Effect of Microwave Irradiation on the Synthesis of Carbonated Hydroxyapatite (CHA) from Chicken Eggshell

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Abstract. The main inorganic constituent of human bone is carbonated-hydroxyapatite (CHA). Chicken eggshells (CES) become potential waste which can be used for biomaterial synthesis because it contains of 94% calcium carbonate (CaCO₃). This research aims to synthesize CHA by microwave assisted methods. CHA was successfully synthesized by mixing a calcium solution from CES with aqueous solution of NaHCO₃ and H₃PO₄. Microwave assist offers many advantages such as fast reaction, easy reproducibility, high yield, high purity, efficient energy transformation, and throughout volume heating. After precipitation process, samples were irradiated in microwave oven for 30, 35, and 40 minutes by using microwave power 40, 200 and 400W. The functional groups of CHA powder was determined by FTIR. There are functional groups of phosphate (PO₄³⁻), hydroxyl (OH⁻), and carbonate (CO₃²⁻). The FTIR spectrum indicates that the prepared sample is B- type carbonated substituted HA because part of phosphate groups in the HA structure is replaced by carbonated groups. The phase composition was evaluated by XRD and indicated the presence of HA and CHA. The quantitative analysis showed that Ca/P ratio is 1.64, which indicates that the obtained product is non-stoichiometric HA. The successful synthesis of CHA is a critical step forward in our efforts to fabricate bone tissue engineering scaffold.

1. Introduction

The research on biomaterial fields are growing rapidly. The development of synthetic bone graft as alternatives graft material become a major advance in biomaterial technology. The need of biomaterials in the medical field is increasing by the number of bone damage cases in many countries. Bone damage is triggered by various factors, such as rampant cases of accidents, age factors, infections, tumors, and others. Bone is a complex tissue that support our body. Natural bone is containing 50-70% of apatite minerals, 20-40% organic material, 5-10% water, and 3% of proteglycan, peptides, and lipid [1]. Currently, biomaterials have been developed by utilizing waste from chicken egg shells (CES) [2]. CES is easy to find because many vendors are utilizing CES in the food industry, while the CES are left to be untapped waste whereas it has high source of calcium contains. Bioceramics which developed from calcium phosphate have been widely used in bone healing applications because they have both biocompatible and osteoconductive properties for implant. Current synthetic bone grafts commonly are made by hydroxyapatite (HA) which has similar composition to mineral apatite. Although HA synthetic has the ability to bond bone, but the rate of osseointegrations are relatively slow. The way to enhance osseointegration is add an incorporate ion that are present in bone mineral such as carbonate ion [3]. Recent research suggests that carbonate
hydroxyapatite (CHA) is a promising material because it has more bioresorbable and bioactive on in-vivo process than stoichiometric HA.

Carbonate is one of the most abundant ions (4-8 wt %) which present in bone mineral. Carbonate ion in HA structure can replace the phosphate group (PO$_4^{3-}$) or hydroxyl group (OH$^-$) to produce a non-stoichiometric apatite mineral with Ca/P ratio in the range 1.6-2.0 [4]. The carbonate group may produce CHA-A which has chemical formula (Ca$_{10}$ (PO$_4$)$_6$(OH)$_{2x}$ (CO$_3$)$_x$ or CHA-B Ca$_{10}$y(PO$_4$)$_6$. 3y(CO$_3$)$_x$(OH)$_{2x}$. It is important to mention that bone mineral in human body contain both A and B type CHA. CHA-B was dominant in young men bone. However, as human get old, CHA-A amount will increase. In this work, we focus to get CHA-B powder by using calcium from CES with irradiation treatment.

