Influence of Microwave Irradiation Time and Cross-Linker Additions on Synthesis of Hydroxyapatite-Alginate (HA-Alginate) Composite

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Abstract. Composite was prepared with hydroxyapatite and commercial alginate by microwave irradiation method. Hydroxyapatite is a calcium phosphate compound which has a similar chemical composition of human bone tissue. In this study, hydroxyapatite-alginate (HA-Alginate) composite with the mass ratio of 80:20 (HA-Alginate) was synthesized using microwave irradiation for 10 min and 15 min. The power source was settled at 360 W. The functional groups of the composites were examined by FTIR (Fourier Transform Infrared Spectroscopy). The crystallite size and phase purity of HA were determined by XRD (X-Ray Diffraction). The FTIR and XRD results confirmed the existence of alginate and HA in the composites. The crystallite size of the HA increased with the increasing microwave irradiation time. This study also investigated the presence of CaCl₂ as cross-linker agent at different concentrations (0.01 M; 0.03 M; and 0.05 M). The results showed the crystallite size of the HA increased by adding cross-linker from 10.806 nm (without cross-linker) to 13.081 nm (with CaCl₂ 0.05 M) and a composite mass increased from 4.84 gram (without cross-linker) to 5.10 gram (with addition 0.05 M CaCl₂).

1. Introduction
The research of nano-composite for bone tissue is very interesting. The previous studies have been done to obtain materials that have the ability to imitate the natural tissues like bone, from its structure, composition, and also the function. The development of new materials especially bone tissue engineering, was motivated by the need for finding alternative to auto-grafts and allografts. The auto-grafts and allografts have some disadvantages including donor limitations, and occurring clinical cases such as autoimmune response and pathological factors like transmissions and contamination [1].

Bone tissue engineering is a composite which usually consists of a natural polymer as organic compound and calcium phosphate ceramics as an inorganic compound. The used common natural polymers were collagen type 1 and alginate, and the used common ceramic was hydroxyapatite. Hydroxyapatite/HA [Ca₁₀(PO₄)₆(OH)₂] is the closest material to the bone mineral due to its chemical and crystallographic parameters [2]. HA has also good mechanical properties to use bone tissue
engineering application. Alginate is a natural polysaccharides and an anionic bio-polymer. It has bio-compatible, hydrophilic, and biodegradable properties. Using alginate in bone tissue engineering is limited because it has weak mechanical properties, lack of cellular interaction and uncontrollable degradation. To improve these limitations, the use of HA as a reinforcing material is proposed to make HA-alginate composite scaffolds [3].

The nano-composite is a composite reinforcing material that has nano-scale. HA in nano scale makes HA very active and strongly affect as the large surface area [4]. One simple method to make nano-HA is a microwave (MW) irradiation method. MW processing could be more efficient than conventional thermal processing because the MW energy can be directly coupled with the material at the molecular level [5].

The present study investigates the influence of microwave irradiation time on the formation of HA-alginate composite. HA and alginate have different hydrophilicity properties, HA is hydrophobic and alginate is hydrophilic. This study also investigated the presence of CaCl₂ as cross-linker agent at different concentrations (0.01 M; 0.03M; and 0.05M) on the formation HA-alginate composite. This preliminary study aimed to make bio-composite via MW method for medical and tissue engineering applications.

2. Materials and Methods
The materials used for synthesis of HA-Alginate composites were divided into three components; the precursor of HA were calcium hydroxide (Ca (OH)₂; E. Merck); di-ammonium hydrogen phosphate ((NH₄)₂HPO₄; E. Merck) that set at 1.67 for Ca/P molar ratio, the matrix of composite was sodium alginate (HIMEDIA, MB114, CAS No. 9005-38-3), and the cross-linker agent was calcium chloride (CaCl₂; E. Merck).

2.1. Synthesis of HA-alginate composite
The alginate solution was added to the calcium suspension then the phosphate solution was titrated to the mixture solution with continuous stirring. The composite solution then added CaCl₂ as a cross-linker agent. The schematic of synthesis process was illustrated in the Figure 1. The concentration of cross-linker agent varied at 0.01; 0.03; and 0.05 M as shown in Table 1. The mass ratio of HA and alginate was 80:20. The composite solution was exposed to microwave irradiation for 10 min and 15 min at 360 W. The sample was dried in an oven. The dried samples were characterized by XRD and FTIR.

