Journal of Ocean, Mechanical and Aerospace
-Science and Engineering-, Vol. 37

November 30, 2016

Synthesize and Characterization of Hydroxyapatite from Freshwater Snail Shell *Sulcospira* Sp. Proceed by Combination of Ball Milling and Heat Treatment

Hidayatul Fajri[a] , Gunawarman[b,*], Jon Affi[b], Rezky Syahemi Putra[b]

[a)]*Biomedical Department, Medicine Faculty, Andalas University, West Sumatera, Indonesia*
[b)]*Mechanical Engineering, Engineering Faculty, Andalas University, West Sumatera, Indonesia*

*Corresponding author: gunawarman@ft.unand.ac.id

Paper History

Received: 31-October-2016
Received in revised form: 14-November-2016
Accepted: 30-November-2016

ABSTRACT

Hydroxyapatite is one of important material in orthopedic application because its features like human bone, and it has potential to replace metal and ceramic in implantation. Hydroxyapatite may get from waste which rich calcium like sea shell and snail shell. In this research, the characterization of raw materials of hydroxyapatite from freshwater snailshell (*Sulcospira* sp.) was investigated. The shell was milled with ball mill in 200 rpm during 15 minute with 4 steps. Between the milling was conducted heat treatment process at temperature 900^0C for 4h. The shell powder was then sorted based on its roughness with siever machine. Powder characterization was done with *Scanning Electron Microscope (SEM), Energy Dispersive X-ray (EDX) and X-ray Diffraction (XRD).* Result of this study show that the minimum size of powder about *67 μm* and percentage of calcium in the powder is increased until *55%.*

KEY WORDS: *Freshwater Snail Shell (Sulcospira), Calcium, Powder, Ball Mill, Heat Treatment.*

NOMENCLATURE

SEM	Scanning Electron Microscope
EDX	Energy Dispersive X-ray
XRD	X-ray Diffraction
HA	Hydroxyapatite

1.0 INTRODUCTION

Hydroxyapatite (HA) is one of the material that has getting attention in tissue engineering because its similarity with mineral fractions in human bone and good biocompatibility in living tissue [1]. Beside, this material has bio-affinity, stimulate osteo-induction, non-toxic, non-inflammatory [2,3] and slowly change with bone of host after implantation. Some method for synthesize HA powder has been proposed like solid-state reaction, hydrothermal reaction, sol-gel synthesized, and mechano chemical synthesized, etc [4,5,6].

Many researchers have investigated on the synthesize mechanisms and parameters for processing HA powder from eggshell, fish bone, and bovine bone. Another source may used to get HA from invertebrate like Mollusc and crustaceae [7,8]. Concentration of calcium in most of the freshwater species as well as terestrial environments is, in general, in low grade and low resources. However, some species of freshwater like Molusc may produced in high concentration of HA such as *Anodontacygnea* [9], *Biomphalariaglabrata* [10], and *Neritinanatalensis* [11].

In other hand, one of the purpose of synthesize hydroxiapatite is to increase bio-affinity and compatibility of metal material that has function as instrument for fracture. Iti well known that some metal had used for implantation and they should have a good biocompatibility, so body have good response and it is not considered as foreign material. If it has low biocompatibility, may result corrosion and deteration when contact with body liquid and it may result inflammation reaction around implantation tissue.

Some disadvantage of implant based on metal materials could be covered by using material which has good biocompatibility and might be used for repairing, filling, adding and reconstructing bone tissue which getting damage and also in soft tissue. Main resource of hydroxyapatite could come from waste like bovine bone, chicken bone, eggshell, shell, shell snail, etc where it available excessively. One of the potential resources for HA is snail shell from freshwater (*Sulcospira* sp) (Fig. 1) which is one of the favorite food in West Sumatera, especially in Padang Beach, and the most abundant also widespread snailshell in West Sumatera. It has a larger number of spiral cords and a smaller apical angle and a broader outer lip [12]. High consumption of snail shell had caused so many wastes around the beach and it could not reused, so it tend to caused pollute the environment around the beach. Therefore, it would be better if the waste of this snail shell used as resource of raw material of hydroxyapatite which has efficient in economic value after processed.

Figure 1: Snail shell freshwater (*Sulcospira* sp).

