of P-O from PO_4^{3-}
4	1033	1047	1200-900*	Bending of P-O from PO_4^{3-}
5	806	805	900-700**	Stretching of Al-O bond
6	565	563	700-500**	Bending of P-O from PO_4^{3-}
7	507	531	525-500*	Stretching of Zr-O bond

* Nakamoto, 1970
** Figueiredo.*et al*., 2010

XRD Analysis

Figures 7 and 8 show X-ray diffractograms of the prepared hydroxyapatite-alumina-zirconia biocomposite samples CC-1 and CC-2. The planes of hydroxyapatite (1 1 2), (2 1 1), (2 0 3) and (3 2 2) were still present in samples CC-1 and CC-2. The planes of alumina (1 1 1) and (3 1 0) were also present in samples CC-1 and CC-2. The planes of zirconia ($\bar{1}$ 1 1) and (1 1 1) were still present in CC-1 and CC-2 samples. The results were in good agreement with literature (Danilchenko *et al*., 2009)

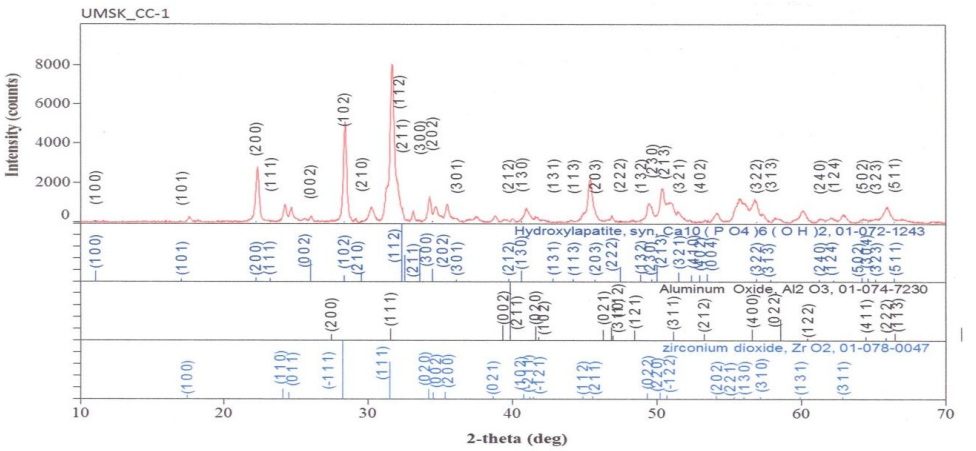


Figure 7: XRD diffractogram of hydroxyapatite-alumina-zirconia biocomposite sample CC 1

