Microwave–assisted precipitation of carbonated hydroxyapatite

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Abstract. Carbonated hydroxyapatite (CHA) is a bone implant material that has good solubility and resorption. In this study, CHA was synthesized and characterized using microwave–assisted precipitation method under different irradiation power and time. CHA powder was synthesized starting from calcium hydroxide, diammonium hydrogen phosphate, and calcium hydroxide. Chemical and physical analyses, XRD, FTIR, SEM, and EDX, were applied to investigate the composition, crystallinity, crystallite size and morphology of CHA powder. XRD patterns showed that CHAs had low characteristic peaks that indicating poor crystallinity. According to the crystallinity index, 450 W 30 minutes CHA with CI = 35.3% and SF = 2.908 was chosen as the best result because it was the closest result to the bone crystallinity reference. The crystallite size for all CHA samples reached 19.386 – 24.019 nm, were almost similar to the crystallite size for bone. FTIR and EDX affirmed that CHA bond existed. The carbonated hydroxyapatite was formed B type CHA with some additional A and AB type. CHA crystal was formed of nanorod particle. Hence the physical and chemical analyses suggest that CHA powder can be obtained using a microwave-assisted precipitation method with good results.

Keywords: Carbonate, Hydroxyapatite, Microwave Irradiation, Biomaterial

1. Introduction

Incidence and diseases that are related to human’s bone is a common event. In 2017, the necessity of bone grafting procedure approximate 700,000 procedures and could reach $2.3 billion in the US market and makes bone grafting a massive global market [1]. Bone graft could be obtained from autograft (patient), allograft (donor), xenograft (another species) and synthetic bone graft. Rather than autograft, allograft, and xenograft, synthetic bone graft most preferred because could decrease operating procedure. Synthetic bone graft could be prepared from calcium phosphate (CaP) synthesizing [2].

Calcium phosphate can be applied in biomedical for bone repair since it is a bioceramic material that has chemical similarity to the mineral bone component, good biocompatibility, and bioactivity [3]. If the ratio of calcium to phosphate is 1.67 so the material named hydroxyapatite (HA) [4]. HA in mineral bone contains impurity ions such as carbonate, magnesium, and fluoride, which carbonate ion is the most abundant species and plays a vital role in bone metabolism. If the carbonate ion substitutes hydroxyl ion or phosphate ion from the crystal structure of HA, then carbonated hydroxyapatite (CHA) is obtained [5]. So, CHA synthesis is needed for bone graft. Therefore, CHA synthesis has been planned.
The preparation of calcium phosphate ceramics by solid-state reaction, co-precipitation, sol-gel, microemulsion, hydrothermal, and microwave irradiation has been reported [6]. The preparation using microwave assisted offers the advantages of heating throughout the volume and transformation of energy efficiency. Ran et al (2007) showed that synthesize CHA using microwave irradiation are faster at forming CHA crystal [7]. Therefore, in this study microwave irradiation was used to synthesize CHA. The variation of power and time irradiating at low numbers was applied to finding the best performance of CHA, to avoid decomposes of carbonate ion and for time efficiency. The best performance of CHA was evaluated by parameters such as crystallinity, crystallite size, and morphology.

2. Materials and methods

2.1. Carbonated Hydroxyapatite Powder Preparation
In this study, we used calcium hydroxide (Ca(OH)$_2$), diammonium hydrogen phosphate ((NH$_4$)$_2$HPO$_4$), and calcium carbonate (CaCO$_3$) for analysis (Merck KgaA Company Inc.) as the starting material for carbonated hydroxyapatite. The wet solution of CHA was carried out by using the precipitation method. Equivolume of 0.6 M diammonium hydrogen phosphate and calcium carbonate (10 mol% from Ca(OH)$_2$) was added simultaneously at rate 5 mL/minute to the 1 M calcium hydroxide under constant stirring. Then, the aqueous suspensions obtained by this process was irradiated using a microwave (Sharp, R-728(W)-IN 900 W) under variation of powers (270, 360 and 450 W) for 10, 20 and 30 minutes, respectively. The aqueous suspension by this process also aged for 24 hours as a control. The samples code as Aging, 270 W 10 minutes, 270 W 20 minutes, 270 W 30 minutes, 360 W 10 minutes, 360 W 20 minutes, 360 W 30 minutes, 450 W 10 minutes, 450 W 20 minutes and 450 W 30 minutes CHA. Then, the suspension was filtered using filter paper (Whatman no. 42) and dried in the oven at 80°C. Then, the dry CHA was ground into fine powder and filtered using powder filter with 100 mesh.

