Synthesis and characterization of hydroxyapatite-chitosan composite in situ by microwave irradiation method

F M Ersal¹, Nurlely¹, Y W Sari²

¹Department of Physics, Universitas Indonesia, 16424, Depok, Indonesia
²Department of Physics, Bogor Agricultural University, 16680, Bogor, Indonesia

Email: nurlely@sci.ui.ac.id

Abstract. Chitosan is a natural polysaccharide which has bioresorbable, biocompatible, and non-toxic properties. The combination between chitosan and hydroxyapatite will form a nanocomposite which improves its mechanical properties and can be applied as a bone implant material. The aim of this work was synthesis and characterization of composite hydroxyapatite-chitosan through in situ way utilized by microwave irradiation with time variation. A composite which was formed by hydroxyapatite and 2% chitosan solution through in situ way were irradiated by 270-watt power for 20-60 minutes with an interval of 10 minutes. The XRD results showed peak hydroxyapatite and chitosan. Meanwhile, the highest crystallite size was found in 50 minutes composite with value 20.87 nm. The FTIR spectroscopy identified the functional group of hydroxyapatite and chitosan (NH). Increase in irradiation time caused reduction of chitosan-based on the existence of NH composition. Highest mass was present at time 20 minutes which means the reduction of mass also occurred with increasing irradiation time. The SEM and EDX results indicated the hydroxyapatite has filled chitosan matrix. The presence of NH cluster proved that microwave irradiation method can be used to synthesize nanocomposites hydroxyapatite and chitosan. Increase in irradiation time caused a reduction of chitosan existence and its mass.

Keywords: Biophysics, Composite, Biomaterial, Microwave Irradiation.

1. Introduction

Calcium phosphate in its clinical applications is an interesting field of research and development in the production of useful biomaterials [1] [2]. Hydroxyapatite with chemical formula Ca₁₀(PO₄)₆(OH)₂ is the most used calcium phosphate to bone and teeth implant fabrication due to the components are the most similar to bone among other calcium phosphates minerals [3]. Furthermore, hydroxyapatite has some advantages such as biocompatible, bioactive, and osteoconductive which is a parameter to determine the quality of biomaterial.

Chitosan is a natural polysaccharide in the deacetylated product of chitin. The availability of chitosan is abundantly found in crustacean shells [4]. Chitosan has some characteristics such as bioresorbable, biocompatible, non-toxicity, non-antigenic, biofunctional [5] and osteoconductive [6]. Besides that, chitosan also has weak mechanical properties, unless if combined with another biomaterial such as hydroxyapatite or bioglass ceramic (BGC) to form a nanocomposite, the mechanical properties will increase and can be used as a bone graft or fracture repair [7].

Research about composites hydroxyapatite-chitosan in situ has been done before. It produced an interconnected porous structure, controlled biodegradation and have better application in tissue engineering because they have a better morphology and allow the cell growth [8].
2. Materials and methods
2.1. Synthesis of the hydroxyapatite-chitosan composite in situ

The following chemicals were purchased and were used without further purification: calcium hydroxide (Ca(OH)\textsubscript{2}, Merck KgaA Company Inc.), diammonium hydrogen phosphate ((NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}, Merck KgaA Company Inc.), acetic acid (CH\textsubscript{3}COOH, 98%), distilled water, chitosan (84% deacetylation, CV, ChiMultiguna).

Generally, this research was about the synthesis of a hydroxyapatite-chitosan composite in situ with 20/80 (wt/wt) composition. The results were irradiated by microwave with 270-watt power for 20-60 minutes with an interval of 10 minutes. The procedure was prepared by dissolve chitosan powder into 2% acetic acid solution. Precursors Ca(OH)\textsubscript{2} and (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} ratio was 1.67 which was both precursors were dissolved in 100 ml distilled water up to 1 M concentration for Ca(OH)\textsubscript{2} and 0.6 M for (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4}. Then the Ca(OH)\textsubscript{2} solution was added to chitosan solution which was stirred using magnetic stirrer. Mixing has been one by slowly adding 0.6 M (NH\textsubscript{4})\textsubscript{2}HPO\textsubscript{4} into solution with a droplet rate of 5 ml/min. The solution was stirred using magnetic stirrer for 15 minutes.

The mixture was irradiated with microwave (SHARP R-728-IN) using power 270 watt. As a control, another mixture was aged for 24 hours. The composite was dried using the oven at 40°C.