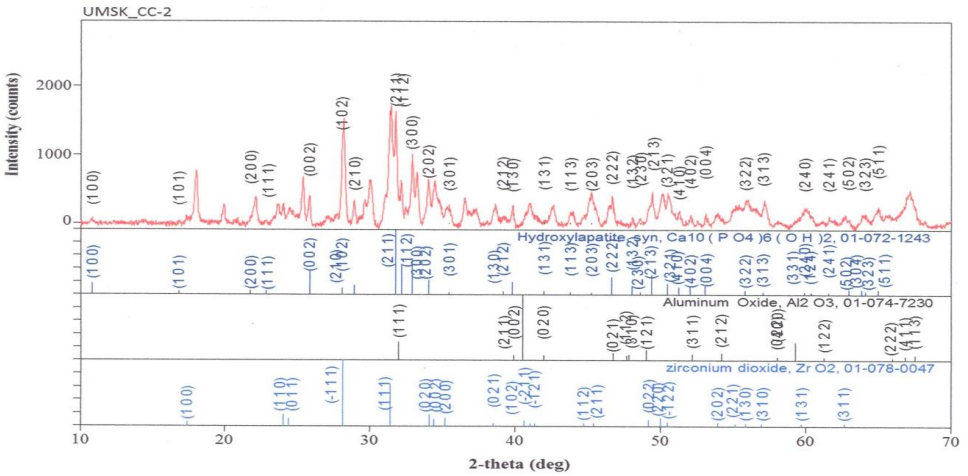
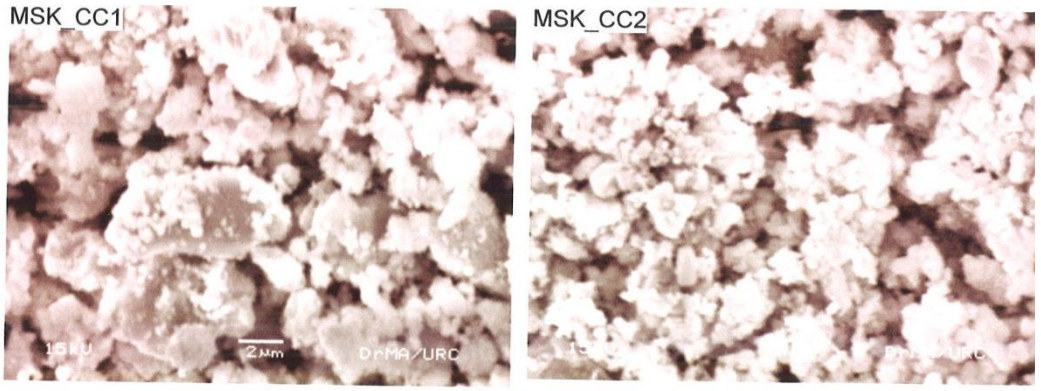


Figure 8: XRD diffractogram of hydroxyapatite-alumina-zirconia biocomposite sample CC 2

SEM Analysis

Figure 9 shows the morphology of prepared hydroxyapatite-alumina-zirconia biocomposite. According to SEM micrographs, CC-1 was seen to be in more porous nature and more agglomerate.



(a) **(b)**

Figure 9: SEM images of hydroxyapatite-alumina-zirconia biocomposite samples (a) CC 1 (b) CC 2

TEM Analysis

Figure 10 shows the morphology of prepared hydroxyapatite-alumina-zirconia biocomposite. According to TEM image, The particle sizes of hydroxyapatite-alumina-zirconia composite CC-2 was found to be 20 nm from TEM image by JRE 6 (Java Runtime Environment) software.

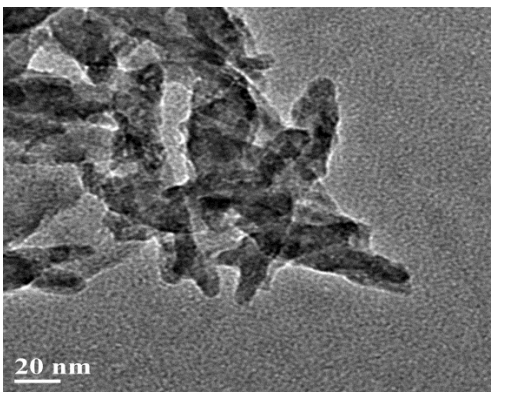


Figure 10: TEM images of hydroxyapatite-alumina-zirconia biocomposite samples CC 2

Conclusion

The present work is based on the utilization of biological waste (clam shells) to produce hydroxyapatite for biomedical applications. The clam shell was found to be a promising source of calcium for preparing hydroxyapatite with excellent properties essential for hard tissue replacement by wet precipitation method. The prepared biocomposites were characterized by EDXRF, FTIR, XRD, SEM and TEM. FT IR spectrum of hydroxyapatite-alumina-zirconia biocomposite confirmed the formation of hydroxyapatite-alumina-zirconia composite. The prepared samples CC-1 and CC-2 were determined by XRD leads to formation of desired phases. CC-1 is more agglomerate than CC 2. Porous nature was observed in CC-2. CC-2 has continuous distribution of particle size. The particle sizes of hydroxyapatite-alumina-zirconia composite CC-2 was found to be 20 nm from TEM image by JRE 6 (Java Runtime Environment) software. It may be concluded that hydroxyapatite-alumina-zirconia composite weight ratio of 3:6:1 and 3:1:6

were successfully prepared from biowastes (clam shell) by wet mixing method.