Apatic carbonate or CHA can be obtained by various methods of synthesis including precipitation, hydrothermal, ultrasonic treatment, sol-gel, and mechanochemical methods [5]. However, these methods are time consuming, expensive, and relatively inefficient process. Microwave synthesis has received special attention from researchers due to its unique characteristics compared with conventional methods. Microwave is an electromagnetic wave which has frequencies range from 300 MHz-300GHz and is located between radio and infrared waves in electromagnetic spectrum [6]. It provides a rapid and shorter synthesis time because the electromagnetic waves can penetrate and excite water and lipid molecules evenly. The waves at this frequency (2.5 GHz) can be absorbed by water, lipid, sugars and will not absorbed by glass, ceramic, and some plastic materials. The advantages of using microwave irradiation are can produce nanometer size of the material, more precision, shorter synthesis time, faster temperature rise, lower energy use, high yield and purity [7].

2. Research Methods

CES, H$_3$PO$_4$, and NaHCO$_3$ were used as starting materials. CES were collected and washed using distilled water and their membranes were peeled off. This step aims to remove macro dirt from CES that we used. The cleaned and dried CES were then calcined using furnace at 900°C for 8 hours to remove the organic components of the sample. CES which is containing CaCO$_3$ will release CO$_2$ during the calcination process and produce CaO for calcium sources in this synthesis. CES then ground to fine powder using mortar and pestle. A calcium solution 1M Ca(OH)$_2$ 50 ml was mixed with an aqueous solution of 50 ml 0.6M H$_3$PO$_4$ and 50 ml 0.3M NaHCO$_3$ at molar ratio Ca$^{2+}$PO$_4^{3-}$:CO$_3^{2-}$ = 1.67:1:0.5 using a magnetic stirrer and the aqueous solution was adjusted to pH 13. The solution was immediately transferred into microwave oven with some treatments (Table 1). Sample control (CHA-C) was made without microwave irradiation treatment. The mixture CHA then aged overnight for 24 hours. The aged mixture was filtered and the resulting was dried into oven at 110°C for 5 hours. This procedure instruction was repeated for two times.

| Table 1. Sample ID and synthesis condition in the synthesis of CHA |
|---------------------------------------------------------------|
| **Power (Watt)** | **Duration (minutes)** |
|------------------|-----------------------|
| Low (40)         | 30                    |
| Middle (200)     | 35                    |
| High (400)       | 40                    |

Table 1 shows the synthesis condition and sample ID. Before samples were used for synthesis, samples were examined by AAS. AAS (Atomic Absorption Spectrometry) used to determine the calcium contains in CES after calcination process. The final dried CHA powders were examined by X-ray diffraction (XRD) to determine phase purity and crystallinity of the sample. The XRD result is an identified phase graph based on the intensity and angle 20. Phase identification was achieved by comparing the diffraction pattern of HA and CHA with Joint Committee on Powder Diffraction
Standard (JCPDS). The substitution type of CHA (A or B type) was identified from wave number by using infrared spectrometry on Fourier transform spectrometer. CHA powder was mixed with 100 mg KBr and pellet was made then measured using FTIR spectrophotometer at 400-4000 cm\(^{-1}\). Morphology analysis is determined by scanning electron microscope (SEM). Scanning electron microscope with energy dispersive (SEM-EDX) is used to know the component of CHA and calculated Ca/P ratio formed in CHA sample.

3. Results and Discussion

Calcium carbonate (CaCO\(_3\)) is calcium salt which is become a major component of CES. It is white powder, odorless, and tasteless. The CaO compound is the decay product of CaCO\(_3\) due to the high temperature combustion or calcination process which causes the release of carbon compounds. The Ca content in CaO compounds is required to determine calcium molarity used for the synthesis. The AAS results show that the average percentage of calcium in CaO powder is about 72.70%. This shows that the calcium content in CES is high enough, so that it can be used as calcium source as well as efforts to utilize organic waste materials in the synthesis of biomaterials.