Obtaining hydroxyapatite with high quality and small size powders are really needed, so its characteristic is to be closer to human bone. That is, the raw materials should pass through a series of processes to be sized as very fine powder. Therefore, it is necessary for processing, synthesizing and characterization method of the powder snail shell using ball milling and heat treatment. Through this process, it is expected to have snailshell powders with high concentration of calsium with size and price ideal as powder of raw material of hydroxyapatite.

2.0 METHODOLOGY

2.1 Sampel Preparation

Sample (*Sulcospira*sp) was collected around the bridge of Purus Beach, Padang, West Sumatera. Snailshell cleaned with water and air drying. Then, snail shell crushed with hammer and ball milling to obtain the first rough-textured powder. Powder with rough texture milled with ball mill (*Pulverisette 6 Classic Line Fritsch Planetary Mono Mill*) with speed of rotation (n) about 200 rpm during 15 minute with 4 repetition. The ball mill comes from agate with diameter about 10 mm and mass about 14,5 g. Furthermore, the powder sieved to obtain fine powder that will use as raw material of hydroxyapatite. Sieving process with siever machine (*Retsch*), standard of ASTM E11 and the number of sieve about 35, 60, 120 and 230 mesh. Sieving process was carried out for 10 minute with amplitude of 80.

This research consist of 4 step of characterization, step 1 (powder from first ball milling process), step 2 (first heat treatment process), step 3 (powder from second ball milling process) and step 4 (second heat treatment process).

2.2 Heat treatment (Calcination) process

The purpose of heat treatment (calcination process) are to reduce the concentration of water, bacteria, and organic component or another substance that does not have function and have a chemical bonding with powder of snail shell. This step was carried out in a furnace (Nabertherm) that has been exposed to air. Temperature for calcination process about 900^{o}C during 4 hour and then slowly cooling in air as can be seen schematically in Fig. 2.

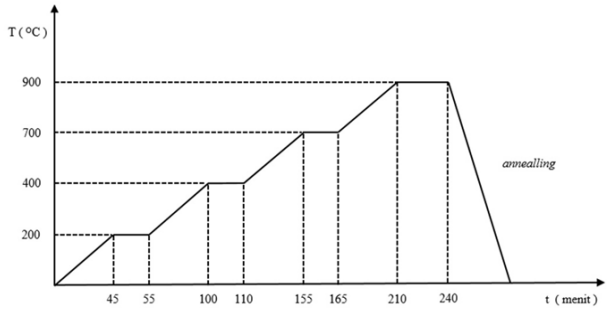


Figure 2: Calcination process step of freshwater snail shell

2.3 Observation with Scanning Electron Microscope (*SEM*)

This step have purposed to monitoring size distribution and morphology of powder from freshwater snail shell. Monitoring is done by SEM (Hitachi S3400N) in low vacuum with parameter, Voltase 15 kV, *objective aperture* 9,8 mm and magnification around 100×, 500×, 2500×, 3500×, and 5000×.

2.4 Observation of Chemical Composition with *energy dispersive x-ray* (EDX)

This process is conducted by *energy dispersive x-ray* (EDX) (Horiba) that connected with *scanning electron microscope* (SEM). Voltage is about 15 kV with deadtime is about 20-30%.

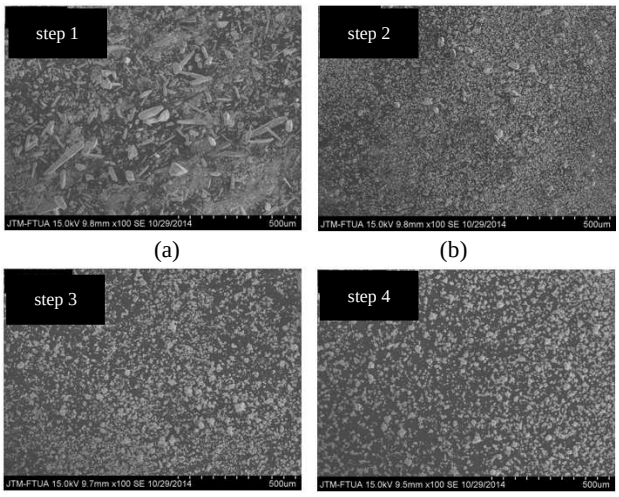
2.5 Characterization with *X-Ray Diffraction* (XRD)

XRD examintation was conducted to evaluate phase or compound, knowing the type of mineral, crystalography structure and to confirm the size of material crystal that used. Type of XRD is *PANalyticalX'Pert Pro X-ray Diffractometer*. Parameter that we used are Anoda = Copper (Cu), K-Alpha1= 1,54060Å, K-Alpha2= 1,54443 Å, K-Beta= 1,39225 Å, Ratio K-A2/K-A1= 0,5, Generator setting = 30 mA and 40 kV.