![Figure 1. Schematic of synthesis HA-alginate composite using microwave irradiation.](image-url)
Table 1. Preparation conditions for HA-alginate composite.

| Irradiation Time (min) | Concentration CaCl₂ (Molar) |
|------------------------|-----------------------------|
| A1                     | 15                          |
| A2                     | 15                          | 0.01 |
| A3                     | 15                          | 0.03 |
| A4                     | 15                          | 0.05 |
| B1                     | 10                          | -   |
| B2                     | 10                          | 0.01 |
| B3                     | 10                          | 0.03 |
| B4                     | 10                          | 0.05 |

2.2. Characterization HA-alginate composite

The lattice parameter, crystallite size, crystal index (CI) and phase purity of HA-alginate composites were characterized by X-ray diffraction (XRD). The crystallite size of HA can be calculated using Scherer’s formula, because the peak broadening of the XRD reflection was used to estimate the crystallite size in a direction perpendicular to the crystallographic plane [4]. The crystal index (CI) or fraction of crystallinity $X_c$ of HA was determined [6] from the following equation:

$$X_c = \left(\frac{K}{B_{002}}\right)^3$$  \hspace{1cm} (1)

Where K is a constant found equal to 0.24 for a very large number of different HA powders, and $B_{002}$ is FWHM (°) of reflection (002). FTIR-ATR diamond (Fourier Transform Infrared Spectroscopy - Attenuated Total Reflection) (Thermo scientific Nicolet iS10) was used to examine the functional groups present in the composite.

3. Result and discussion

3.1. XRD analysis

Figure 2 presented XRD patterns of pure HA synthesized by microwave method for 10 min and 15 min. The patterns of pure HA synthesized were compared with HA reference (96-100-1234) via High Score software. The similar scores were 58% (pure HA synthesized for 10 min) and 55% (pure HA synthesized for 10 min). The XRD patterns of HA-alginate composites were shown in Figure 3. The patterns of composites were compared to pure HA synthesized and commercial alginate. The result showed that all composites had two components; a similar pattern to pure HA synthesized and the XRD patterns of all of the composites at two theta below 20° show the presence alginate in composites. Table 2 presents the structural features of HA-alginate composites from XRD analysis and mass of the composite. The crystallite size and crystal index of the HA increase with longer irradiation time. The crystallite size of HA increased from 10.806 nm (irradiation for 10 min) to 12.047 nm (irradiation for 15 min). Crystal index (CI) increased from 0.028 (irradiation for 10 min) to 0.039 (irradiation for 15 min). Increasing irradiation time enhances the crystal index and the crystallite size of HA [7]. HA has hexagonal structure, whose atoms occupy special position, needs longer time for preparation of intact crystal [8].

The composite which was prepared at different concentration of CaCl₂, the crystallite size and the crystal index values increased with increasing concentration. The crystallite size of HA increased from 12.660 nm (CaCl₂ 0.01M and irradiation for 10 min) to 13.081 nm (CaCl₂ 0.05M and irradiation for 10 min) and from 12.756 nm (CaCl₂ 0.01M and irradiation for 15 min) to 13.196 nm (CaCl₂ 0.05M and irradiation for 15 min). The crystal index (CI) increased from 0.045 (CaCl₂ 0.01M and irradiation for 10 min) to 0.049 (CaCl₂ 0.05M and irradiation for 10 min) and from 0.046 (CaCl₂ 0.01M and irradiation for 15 min) to 0.052 (CaCl₂ 0.05M and irradiation for 15 min). The crystallite size of HA increased because of ionic interaction between divalent cations (Ca²⁺) of CaCl₂ and carboxylate group anions (COO⁻) of alginate polymeric backbone [9]. Increasing concentration of CaCl₂ decreased
interaction between divalent cations (Ca$^{2+}$) of Ca(OH)$_2$ as precursor of HA and carboxylate group anions (COO$^-$) of alginate.

Figure 2. XRD patterns of pure HA synthesized which exposed in MW (a) for 15 min and (b) for 10 min.

Figure 3. XRD patterns of HA-alginate composites which exposed in MW (a) for 15 min and (b) for 10 min.