The synthesis step begins with the preparation of calcium, phosphate, and carbonate solution [8]. Synthesis is performed by precipitation method by mixing slowly 0.6M H\(_3\)PO\(_4\) solution and 0.3M NaHCO\(_3\) solution into 1M Ca(OH)\(_2\) while stirring 300 rpm for 30 minutes and also directly transferred into microwave. In this study we compared the mass effectiveness of precipitants formed with and without microwave irradiation treatment. Microwave irradiation treatment aims to replace the aging process that is usually needs 24 hours in calcium phosphate synthesis. By using microwave, the synthesis duration will be shorter. The solution which included in the microwave oven gets irradiated electromagnetic waves directly so there is effect in physical mechanism that aids the synthesis process. This process does not depend on the thermal conductivity of the container used, so that heating immediately interacts with the substance contained in the sample solution, rather than heating the container [9]. Therefore microwave heating is more selective, homogeneous, and effective in assisting CHA synthesis.

| Sample ID | CHA Mass (g) | Average (g) | Standard Deviation |
|-----------|--------------|-------------|--------------------|
| 1         | 5.89         | 5.51        | 5.70               | 0.27               |
| 2         | 5.51         | 5.40        | 5.45               | 0.08               |
| 3         | 5.88         | 5.70        | 5.79               | 0.13               |
| 4         | 5.61         | 4.75        | 5.18               | 0.61               |
| 5         | 4.93         | 4.87        | 4.90               | 0.04               |
| 6         | 5.79         | 5.07        | 5.43               | 0.51               |
| 7         | 5.82         | 6.28        | 6.05               | 0.32               |
| 8         | 6.07         | 6.49        | 6.28               | 0.29               |
| 9         | 7.38         | 6.30        | 6.84               | 0.76               |

The result of CHA mass can be seen in Table 2. The average CHA mass increases by increasing power and time duration. In the sample L1, L2, and L3 the sample still contains a lot of water, so samples were being filtered and dried because the microwave power used is not able to absorb more water molecules contained in the sample. Meanwhile, in samples M1, M2, and M3 the water content is less than L sample. While on samples H1, H2, and H3 that using maximum irradiation power and
time, the sample was dried because of the faster vibration and heat generated from the irradiation process becomes higher. Samples with high irradiation treatments produce larger average mass than other samples and we don’t need filtering and drying processes, so the synthesis duration will be shorter and more effective.

The XRD pattern of CHA is shown in Fig.1 which matched well with the JCPDS data for HA (JCPDS file no. 9-432), CHA-A (JCPDS file no. 35-180) and CHA-B (JCPDS file no. 19-272). It suggested that the crystalline phase present in the CHA-B is a hexagonal crystal system with lattice constants $a = b = 9.309 \text{ Å}$, and $c = 6.927 \text{ Å}$.

The XRD pattern in Figure 1 shows the same diffraction pattern dominated by the peaks of HA, and A or B type CHA. Sample C and L1 indicate the dominant phase of HA but at certain angle indicates CHA-B peaks at angle $2\theta = 29.46^\circ$, $33.11^\circ$, $34.16^\circ$ and $47.19^\circ$, and for sample L1, CHA-B detected at angle $2\theta = 33.04^\circ$. Meanwhile, in higher irradiated samples (M1 and H1) showed more CHA peaks than C and L1 samples. Sample M1 shows that the CHA-B is dominant and there are two HA phases at $49.43^\circ$ and $50.79^\circ$. While the H1 sample which treated by maximum irradiation showed the peaks of CHA-A at $28.60^\circ$ and $33.97^\circ$, CHA-B at $25.60^\circ$ and $47.01^\circ$, HA at $32.93^\circ$, $50.67^\circ$, and $54.23^\circ$. From Figure 1 it can be seen that the H1 sample shows higher peak intensity than other samples. The sharper intensity is obtained because the power used is higher and this shows the higher crystallinity. Efficiency of energy transformation and microwave heating can produce well-crystallize CHA without sintering process or further heating as has always been reported by other studies.