3.0 RESULT AND DISCUSSION

Based on the observation with SEM, size distribution and morphology of the powder are shown in Fig 3. Size distribution becomes small and soft for each step. In step 1, it can be seen that each grain of powder has a great distance and few grain of powder still rough textured (Fig 3a). In step 2, distribution of each powder looks better than before but few grain of powder still rough textured (Fig. 3b). Furthermore, size istribution of step 3 has proved that milling process giving an effect of reducing size

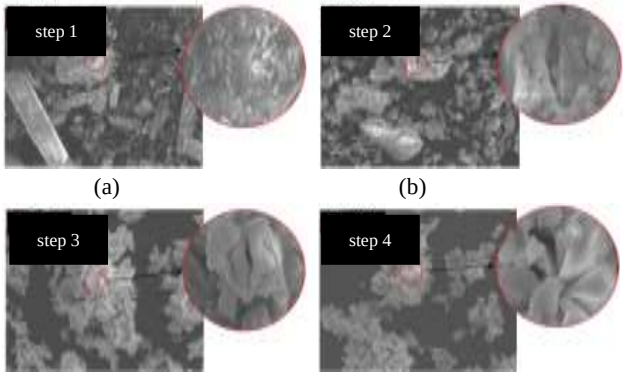
distribution. The powder looks more refined than previous (Fig. 3c). In step 4, increase of size distribution better than before because of calcination process (Fig.3d).



(a) (b) (c) (d)

Fig. 3: Size distribution of powder of freshwater snail shell with magnification about 100x; (a) powder of step 1, (b) powder of step 2, (c) powder of step 3, (d) powder of step 4.

Based on the result, we could explain that ball milling process has given an effect to the level of refinement and size distribution of powder. It happened because of the principle of how ball mill work. Ball mill and material in cup of milling have a centrifugal force in its axis. The milling cup and its bearing plate rotate in opposite, so centrifugal force working alternately and made a balance force. Balance of force obtained from *counterweight* that combined in bearing plate. Milling process produces friction force and impact load [13].



(a) (b) (b) (d)

Fig. 4: Typical SEM micrograph of the powders with magnification of 1000x and 5000x (insert); (a) powder of step 1, (b) powder of step 2, (c) powder of step 3, (d) powder of step 4.

Morphology observation of freshwater snail shell powder with SEM and magnification about 1000x become 5000x has given result in more detail and distinct. In step 1, powder morphology still in solid state and looks like chunk because blow of the balls of ball milling (Fig. 4a). In step 2, there were reduction of sectional and a hole has formed in powder because heat treatment process in high temperature (Fig. 4b). In step 3, size of powder becomes refine than before because of bowl of the balls of ball milling (Fig. 4c). Finally in step 4 (Fig. 4d), the powder becomes more refined because of reduction of organic substance in heat treatment process.

Table 1: Result of sieving process of freshwater snail shell powder (Sulcopira)

No. Sieve (size of powder)	**Spesification of powder**			
	Step 1	**Step 2**	**Step 3**	**Step 4**
35 (500 µm)	0,00	0,00	0,00	0,00
60 (250 µm)	7,93	1,75	0,02	0,00
120 (125 µm)	25,71	10,64	3,08	0,38
230 (63 µm)	3,77	1,82	3,52	0,36
Base	**0,27**	**0,14**	**0,05**	**13,49**
Σ weight	**37,68**	**14,35**	**6,67**	**14,23**
Number of powder softness	**61,95**	**66,21**	**92,86**	**222,68**
Means of size (µm)	**245,94**	**237,06**	**181,54**	**67,125**

The product of powder then sieved with various of sieve size. The result showed in table1. Result of sieving process showed that process for each step has effected for powder size becomes more refined and target to obtain fine powder has been done. Alteration level of powder size can be seen in Fig. 5.

Calcination process for step 2 and step 4 has given an effect where calcination done through the process of burning organic substances or waste substances in desired material such as C, H, and O as seen in table 2. In Table 2, it can be seen that the heat treatment process for organic substance and bacteria in raw material about 32,2% for first calcination and 16,8% for second calcination.