The lattice parameter of all composites at a value a ~ 9.42 Å and c ~ 6.88 Å, indicated to a hexagonal standard HA pattern (JCPDS 9-432) [1]. The mass of composite increased as the increasing concentration of CaCl$_2$, but it is not significant. The mass of composite increased from 4.84 gram (without cross-linker and irradiation for 15 min) to 5.10 gram (CaCl$_2$ 0.05M and irradiation for 15 min) and from 4.94 gram (without cross-linker and irradiation for 10 min) to 5.11 gram (CaCl$_2$ 0.05M and irradiation for 10 min). The increase of CaCl$_2$ concentration raised the amount cation (Ca$^{2+}$) of Ca (OH)$_2$ to form HA.
Table 2. Structural features from XRD analysis and mass of the composite.

|       | Crystallite size (nm) | HA crystal lattice parameter (Å) | CI  | Mass (gram) |
|-------|-----------------------|----------------------------------|-----|-------------|
| A1    | 12.047                | 9.432                            | 6.887 | 0.039       | 4.84 |
| A2    | 12.756                | 9.426                            | 6.889 | 0.046       | 5.00 |
| A3    | 12.818                | 9.421                            | 6.888 | 0.047       | 5.04 |
| A4    | 13.196                | 9.428                            | 6.883 | 0.052       | 5.10 |
| B1    | 10.806                | 9.425                            | 6.886 | 0.028       | 4.94 |
| B2    | 12.660                | 9.423                            | 6.885 | 0.045       | 5.01 |
| B3    | 12.780                | 9.428                            | 6.880 | 0.047       | 5.05 |
| B4    | 13.018                | 9.427                            | 6.888 | 0.049       | 5.11 |

3.2. FTIR analysis

FTIR spectra of all samples are shown in Figure 4. The spectra of all composites show that the presence of alginate with HA. Alginate has three characteristic absorption peaks: hydroxyl group (OH$^-$) at 3500 cm$^{-1}$ [10], asymmetric COO$^-$ stretching vibration ($\nu_3$COO$^-$) at 1635 cm$^{-1}$, and symmetric COO$^-$ stretching vibration ($\nu_1$COO$^-$) at 1419 cm$^{-1}$ [11]. HA has four group functional absorption peaks: hydroxyl group (OH) 3440 cm$^{-1}$, CO$_3^{2-}$ at 1459-1651 cm$^{-1}$, asymmetric PO$_4^{3-}$ stretching vibration ($\nu_3$PO$_4^{3-}$) at 1044 cm$^{-1}$, and symmetric PO$_4^{3-}$ bending vibration ($\nu_4$PO$_4^{3-}$) at 568 cm$^{-1}$ [12]. Table 3 presents the wave number of alginate and HA in the HA-alginate composites.

Figure 4. FTIR spectra of HA-alginate composites which were exposed in MW (a) for 15 min and (b) for 10 min.

Table 3. Characteristic infrared bends for HA-alginate composites.

| Assignment | Assignment | Assignment | Assignment | Assignment | Assignment |
|------------|------------|------------|------------|------------|------------|
| OH         | $\nu_3$COO$^-$ | $\nu_1$COO$^-$ | CO$_3^{2-}$ | $\nu_3$PO$_4^{3-}$ | $\nu_4$PO$_4^{3-}$ |
| A1         | 3446.21    | 1620.18    | 1421.31; 1620.18 | 1031.9    | 563.52     |
| A2         | 3339.29    | 1602.82    | 1414.58; 1602.82 | 1014.58   | 559.05     |
| A3         | 3340.23    | 1601.88    | 1415.53; 1601.88 | 1013.64   | 559.05     |
| A4         | 3331.76    | 1601.88    | 1414.76; 1601.88 | 1022.17   | 556.23     |
| B1         | 3379.27    | 1600.94    | 1415.80; 1600.94 | 1018.28   | 558.11     |
| B2         | 3363.76    | 1599.05    | 1416.47; 1599.05 | 1017.41   | 557.17     |
| B3         | 3380.70    | 1600.94    | 1413.64; 1600.94 | 1022.12   | 558.11     |
| B4         | 3348.70    | 1600      | 1415.53; 1600.53 | 1015.53   | 556.23     |
4. Conclusion
Hydroxyapatite-alginate (HA-Alginate) composites were successfully synthesized by microwave irradiation method. The XRD and FTIR results confirmed the presence of alginate with HA in the composites. The increase of irradiation time and addition of cross-linker enhanced the crystallite size of HA in the composites. The biggest crystal size and crystal index of HA in the composites were 13.196 nm and 0.052, when the HA-alginate composite was prepared by adding 0.05 M CaCl₂ and irradiation for 15 minutes. Composites mass increased by adding cross-linker and longer irradiation time, from 4.84 gram to 5.10 gram when the HA-alginate composite irradiation was 10 minutes, and from 4.94 gram to 5.11 gram when the HA-alginate composite irradiation was 15 minutes. The irradiation time and the presence of cross-linker affected crystallite size and crystal index of HA in the composites and composites mass obtained.

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