The structure of a crystal is constructed by unit cell which is the set of atoms arranged periodically in a lattice. The phases indicated at the $2\theta$ angle can provide information related to the direction in $hkl$ plane. Table 3 shows the call parameters that were calculated by Cohen methods for hexagonal structure. Based on these results, CHA samples obtained precision above 90%. The XRD results thus indicate the formation of CHA-B in all samples. Table 4 shows the average crystallite size. The size of the crystal is calculated by using Scherrer equation. Based on the calculations, it was found that the crystallite size ranged from 40 nm and increased by increasing microwave irradiation power.
Table 3. Lattice parameters of CHA samples

| Sample ID | Lattice parameters (Å) | (%) |
|-----------|------------------------|-----|
|           | a=b   | C   | a=b   | C   |
| C         | 9.626 | 7.123 | 96.59 | 97.17 |
| L1        | 9.956 | 7.325 | 93.04 | 95.10 |
| M1        | 9.528 | 7.167 | 97.64 | 96.53 |
| H1        | 9.593 | 6.955 | 96.94 | 99.59 |

Table 4. Crystallite size of CHA samples

| Sample | Crystallite size (nm) |
|--------|-----------------------|
| C      | 41.56                 |
| L1     | 41.93                 |
| M1     | 43.41                 |
| H1     | 47.71                 |

The FTIR spectroscopy show further details the carbonate substitution in phase. FTIR spectrophotometer is one tool that can be used for identification of functional groups. FTIR spectra of CHA are shown in Figure 2. Generally, HA is characterized by various vibrational modes of the hydroxyl (OH\(^-\)) and phosphate (PO\(_{4}^{3-}\)) groups. The FTIR spectra is shown in Table 5, which indicates that the prepared sample is B-type carbonate substituted HA because that is part of the phosphate groups in the HA structure is replaced by carbonate groups.

![FTIR Spectra of CHA sample H1,C,M1](image-url)
Table 5. Result of FTIR Analysis for C, H1, and M1 samples

| Functional Group | Wave number (cm⁻¹) | C    | H1   | M1   |
|------------------|-------------------|------|------|------|
| Phosphate (PO₄³⁻) |                   | 468.56 | 464.56 | 469.09 |
|                  |                   | 566.26 | 568.62 | 566.33 |
|                  |                   | 604.17 | 605.31 | 604.46 |
|                  |                   | 1032.51 | 1036.93 | 1032.48 |
| Hydroxyl (OH⁻)   |                   | 3641.15 | 3443.18 | 3419.84 |
|                  |                   | 3443.18 | 3641.15 | 3642.64 |
|                  |                   | 3696.21 | 3696.21 | 3695.93 |
| Carbonate (CO₃²⁻) |                   | 873.65 | 875.60 | 874.00 |
|                  |                   | 1421.54 | 1424.46 | 1420.23 |

All samples show vibration modes corresponding to the phosphate, hydroxyl, and carbonate groups. The PO₄³⁻ group has four vibration modes: symmetry stretching (υ1) at wave numbers around 956 cm⁻¹, bending symmetry (υ2) at 430-460 cm⁻¹, stretching asymmetry (υ3) at 1040-1090 cm⁻¹, and bending asymmetry (υ4) at 575-610 cm⁻¹ [10]. FTIR analysis results show that phosphate vibrate υ2 at 468.56 cm⁻¹, 464.56 cm⁻¹, and 469.09 cm⁻¹. The transmittance band at 1032.51 cm⁻¹, 1036.93 cm⁻¹, and 1032.48 cm⁻¹ is indicating the presence of amorphous and crystalline phase mixtures. The transmittance band υ4 lies at the wave number 560-605 cm⁻¹. The band of υ3 and υ4 phosphate is an asymmetric band indicating that the sample is not entirely amorphous. The hydroxyl bond stretch is observed at the range of 3400-3700 cm⁻¹ in all samples. The carbonate substitution in apatite is identified by the characteristic peak of carbonate ions around 870 cm⁻¹ (asymmetry stretching) and 1420 cm⁻¹ (symmetry bending). The carbonate group at location B is supposed to replace the phosphate group and cause contraction in the lattice a parameter [11]. The CHA type B is easier to form than CHA type A because hydroxyl band in HA requires more energies for release the bond than phosphate.

Scanning electron microscope observations of synthesized CHA powders can be seen in Figure 3. It can be seen that CHA was agglomerated and has irregular round shape. Nanostructure CHA can be achieved by using microwave irradiation treatment. It is important to get nanosized particles because it can exhibit many advantages such as enhance biocompatibility, bioactivity, and flexibility which provides homogenous shapes [12].