2.2. Characterization

The composite powders were characterized using several analysis techniques. Fourier transform infrared spectroscopy (FTIR, Bruker Tensor 37) was used for functional groups analysis of composite at a range of 400–4000 cm\textsuperscript{-1} (mid-IR-spectrum) with the resolution of 4 cm\textsuperscript{-1}. The crystal phase analysis by X-ray diffraction (XRD, Panalitical X'Pert Pro MPD) using 0.02 mm Ni-filtered with monochromated Cu Kα (λ\textsubscript{CuKα} = 1.540598 Å, setting the radiation at 40 kV and 30 mA). Particles were examined over the 20 range of 20°–60° with the size of step 0.4°/s. The crystallite size was determined by using Scherrer formula [14] and the crystal index of the composites was also evaluated [15]. The morphology and element composition of composites were investigated by scanning electron microscopy (SEM, FEI inspect F10) coupled with energy dispersive x-ray (EDX, EDAX Apollo) spectroscopy.

3. Results and discussion
3.1. Mass efficiency and X-ray diffraction

The XRD patterns of the composite are shown in Fig.1. The patterns were matches with Crystallography Open Database code number 2105283 with crystal system hexagonal, space group of P 6\textsubscript{3}m, and space group number of 176 [16]. The results of lattice parameters, crystallite size, and crystallinity index of the composite with a variation of the time were calculated using XRD data are shown in Table 1.

The largest mass was found in Aging composite with a value of 48.87 grams. Meanwhile, for composites with microwave radiation, the largest mass was found in a sample of 20 minutes with a value of 40.01 grams. Furthermore, the mass continues to decrease until the 60 minutes composite with a value of 33.37 grams. This showed that increasing radiation time caused the reducing of composite mass. This was allegedly due to the reduced number of chitosan which was an organic compound. For the Aging composite, chitosan was not reduced due to the absence of radiation. Increased radiation time with 270 W power showed an increase in the size of crystallite size as shown in Table 1. The crystal size and crystal index in Aging composites was relatively low because in the in situ method the apatite formation process was carried out in the chitosan matrix so that chitosan spreads more and caused more amorphous composites. Meanwhile on composites that were microwaved, the crystal size in 20 minutes composite with a value of 16.71 nm the crystal size was increased up to 50 minutes composite with a value of 20.87 nm, this was reported in another study which stated that the increase in radiation time results in an increase in crystal size and crystal index. [9].
Figure 1. XRD pattern of obtained composites for Aging, 20, 30, 40, 50, and 60 minutes of radiation time.

Table 1. Calculated lattice parameter, crystal size, mass and crystal index at 270 W microwave power for aging, 20, 30, 40, 50, and 60 minutes.

| Time (minutes) | Lattice Parameter | Crystallite Size | Crystal Index | Mass (gr) |
|----------------|-------------------|-----------------|---------------|-----------|
|                | a (Å)             | c (Å)           | (nm)          |           |
| Aging          | 9.410             | 6.868           | 15.67         | 0.09      | 48.87     |
| 20             | 9.410             | 6.872           | 16.71         | 0.11      | 40.01     |
| 30             | 9.406             | 6.866           | 17.18         | 0.12      | 34.57     |
| 40             | 9.409             | 6.870           | 19.51         | 0.19      | 34.03     |
| 50             | 9.408             | 6.866           | 20.87         | 0.23      | 34.02     |
| 60             | 9.476             | 6.848           | 12.54         | 0.05      | 33.37     |

Increasing of crystal index were caused by the energy of microwave radiation transferred to the molecule, causing the molecules to move rapidly and collide each other with high energy [10]. The rapid temperature changed, accelerated atomic motion and decreased the diffusion barrier, thus making it easier for atoms to be transported to the lattice [11]. However, at a composite of 60 minutes, the crystal size and crystal index decreased significantly, based on the XRD graph which showed a peak at 2θ (002), (300), and (310) planes was decreased. This occurred to be suspected denaturation of chitosan matrix which also affected the apatite so that the nucleation process was inhibited.

The lattice parameter of the Aging composite at value a=9.410 Å and c=6.868 Å, from all the composite results with microwave radiation, the closest composite to Aging composite was 50 minutes composite with a value of a=9.408 Å and c=6.866 Å and 40 minutes composite with a value of a=9.409 Å and c=6.870 Å.
3.2. Fourier transform-IR

The FTIR spectrum of composites are shown in Fig. 2 and the structure of hydroxyapatite and chitosan are shown in Table 2. The results showed all composites have properties of hydroxyapatite ($\text{PO}_4^3-$, OH$^-$) [12] and chitosan (NH$_2$, CH) [8]. Mixed characteristic peaks of hydroxyapatite and chitosan were observed clearly indicated that hydroxyapatite particle was dispersed in the chitosan matrix. The chitosan spectrum band in composites decreased, which indicated that there was some intermolecular and coordination chemical interaction between chitosan and calcium ion of hydroxyapatite particles. These chemical interactions made the composite more rigid and stable [8]. The results of splitting factor showed that the behavior oh crystal index that was increased up to 50 minutes composite.