2.2. Chemical and Physical Analysis
X-ray diffractometer (XRD, Panalitycal X’pert Pro MPD) was used for the crystal phase and crystallite size analysis of CHA. The data was collected in the 20 range of 20°–40° with rate 0.4 degrees/second using 0.02 mm Ni-filtered with monochromated Cu Kα (λCuKα = 1.540598 Å, setting the radiation at 40 kV and 30 mA). COD database code: 9003553 (CHA reference) was used for the phase analysis by matching with XRD patterns. The crystallite sizes were calculated by using the Scherrer equation at (002) Miller’s plane. Crystallinity can be interpreted mathematically using the equation:

\[
CI = \frac{(0.24/\beta_{002})^3}{(1)}
\]

where $\beta_{002}$ is FWHM of (002) reflection [9]. The results would be compared and supported by Splitting Factor (SF) method [10].

FTIR (Bruker Tensor 37) was used to investigate the CHA bond. The FTIR spectra were collected in the range 400-3750 cm$^{-1}$ (mid-IR-spectrum) with a resolution of 4 cm$^{-1}$. The ratio of calcium to phosphate (Ca/P) was calculated from EDX (Apollo X from EDAX) results. And the surface morphology of CHA powder was observed by SEM (FESEM: Inspect F10, FEI).

3. Results and discussion

3.1. XRD
The XRD patterns of the samples are shown in Fig 1. Correspond to the COD database code: 9003553, samples had similar apatite to the hexagonal carbonated hydroxyapatite at about diffraction degree 26.00°, 31.84°, 32.27°, 32.96°, and 34.181°. All the patterns showed the low characteristic peaks indicating poor crystallinity and the results are shown in table 1 and figure 2. Crystallinity index of CHA that assisted by microwave showed higher crystallinity than using conventional method (aging). The results of the crystallinity index using microwave showed that CI increase with higher power and time irradiating. These results can be obtained because when CI increased with an increase
of time irradiating, the atoms in carbonated hydroxyapatite neatly arranged [11]. Crystallinity index results showed value in a range 18.6 – 35.3 %, where from these results only sample with code 450 W 30 minutes that fit the bone crystallinity index reference, i.e. 33 – 37% [12]. Crystallinity from SF

![Figure 1. XRD patterns of CHA powders at power (a) 270 Watt (b) 360 Watt and (c) 450 Watt](image)

method showed a range of 2.414 – 2.908, these value lower than bone crystallinity reference, i.e., 2.96 – 5.92 [13]. However, the sample with code 450 W 30 minutes is the closest to the bone crystallinity reference. Hence, in this study, CHA 450 W 30 minutes was chosen as the best result.

Lattice parameters were calculated and showed in table 1. Aging’s lattice parameter revealed almost similar number from reference, COD database code: 9003553 (a = 9.43 Å; c = 6.88 Å). The means score for lattice parameter of CHA using microwave was lower than reference and aging’s sample.

Crystallite sizes were calculated by Scherrer equation at (002) reflection Miller’s plane and showed in table 1. The crystallite size for all CHA samples reached 19.386 – 24.019 nm, were almost similar to the crystallite size for bone, i.e. 25 nm [12]. Crystallite size increased with an increased of time irradiating. However, crystallite size had nonlinear relation with an increase of power irradiating because, at 360 W, the nuclei formation may have consumed most of the microwave energy and decreasing crystal growth[14]

![Figure 2. Crystallinity index of CHA samples that calculated using (a) formula CI = (0.24/β₀₀₂)³ and (b) splitting factor method.](image)
Table 1. Crystallinity index, lattice parameter, and crystallite size from all samples

| Sample | CI (002) (%) | L (002) (nm) | SF | Lattice Parameter (Å) | a | c |
|--------|-------------|--------------|----|-----------------------|---|---|
| Aging  |             |              |    |                       |   |   |
| 10     | 270         | 18.6         | 2.414 | 9.406               | 6.864 | 19.386     |
|        | 360         | 24.8         | 2.632 | 9.361               | 6.824 | 21.357     |
|        | 450         | 29.7         | 2.879 | 9.381               | 6.866 | 23.509     |
| 20     | 270         | 24.4         | 2.455 | 9.375               | 6.830 | 21.246     |
|        | 360         | 27.3         | 2.567 | 9.371               | 6.844 | 22.047     |
|        | 450         | 28.0         | 2.622 | 9.410               | 6.823 | 22.473     |
| 30     | 270         | 27.5         | 2.667 | 9.394               | 6.843 | 22.103     |
|        | 360         | 34.1         | 2.838 | 9.364               | 6.829 | 23.324     |
|        | 450         | 35.3         | 2.908 | 9.373               | 6.841 | 24.019     |