Figure 3. Scanning electron micrograph of CHA-H1 at magnification of 1000x
Figure 4 shows the EDX spectrum that are give the information about Ca, P, O and C present in samples. EDX spectrum of the synthesized CHA is shown contains of Ca (16.09%), P (8.97%), C (2.83) and O (72.11%) were present in the synthesized product. The quantitative analysis showed that Ca/P is 1.64, which indicates that the obtained product is non-stoichiometric HA.

**Figure 4.** EDX Result of element analysis (CHA-H1)

4. Conclusion

Apatite carbonate (CHA) was successfully synthesized by utilizing calcium from chicken egg shells. Ca content in chicken egg shell that has been calcined is 72.70%. FTIR results indicate the content of the OH\(^{-}\) and PO\(_4^{3-}\) groups that characterizes the presence of calcium phosphate compounds. In addition there is also a group of CO\(_3^{2-}\) which replaces the group PO\(_4^{3-}\) to form CHA type B. The XRD results show that the phase formed is HA and CHA. The CHA phase is increasingly dominant in high irradiated samples. Microwave irradiation assists the synthesis process and affects the final product of CHA. The effect of microwave irradiation in CHA type B synthesis may shorten synthesis time by cutting aging, filtering, and drying step. Moreover, it can produce higher relative masses, precision lattice parameters above 90%, increase crystallinity and crystallite size in the sample.

References

[1] Garškaite E, Alinauskas L, Drienovsky M, Krajcovic J, Cicka R, Palcut M, Jonusauskas Malinauskas, Stankeviciute, Kareiva 2016 Polylactic acid-nanocrystalline carbonated hydroxyapatite (PLA-cHAP) composite : preparation and surface topographical structuring with direct laser writing (DLW) *Journal the royal society of chemistry*

[2] Dahlan K, Dewi SU 2013 Pengaruh sintering dan penambahan senyawa karbonat pada sintesis senyawa kalsium fosfat *Prosiding Semirata FMIPA Unila* 153-157

[3] Gibson IR Bonfield W 2001 Novel synthesis and characterization of an AB-type carbonated substituted hydroxyapatite *IRC in Biomedical Materials* 697-70

[4] Pramuditya Ryan C 2013 Perbandingan kuantitas dan kualitas kapsul pasca implantasi subkutan karbonat apatit dan hidroksiapatit [Skripsi] Yogyakarya (ID) Universitas Gadjah Mada

[5] Sajahan Nor A, Ibrahim Wan Mohd 2014 Microwave Irradiation of Nanohydroxyapatite from Chicken Eggshells and Duck Eggshells *The Scientific World Journal* Hindawi Publishing Corporation 1-7

[6] Hassan MN, Mahmoud MM, El-Fattaha AA, Kandila S 2016 Microwave-assisted preparation of nano-hydroxyapatite for bone substitutes *Journal Carmines International* 42 3725-3744
[7] Kumar TS, Manjubala, Gunasekaran 2000 Synthesis of carbonated calcium phosphate ceramics using microwave irradiation ELSEVIER Biomaterial 21 1623-1629
[8] Zhoou WY, Wang M, Cheung W, Guo BC, Jia DM 2008 Synthesis of carbonated hydroxyapatite nanospheres through nanoemulsion Journal material science 119 103-110
[9] Surati M, Jauhari S 2012 A brief review: Microwave assisted organic reaction. Archives of Applied Science Research 4(1) 645-661
[10] Lesmana Taofik J 2007 Pembuatan komposit polimer-kalsium fosfat karbonat : Spektroskopi serapan atomic, ultraviolet, dan fourier transform infrared [Skripsi] Bogor (ID) Institut Pertanian Bogor
[11] Suryadi 2011 Sintesis dan karakterisasi biomaterial hidroksiapatit dengan proses pengendapan kimia basah [Tesis] Depok(ID) Universitas Indonesia
[12] Kalita, Verma 2010 Nanocrystalline hydroxyapatite bioceramic using microwave radiation: synthesis and characterization Materials science and engineering 30(2) 295-303