Meanwhile, all composites have a considerable amount of H$_2$O, according to the wide OH spectrum, with the Aging composite having the most H$_2$O. However as the radiation time increases, the H$_2$O of 20, 30, 40, 50, and 60 composite was decreased based on OH$^-$ spectrum which was not as wide as the Aging composite.

![Figure 2. IR spectrum of composites for Aging, 20, 30, 40, 50, and 60 minutes time radiation.](image)

### Table 2. Observed IR bands and splitting factor assigned to composite for Aging, 20, 30, 40, 50, and 60 minutes.

| Assignments | $v_2^{\text{PO}_4^3-}$ | $v_4^{\text{PO}_4^3-}$ | OH$^-$ | $v_1^{\text{PO}_4^3-}$ | $v_3^{\text{PO}_4^3-}$ | OH$^-$ | CH | NH$_2$ | \(\text{Splitting Factor}\) |
|-------------|-----------------|-----------------|-------|-----------------|-----------------|-------|-----|-------|-----------------|
| Aging       | 469             | 564             | 661   | 961             | 1079            | 1158  | 3402| 2923  | 1565            | 2.98             |
| 20 minutes  | 472             | 565             | 662   | 961             | 1034            | 1158  | 3426| 2924  | 1564            | 3.14             |
| 30 minutes  | 472             | 565             | 662   | 962             | 1034            | 1153  | 3419| 2923  | 1567            | 3.23             |
| 40 minutes  | 472             | 565             | 662   | 962             | 1058            | 1152  | 3405| 2923  | 1565            | 3.33             |
| 50 minutes  | 472             | 566             | 662   | 962             | 1041            | 1154  | 3432| 2924  | 1566            | 3.55             |
| 60 minutes  | 472             | 566             | 661   | 962             | 1049            | 1152  | 3395| 2924  | 1564            | 2.58             |
3.3. SEM & EDX

The morphology and element composition pure chitosan and 50 minutes composite are shown in Fig. 3 and Fig. 4. Chitosan has dense and wavy structure when it combined with hydroxyapatite, a distribution of hydroxyapatite on the surface of the chitosan was observed which means that the hydroxyapatite has filled the chitosan matrix in form of the fine crystal due to the homogeneous composite surface could give more areas for bone cell deposition. Hydroxyapatite particles are also crystals that seem to have growth direction given by the microwave irradiation [13], it is known by the existence of hydroxyapatite and the porosity structures of chitosan were found in this composite. The particle size was 94.72 nm, indicated the particle size is the same as the particle of hydroxyapatite which has range 60 nm-300 μm [17].

The Ca/P measurement ratio obtained by EDX analysis in 50 minutes composite tended to be smaller as 1.37 compared to the pure Ca/P hydroxyapatite ratio of 1.67 due to the composition of hydroxyapatite is less than chitosan. The presence of chitosan caused the Ca/P ratio not to be 1.67. The EDX results also showed the elements in the composite, namely Ca, P, N, C and O where chitosan was dominated by O and C because of their presence on the structure of chitosan added with the presence of a large amount of water absorption from FTIR analysis.

Figure 3. The SEM image and EDX spectrum of pure chitosan at the 50.000x magnification.

Figure 4. The SEM image and EDX spectrum of 50 minutes composite at the 50.000x magnification.
4. Conclusions

Hydroxyapatite-chitosan composites were successfully synthesized via microwave irradiation method. The X-ray diffraction results showed the existence of hydroxyapatite and chitosan peak which means microwave irradiation could form the crystalline phase of hydroxyapatite without removing the chitosan phase. However, 50 minutes composite has the most similar crystalline phase with hydroxyapatite and an increase in radiation time also causes a decrease in mass. The FTIR results showed that microwave irradiation did not remove the existence of hydroxyapatite and chitosan in the composite through their spectrum. The SEM and EDX result showed the presence of hydroxyapatite distribution on the surface of chitosan and pores in 50 minutes composite. The particle size was 94.72 nm, indicated the particle size is the same as the particle of hydroxyapatite. However, this composite has still to be evaluated due to the Ca/P ratio is less than 1.67 which is ratio of pure hydroxyapatite.

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