3.2. FTIR

The carbonate bands and other major vibration bands from FTIR spectra for all the CHA samples are presented in table 2. All the samples showed similar bands except for sample with the lowest time and power irradiating, 270 W 10 minutes CHA. The sample with the lowest time and power irradiating, 270 W 10 minutes CHA, had non-apatitic phosphate band at 617 cm⁻¹. Hydroxyl stretches at 3570 and 630 cm⁻¹ disappeared for all the samples was due to the fact that carbonate ions are substituted A-site hydroxyapatite crystal, hydroxide (OH⁻) ions. All the samples also showed broad H₂O absorption as carbonate apatite characteristic [17,18]. The carbonate band at 872-873 cm⁻¹ was the signature band of type-B substitution, carbonate ions substituting for phosphate ions. There is also appear type AB substitution as unstable carbonate hydroxyapatite form, at 1421 and 1455 cm⁻¹. Hence the carbonated hydroxyapatite was form A, B and AB type of CHA.

Table 2. Wavenumber of CHA samples

| Assignment | Wavenumber (cm⁻¹) | Aging | 270/ 10 | 270/ 20 | 270/ 30 | 360/ 10 | 360/ 20 | 360/ 30 | 450/ 10 | 450/ 20 | 450/ 30 |
|------------|------------------|-------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| v₂PO₄³⁻    |                  | 471   | 471     | 471     | 471     | 471     | 471     | 471     | 471     | 472     | 471     |
| v₂PO₄³⁻    |                  | 567   | 553     | 566     | 565     | 565     | 566     | 567     | 566     | 566     | 566     |
|            |                  | 604   | 582     | 604     | 603     | 604     | 604     | 605     | 604     | 604     | 604     |
|            |                  | -     | 617     | -       | -       | -       | -       | -       | -       | -       | -       |
| v₂CO₃³⁻    |                  | 872   | 873     | 873     | 874     | 873     | 874     | 873     | 873     | 874     | 874     |
| v₃PO₄³⁻    |                  | 1035  | 1034    | 1033    | 1026    | 1041    | 1033    | 1035    | 1036    | 1037    |         |
|            |                  | -     | 1063    | -       | -       | -       | -       | -       | -       | -       |         |
|            |                  | -     | 1134    | -       | -       | -       | -       | -       | -       | -       |         |
| v₃CO₃³⁻    |                  | 1422  | 1421    | 1421    | 1421    | 1422    | 1421    | 1421    | 1421    | 1421    | 1421    |
|            |                  | 1454  | 1456    | 1456    | 1455    | 1455    | -       | 1455    | 1455    |         |         |
|            |                  | 1643  | 1641    | 1650    | 1641    | 1642    | 1649    | 1640    | 1640    | 1630    |         |
| H₂O        |                  | 3420  | 3396    | 3430    | 3427    | 3429    | 3430    | 3428    | 3428    | 3431    |         |
3.3. SEM & EDX

The physical characterization for 450 W 30 minutes CHA was analyzed using SEM, magnification 100,000x (Fig. 4), to investigate the morphology of the sample. As shown in figure 4, CHA crystal was formed nanorod morphology from agglomerated particles. The EDX analysis for CHA is shown in Fig. 4. The results show that CHA only contains carbonated hydroxyapatite elements (i.e Ca, C, P, and O). Hydrogen elements aren’t shown in this analysis because has low energy. Ratio calcium to phosphate was calculated to be 1.60. Type-B substitution of CHA had higher ratio Ca/P than hydroxyapatite (Ca/P HA: 1.67) because it was reducing phosphate content. Thus, Ca/P from 450 W 30 minutes CHA was lower than it must be, the probability could be from the interference of hydrogen phosphate ions (HPO$_4^{2-}$) from the starting material diammonium hydrogen phosphate ((NH$_4$)$_2$HPO$_4$) that had an ability to form calcium deficient hydroxyapatite which has Ca/P ratio 1.5 – 1.67 [12].
4. Conclusions
XRD patterns show that CHAs had similar apatite to the hexagonal carbonated hydroxyapatite and had low characteristic peaks that indicating poor crystallinity. According to the crystallinity index, 450 W 30 minutes CHA with CI = 35.3% and SF = 2.908 was chosen as the best result because it was the closest result to the bone crystallinity reference. The crystallite size for all CHA samples reached 19.386 – 24.019 nm, were almost similar to the crystallite size for bone. FTIR and EDX affirmed that CHA bond existed. The carbonated hydroxyapatite was form A, B and AB type of CHA. CHA crystal was formed nanorod morphology. Hence from the physical and chemical analyses suggest that CHA powder can be obtained using a microwave-assisted precipitation method with good results.

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