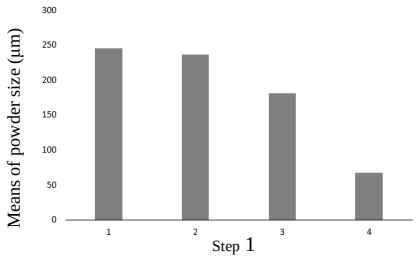


Figure 5: Alteration of powder size in each step of process.

Table 2: Percentage of loosing weight powder caused of calcination.

Calcination Process	Powder weight (gram)			Percentage of loosing weight (Wloss = ΔW/W1 x 100%)
	Before calcination (W1)	After calcination (W2)	Different of powder weight (ΔW = W1-W2)	
First calcination	40	27,11	12,89	32,2%
Second calcination	15	12,47	2,53	16,8%

Measuring the weight of each element in every step is done with investigation chemical composition with EDX connected with SEM. The result is shown in Fig. 6.

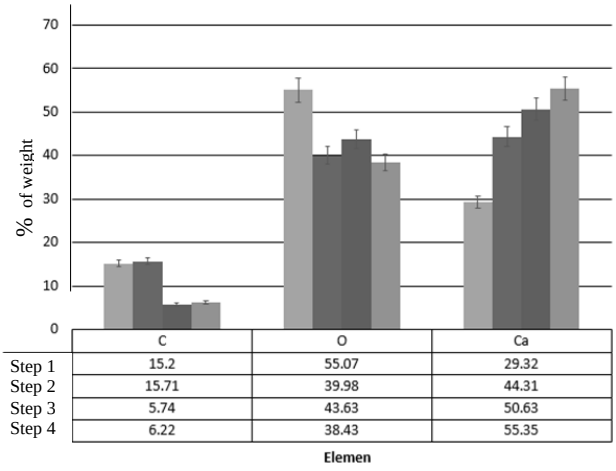


Figure 6: element contens in freshwater snail shell powder.

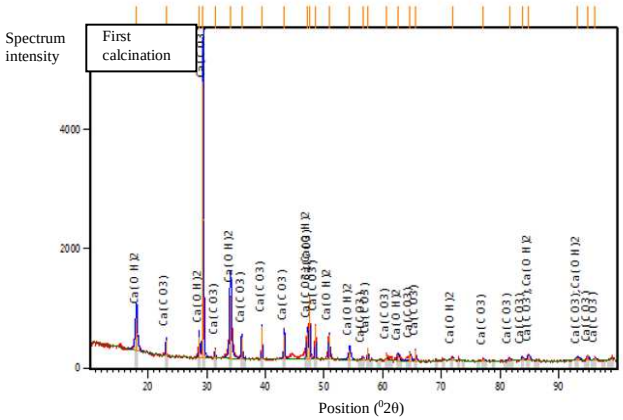
Fluctuation in the value element C for each step is caused by unreadable carbon tape when preparation sampel was being carried out where carbon tape used as place for samples, so percentage of C in material increase. Percentage of element O tended to be large. Loss element whenheat treatment process was conducted to obtain high value of powder. The resulting Ca (Calcium) contain from freshwater snail shell powder as raw material of hydroxyapatite increased significantly in each step. In this powder also there are also percentage of unidentified chemical composition like H and Na. This is because of its element has minor atom mass and hard to read in EDX.

Based on this percentage of C value, we can compare the results with another result before which uses different biomaterial implant [13,14]. Percentage of Ca in this research is higher compared to previous biomaterial, can be seen in table 3.

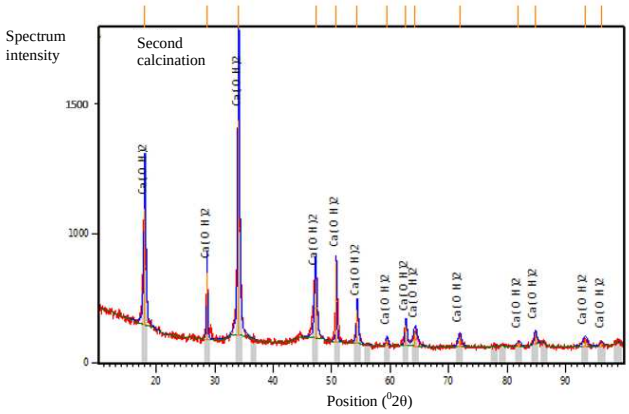
Table 3.comparison of pencentage value of calcium weight

Kind of material	Calcium percentage (% Ca)
Lime stone [13]	37,96
Egg shell [13]	37,60
AnadaraAntiquata [14]	49,67
***Sulcospira*sp.**	**55,35**

This result showed that ball milling and heat treatment method have an effective value to obtain high calcium content. Observation about characterization of powder like phases of compound and crystalography that acquired form each step in calcination process was done by XRD (Fig. 7). We can see that there are change in powder content like $CaCO_3$ in first calcination becomes $Ca(OH)_2$ in second calcination process.