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References

Agnieszka, S., Zygmunt, K. and Zbigniew, W. (2009). "Preparation of Hydroxyapatite from Animal Bones". *Journal of Bioengineering and Biomechanics*, vol.11 (4), pp. 23-28

Choi, S.R. and Bansal, N.P. (2005). "Mechanical Behavior of Zirconia/alumina Composites". *Ceramics International*, vol. 31, pp. 39-46

Cho Lwin Lwin Khine, San Nwe Zin and Ni Ni Sein. (2017 a). "Effect of Calcination on Morphological, Structural and Thermal Properties of Hydroxyapatite (HAp) Prepared from Cow Bone". *J.Myan. Acad. Tech*, vol. 17(1), pp. 68-81

Danilchenko, S.N., Kalinkevich, O.V. and Pogorelov, M.V. (2009). "Chitosan-hydroxyapatite Composite Biomaterial Made by a One Step Co precipitation Method: Preparation, Characterization and *in vivo* Tests". *Journal of Biological Physics and Chemistry*, vol. 9, pp. 119-126

Figueiredo, M., Fernando, A., Martins, G., Freitas, J., Judas, F. and Figueiredo, H. (2010)."Effect of Calcination Temperature on the Composition and Microstructure of Hydroxyapatiten Derived from Human and Animal Bone". *Ceramics International*, vol. 36, pp. 2383-2393

Gergely, G. (2010). "Preparation and Characterization of Hydroxyapatite from Egg Shell".*Ceramics International*, vol. 36, pp. 803-806

Hasan, M., Gafur, M.A., Qadir, M.R. and Karim, M.M. (2015). "Chemical Characteristics of Hydroxyapatite from Oyster Shell by Thermo-Chemical Processes". *Int. J. of Innovative Research in Science, Engineering and Technology*, vol.4(7), pp. 5039-5045

Khin Thu Thu Min. (2006). *Studies of Bioceramics Iron and Zinc Substitute Hydroxyapatites*. PhD Dissertation. Myanmar; Department of Chemistry, University of Yangon

Kim, H., Kong, Y., Kim, S. and Kim, H. (1999). "Reinforcement of Hydroxyapatite Bioceramic by Addition of ZrO_2 Coated with Al_2O_3". *Journal of American Ceramic Society*, vol. 82(11), pp. 2963-3031

Nakamoto, K. (1970). *Infrared Spectra of Inorganic and Coordination Compounds*. New York: 2nd Edition, Wiley Interscience Publication, pp. 233-235

Piconi, C. and Maccauro, G. (1999). "Zirconia as a Ceramic Biomaterial". *Biomaterials*, vol. 20(1), pp. 1-25

Than Than Khaing. (2006). *Process Development of Biocompatible Calcium Phosphate Powders from Waste Material (Egg shell)*. PhD Dissertation, Myanmar; Department of Chemistry, University of Yangon

Thin Thin Nwe, Kyaw Naing, Khin Mar Tun and Nyunt Wynn. (2005). "Preparation and Characterization of Apatite Calcium Phosphate". *Jour. Myan. Acad. Arts and Sc.*, vol. 3 (1), pp. 1-7

Wang, P.P. (2010). "Effects of Synthesis Conditions on the Morphology of Hydroxyapatite Nanoparticles Produced by Wet Chemical Process". *Powder Technology*, vol. 203(2), pp. 315-321

Weng, W. and J.L. Baptista. (1997). "A New Synthesis of Hydroxyapatite". *Journal of the European Ceramic Society*, vol. 17, pp. 1151-1156