(a) first calcination prosess



(b) second calcination process.

Figure 7. Difractogram of powder of freshwater snail shell.

In Fig. 7(a), difractogram shows that first calcination made high peak of $CaCO_3$ (calcium carbonat) with reflection of peak about 29,36° and index Miller 104. This result also supported by confidence interval in pattern list about 68 point. Also showed that there was an initiation of increasing of $Ca(OH)_2$ (calcium hydroxide) value with confidence interval in pattern list about 53 point. In Fig. 7(b) showed that $CaCO_3$ in first step had minimized so we cannot find it anymore in second step, resulting $Ca(OH)_2$ with main grid reflection position about 34,05°, index Miller 101, also confidence interval in pattern list about 84 point. Camparison of the changes that occured in powder for calcination process showed in table 4.

Table 4: Comparison of powder mineral alteration because calcination

Comparison Parameter	First Calcination	Second Calcination
Compound	*Calcium Carbonate* (*68)	*Calcium Hydroxide* (*84)
	Calcium Hydroxide (*53)	
Crystal size (τ)	63,5 nm	21,5 nm

*Score in pattern list (confidence interval)

Value of crystal size that resulted from freshwater snail shell powder, measured with Scherrer equation to determined how big of nano-cyrstalinis caused of some step that have done.

$$Crystal\ size\ (\tau) = \frac{K\lambda}{\beta cos\theta} \qquad (1)$$

Determining powder size needed for some parameter with XRD. In first calcination, the result showed that crystal size about 63,5 nm and diffraction constant parameter (K) about 0,89, wave length (λ) about 1,54 Å, enlargement line in half of maximum intensity (FWHM) (β) about 0,00223 radian and Bragg angle (θ) about 14,68°. In second calcination, crystal size about 21,5 nm used parameter with diffraction constant value (K) about 0,89m wave length (λ) about 1,54 Å, enlargement line in half of maximum intensity (FWHM) (β) about 0,00669 radian and Bragg angle (θ) about 17,025°. So we got different size of powder crystal in first calcination and second calcination.

Changing in mineral composition in each step is caused by effect of calcination process in ball milling, sieving process, etc. such as increasing the calcium hydroxide value in second calcination caused by diffusion of H atom from air to powder when ball milling process was doing. Alteration of compound from calcium carbonat becomes calcium hydroxide was one of the effectivity of ball milling process and heat treatment in freshwater snail shell (*Sulcospira* sp.).

Mineral phase of raw material powder hydroxyapatite that resulted in this research would compare with another research before (14, 15) like in table 5. The result in this table showed that there was an activity in this research so we can increase the percentage of mineral phase of freshwater snail shell powder as raw material of hydroxyapatite for bone implant.

Table 5: Comparison of increasing of powder mineral from raw material of hydroxyapatite

Kind of material	Compound of raw material	Increment percentage (%)
Lime stone [13]	CaO	52,98
Egg shell [13]	$CaCO_3$	94,00
AnadaraAntiquata [14]	$Ca(OH)_2$	91,89
***Sulcospira*sp.**	**$Ca(OH)_2$**	**102,39**

CONCLUSION

Based on the result, it could be concluded that effectivity of ball milling process and heat treatment in freshwater snail shell were good and high value because resulting size distributions of powder. They were also producing superfine powder with uniform morphology. Ball milling and heat treatment process are important way for plane away freshwater snail shell (*Sulcospira* sp.) where it could make powder size changed from 246 µm to 67 µm. Furthermore, this processes had reduced element organic like C and O, so Ca level increased from 29% to 55% and $Ca(OH)_2$ calcium hydroxide contain increases up to 102 % during combination of ball milling and heat treatment process.

ACKNOWLEDGEMENTS

First and second authors thank to Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for PMDSU scholarship and PMDSU research grant no. 324/SP2H/LT/DRPM/IX/2016. However, this work is mainly supported by PUPT grant with contract no. No. 017/UN.16/UPT/2016. Thanks to Ministry of Research, Technology, and Higher Education, Andalas University for the grant.

REFERENCES

1. Mondal, S., Bardhan, J. Mondal, B. Dey, A.,Mukhopadhyay, S.S., Roy, S.,Guha, R.., and Roy, K. (2012). *Synthesis, characterization and in vitro cytotoxicity assessment of hydroxyapatite from different bioresources for tissue engineering application*. Bulletin Material Science. 35(4), pp: 683–691.
2. Kuriakose, T.A.,Kalkura, S.N.,Palanichamy, M.,Arivuoli, D.,Dierks, K..,Bocelli, G.,Betzel, C. *Synthesis of stoichiometric nano crystalline hydroxyapatite by ethanol-based sol–gel technique at low temperature*. Journal of Crystal Growth. Vol*263* (1-4), pp: 517-523.
3. Saranya, K.,Kowshik, M.,Ramanan, S.R. (2011). Synthesis of hydroxyapatite nano powders by sol–gel emulsion technique. Bulletin of Material Science. vol*34* (7), pp:1749-1753.
4. Kim W., Zhang, Q.,and Saito, F.(2000). *Mechanochemical Synthesis of Hydroxyapatite from $Ca(OH)_2$-P_2O_5 and CaO-$Ca(OH)_2$-P_2O_5 mixtures*. Journal of Material Science. pp:35 5401.
5. Balamurugan A.,Kannan, S., and Rajeswari, S.(2002). *Bioactive Sol-Gel Hydroxyapatite Surface For Biomedical Applications-In Vtiro Study*.Trends Biomater. Artif. Organs. Vol16 (18), pp: 18-20.
6. Tadic, D and Epple, M.(2003). *Mechanically stable implants of synthetic bone mineral by cold isostatic pressing*. Biomaterials. Vol 24, pp: 4565-4571.
7. Ziegler, A., Hagedorn, M., Ahearn G. A., Carefood, T. H. (2007). *Calcium translocations during the moulting cycle of the semiterrestrial isopod Ligiahawaiiensis (Oniscidea, Crustacea)*. J Comp Physiol B. Vol 177, pp:99–108.
8. Lopes-Lima M., Rocha, A., Gonc¸alves, F., Andrade, J., Machado, J. 2010. *Microstructural characterization of inner shell layers in the freshwater bivalve Anodontacygnea*. J Shellfish Res. Vol 29, pp: 969–973.
9. Hinzmann, M. F., Domingues, B., Lopes-Lima, M., and Machado, J. (2014).*Morphological and Chemical Characterization of Mineral Concretions in the Freshwater Bivalve Anodontacygnea (Unionidae).* Journal Of Morphology. Vol 00, pp:1–12.
10. Marxen, J. C. and Becker, W. (2000). *Calcium binding constituents of the organic shell matrix from the freshwater snail Biomphalariaglabrata.Comparative*. Biochemistry and Physiology Part B. Vol 127, pp: 235-242.
11. Ekren, N., Gunduz, O., Celik, S., *et*al. (2016). *Production of Apatite from Snail Shells for Biomedical Engineering Aplications*. Key Engineering Materials. Vol 696, pp: 51-56.
12. Marwoto, R. M. and Isnaningsih, N. R. (2012), The freshwater snail genus *Sulcospira* Troschel, 1857 from Java, with description of a new species from Tasikmalaya, west Java, Indonesia (Mollusca: Gastropoda:Pachychilidae). The Raffles Bulletin of Zoology, 60(1), pp: 1–10.
13. Fritsch GmbH. (2012). *Operating Manual, Translation of the Original, ClassicLinePulverisette*. Idar-Oberstein: Manufacturers of Laboratory Instruments Industriestraβe 8.
14. Gunawarman, Affi, J.,Ilhamdi, Gundini, Resti and Ahli, Amalul. (2014). *Characterization of Bioceramic Powder from Clamshell (AnadaraAntiquata) Prepared By Mechanical and Heat Treatments for Medical Application*. Seminar Nasional Tahunan Teknik Mesin Indonesia XIV, 2015, Banjarmasin
15. P. Dasgupta, A. Singh, S. Adak and K. M. Purobit,(2004). *Synthesis and Characterization of Hydroxyapatite Produced from Eggshell*. International Symposium of Research Student of Material Science and Engineering.